

## ORIGINAL ARTICLE



# Arbuscular mycorrhizal fungi in *Melocanna baccifera* from disturbed and undisturbed sites in Mizoram, India

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*Melocanna baccifera* is a well-known native bamboo species of India belonging to the grass family Poaceae. A study on the presence of arbuscular mycorrhizal fungi (AMF) association with *M. baccifera* was conducted from a disturbed and undisturbed bamboo forest sites within Mizoram University, Mizoram, India. Soil from the rhizosphere region as well as physico-chemical properties of the soil were taken to study AMF diversity. The roots were observed for percentage colonization by AMF. It was found that undisturbed site had higher colonization percentage (56%) than the disturbed site (46%). The Shannon's diversity index showed that undisturbed site (1.46) had more diversity than the disturbed site (1.59) while disturbed site showed lower index of dominance (3.34) which indicates higher shared dominance of AMF species than undisturbed site (4.66).

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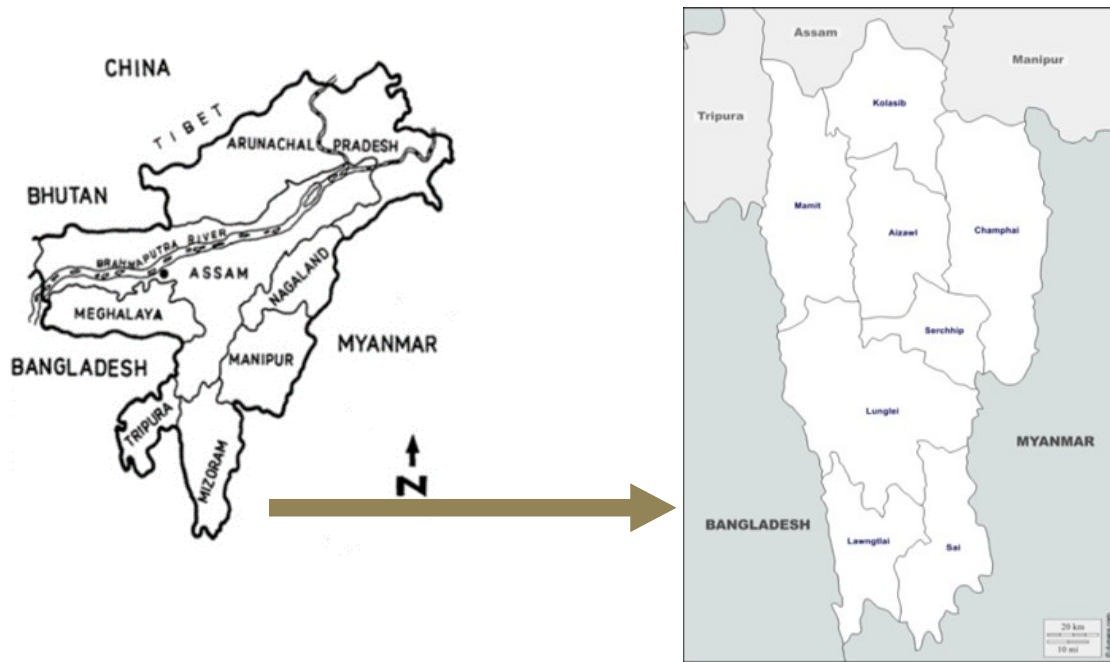
## Introduction

A mycorrhiza is a symbiotic (generally mutualistic, but occasionally weakly pathogenic) association between a fungus and the roots of a vascular plant. Mycorrhiza is a composite structure consisting of fungus and higher plants roots. In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF or AM), or extracellularly as in ectomycorrhizal fungi. They are an important component of soil life and soil chemistry. Mycorrhizas form a mutualistic relationship with the roots of most plant species. While only a small proportion of all species has been examined, 95% of those plant families are predominantly mycorrhizal.<sup>1</sup> This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose.<sup>2</sup>

*Melocanna baccifera* (Roxburgh) Kurz. is a bamboo species native to India, Bangladesh, Myanmar (Burma) and Nepal belonging to the grass

family Poaceae.<sup>3</sup> It has prime economical as well as ecological significance. It spreads aggressively and dominates over other vegetation in a short time.<sup>3,4</sup> *M. baccifera* is an aggressive bamboo, easily occupying large open areas, due to its vigorous long rhizomes and, when fruiting, due to its easily germinating fruits. It flowers gregariously, with a flowering cycle of 30-45 years,<sup>5</sup> while other worker reported that the flowering cycle to be 48 years.<sup>6</sup> The root system of bamboos, in general, is superficial and does not grow to more than a meter deep.<sup>7</sup> Bamboo forests have ecological and environmental functions in terms of control of soil erosion, land rehabilitation, water conservation and carbon sequestration. Bamboos also play an important role in biodiversity conservation.<sup>8</sup> They are reported to be found in diverse land areas such as calcareous grasslands, arid/semi-arid grasslands, several temperate forests, tropical rain forests, shrub and degraded lands in different parts of the world.<sup>9-11</sup>

The information of mycorrhiza on bamboo is very limited from India.<sup>12,13</sup> There is no information record



**Figure 1** | Map of northeast India showing the study site in Mizoram. Δ represents the two study sites in Mizoram University.

about mycorrhizal study on bamboo from Mizoram. Thus, this study aims to investigate the diversity of the species present in disturbed and undisturbed stands of *M. baccifera* as well as the differences in the root colonization and spore number of the AMF species in the respective soil.

**Materials and Methods**

**Study sites**

The study was conducted in Mizoram University,

Tanhril, Aizawl, Mizoram, India (Fig. 1). It lies at latitude a of 23° 44'22"N and a longitude of 92° 39'54"E. The disturbed site is a roadside plantation with continuous anthropogenic disturbances and the undisturbed site is located inside the campus forest which is not under any anthropogenic activity. The two study sites of bamboo stands are inside the Mizoram University Campus as shown in the map (Fig. 2).

**Soil analysis**

Sampling of soil was done once every month



**Figure 2** | A. Disturbed site; B. Undisturbed site.

from February to June 2018 by taking about 200 mg of soil to study the physico-chemical properties of the soil. Soil moisture was determined by drying 10 g fresh soil at 105°C for 24 h in a hot-air oven. Soil bulk density was estimated by using soil corer method.<sup>14</sup> Soil pH was measured by using pH meter. Organic carbon was analyzed by colorimetric method.<sup>15</sup>

### AMF assessment

**Root colonization:** For the analysis of mycorrhizal colonization in the plants, the root samples were washed free of soil and cut into 1 cm long bits, cleared in 2.5% KOH at 90°C for 20-30 minutes, rinsed in water, acidified with 5N HCl and stained in lactophenol containing 0.05% trypan blue.<sup>16</sup> Fifty (50) segments of stained root samples were mounted on slides and examined for AMF colonization under a compound microscope at 10x10 magnifications. Percent root colonization was calculated.<sup>17</sup> Percent root colonization was determined using the following formula:

$$\% \text{ Root Colonization} = \frac{\text{No of Positive segments}}{\text{No of segments observed}} \times 100$$

**Spore analysis:** The soil samples were collected from the two study sites inside Mizoram University campus, India. Three soil replicates of the rhizosphere region from the two study sites were sampled to examine the sporocarpic species of AMF. The soil sample from each site was made into one composite soil sample and transported to laboratory for analysis. Spore extraction was done from 100 g of soil samples following wet sieving and decanting method.<sup>18</sup>

The isolated spores were picked up with needle under a dissecting microscope and were mounted in Meltzer's reagent for identification. The complete and broken spores were examined using a compound microscope with a transmitted light illumination. Images of all the spores were taken. Taxonomic identification of spores to species level was based on sporocarpic size, colour, ornamentation and wall characteristics by matching original descriptions.<sup>19-27</sup>

### Statistical analysis

The individual soil parameters and AMF colonization were analyzed for mean, standard

deviation and standard error using MS Excel. Pearson's correlation coefficient was analyzed between mycorrhizal colonization, spore number and the selected soil parameters. Simpson dominance index and Shannon diversity index were calculated using MS Excel.

### Results and Discussion

The soil physico-chemical properties are shown in Table 1. Soil carbon and moisture content were found to be higher in undisturbed area than disturbed site whereas bulk density was found to be higher in disturbed site. The disturbed site was more acidic ( $5.76 \pm 0.351$ ) than undisturbed area ( $6.74 \pm 0.327$ ).

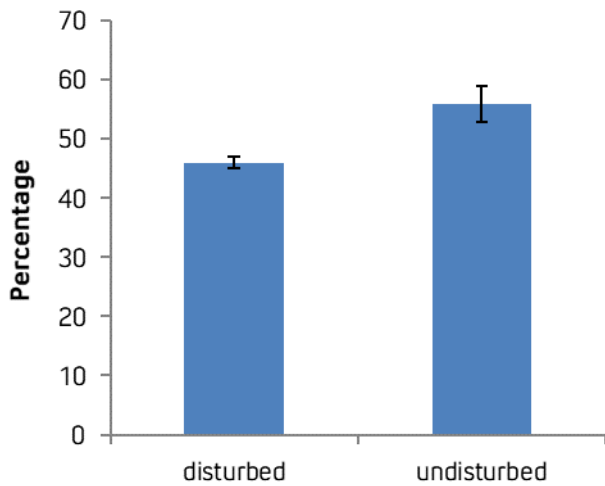
*M. baccifera* maintains a characteristics arbuscular mycorrhizal fungi plants association in both the study sites. The mean percentage of colonization in the disturbed site was lower (46%) than the undisturbed site, i.e. 56% (Fig. 3). The population of spores is relatively higher in undisturbed area as compared to disturbed area. The average numbers of spores extracted from the rhizosphere soil was also lower in the disturbed site (27 per 100 g dry soil) than the undisturbed site (43 per 100 g dry soil) (Fig. 4). There was a reduction in the rate of host root infection, formation and colonization of VAM fungi from the disturbed site which was also reported by several workers.<sup>12,28,29</sup> The ability of the AM fungus to spread and form a hyphal network in the bamboo roots may have been influenced by the soil physical properties, such as compaction and water retention as reported by Gaur and Adholeya.<sup>30</sup>

Pearson correlation was done between percentage of AMF colonization and spore numbers with different soil parameters. A significant correlation (0.992\*\* and 0.934\*) was found between number of spores and soil carbon in both the study sites of undisturbed and disturbed respectively while a negative correlation (-0.958\* and -0.925\*) was found between spore numbers and bulk density in both the study sites respectively. Effect of reduction of AMF propagules due to compaction of soil from 30% to 50% when a forest soil is severely disturbed or exposed have also been recorded by Ahmad.<sup>31</sup> Soil moisture content and percentage of root colonization shows positive correlation (0.900\* and 0.960\*) in disturbed area and undisturbed sites respectively (Table 2).

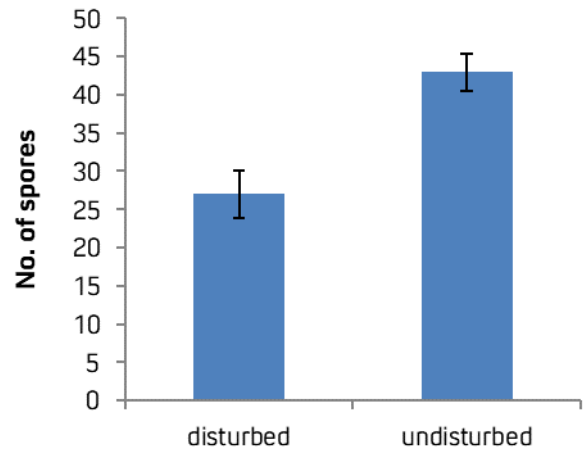
Among the ten species, four different genera

**Table 1 |** Soil physico-chemical properties from the two study sites;  $\pm$  indicates standard error.

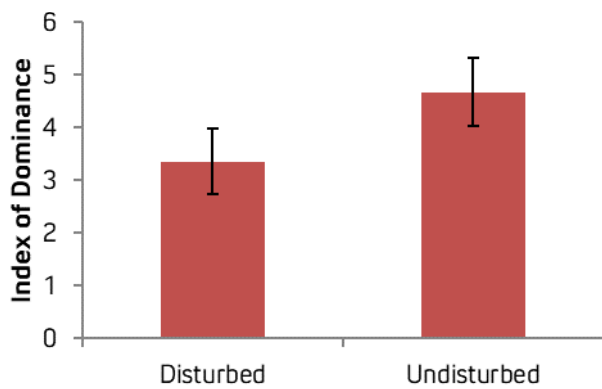
Site	pH	Bulk density	Carbon (%)	Moisture (%)
Disturbed	5.76 $\pm$ 0.351	64.7 $\pm$ 3.184	2.1 $\pm$ 0.380	25.32 $\pm$ 3.159
Undisturbed	6.74 $\pm$ 0.327	57 $\pm$ 3.295	2.8 $\pm$ 0.478	27.11 $\pm$ 2.420



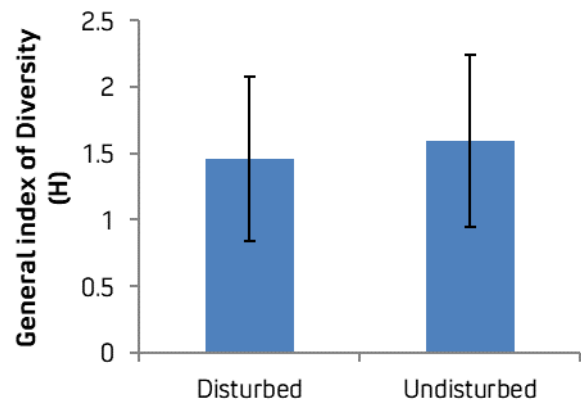
**Figure 3 |** Root colonization percentage in *Melocanna baccifera* from the two study sites.



**Figure 4 |** Number of spores in *Melocanna baccifera* from the two study sites.



**Figure 5 |** Simpson index of dominance from the two study sites.



**Figure 6 |** Shannon's general index of diversity from the two study sites.

were recorded in which six species belong to *Glomus*, two species belong to *Acaulospora* and one species from both *Diversispora* and *Funneliformis*. *Glomus* species is the most dominant which indicated that this species may have established a permanent favorable condition for them to generate their life cycle in the rhizosphere with their host plants. While all the ten species were recorded from undisturbed site two species *Diversispora globifera* and *Glomus boreale* were not recorded from disturbed site.

The lower index of dominance (3.34) in the disturbed site indicates shared dominance of AMF species is higher than the undisturbed site which has the higher value of 4.668 (Fig. 5). Species dominance in the disturbed site was attributed to two AMF species *Funneliformis mosseae* and *G. fasciculatum* with a percentage frequency occurrence of 70% and 57% respectively. Similarly, the species dominance in the disturbed site was also attributed to the AMF species *F. mosseae* and *G. fasciculatum* with a

percentage frequency occurrence of 44% and 55% respectively. In case of the Shannon's diversity index, a value of 1.46 and 1.59 was found for disturbed and undisturbed site respectively suggesting a greater diversity of AM fungi species in the undisturbed site than in disturbed site. There was a lesser number of AMF species in the disturbed site in comparison to the undisturbed site with a total of 8 and 10 respectively. A lesser number of AM fungal species in disturbed jhum land than a natural forest was also reported by Singh *et al.*<sup>12</sup> There are several reports about AM fungi being affected by different disturbances like difference in precipitation,<sup>32-34</sup> plant community composition,<sup>35,36</sup> and soil characteristics, especially soil pH.<sup>33,37</sup> However, the AMF species present at the disturbed site might be due to the ability to overcome these various stresses.

**Conclusion**

The present study shows a lesser AMF species



**Table 2** | Correlation between different soil properties with percentage of colonization and spore number.

Soil properties	Disturbed		Undisturbed	
	Colonization	Spore number	Colonization	Spore number
Bulk Density	0.583	-.925*	-.761	-.958*
Soil Moisture	0.900*	0.047	0.960*	0.451
pH	-.248	-.366	-.430	0.354
Carbon	0.225	0.934*	0.670	0.992**

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*\*Correlation is significant at the 0.05 level (2-tailed).

**Table 3** | Percentage frequency of occurrence (%) of AM fungal spores in disturbed and undisturbed sites.

Species	Disturbed	Undisturbed
<i>Acaulospora scrobiculata</i>	5	17
<i>A. tuberculata</i>	8	14
<i>Diversispora globifera</i>	-	6
<i>Funnelformis mosseae</i>	44	70
<i>Glomus verruculosum</i>	11	10
<i>G. fasciculatum</i>	58	57
<i>G. constrictum</i>	5	14
<i>G. clarum</i>	3	8
<i>G. magnicaulis</i>	1	9
<i>G. boreale</i>	-	8

abundance in an undisturbed stand as compared to a disturbed *M. baccifera* stand. Long term anthropogenic activity in the disturbed site might have caused adverse effect on the survival and distribution of AMF species which may in turn affect the AMF population and soil fertility. It was also observed that there was an absence or loss of two AMF species which may have been eliminated temporarily or perhaps permanently from the disturbed site.

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### Conflict of interest

None declared.

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