4. RESULTS

4.1 Description of study sites

The study site was located in Karbi Anglong Hills (92°45' and 93°54' East Longitude and 25°45' and 26°35' North Latitude) of Assam, India (Figure 4). Study sites showing different degree of disturbances were selected for the present study. Degree of disturbances at selected sites was assessed from plant coverage, vegetation types and amount of forest floor organic matter removal. Three forests representing undisturbed forest (UF), monoculture forest (MF) and slash-and-burn field (SPF) were consequently selected for the present study. The three sites- UF, SBF and MF were located adjacently on a hilly slope at an altitude of 232 m above sea level. The climate of the hill district is typical sub-tropical monsoonal characterized by high relative humidity (73-84%) throughout the year. The annual means for rainfall and temperature based on historic records from January 2000 to December 2012 in the Karbi Anglong hills show that rainfall occurs all through the year, with predominance during spring and summer (Figure 5a). The average mean annual precipitation (2000-2012) was 123.5 cm. Annual mean air temperature (2000-2012) was 23.8°C. Though pre-monsoon showers were experienced in April, proper monsoon began from June, which peaked in July-August and tapered off by October. The highest temperatures occurred during June to September. For the period of the present study (2009-2011), the average temperature and rainfall were highest during spring and summer, in contrast with autumn and winter (Figure 5b). During winter mist and fog occurred regularly without frost. (Records for rainfall and temperature courtesy of Assam Agriculture Research Station, Diphu, Assam, India).

4.1.1 Undisturbed forest

The undisturbed forest was located at about 60 km north-west of Diphu, the district headquarter of Karbi Anglong, Assam. It is one of the most luxuriant forests with a rich floral diversity and a complex forest canopy and structure. The vegetation of the undisturbed forest is moist semi-evergreen (Rao 2004). The top canopy comprised mainly of Stereospermum personatum, Duabanga sonneretiodes Ham, Terminalia chebula Retz.,
Figure 4. Map showing the study area.
Figure 5a. Monthly mean rainfall (columns) and temperature (line) based on records from January 2000 to December 2012.

Figure 5b. Monthly mean rainfall (columns) and temperature (continuous line) from January 2009 to December 2011.
Tetrameles nudiflora R.Br, Amoora wallichi King., Pterospermum acerifolium Willd., Tectona grandis Linn, etc. The middle storey was dominated by plants like Lannea grandis A.Rich, Sterculia villosa Roxb, Dysoxylum binectariferum HK.f. et Bedd, Premna bengalensis Clarke, Mallotus philippinensis Muell.- Arg, Magnolia sp. and Michelia sp. Shrubs and epiphytes were prevalent in these forests (Figure 6 and 7).

4.1.2 Slash-and-burn field

Forest lands were cleared by felling and burning the trees for cultivation. Burning, a common practice in shifting cultivation, releases minerals from the vegetation into the soil. Forest land, in the vicinity of undisturbed forest site, was converted 6 month before soil sampling, to slash-and-burn field by clear-cutting and burning of the slashed dry biomass by local tribal people. A local variety of rice was cultivated as the crop after forest clearance (Figure 6 and 7).

4.1.3 Monoculture forest

Reserve forest areas subjected to jhum cultivation or abandoned deforested areas are artificially regenerated by Forest Departments under the jhum area rehabilitation programme to improve and restore the fragile ecosystem of the hills. The study site MF was about 20 years post-planting and consisted of artificially regenerated fast growing teak (Tectona grandis). The under storey or forest floor was relatively barren and covered with fallen twigs and leaves of the plant itself with very little or no herbaceous plant cover (Figure 8 and 9).

4.1.4 Physicochemical properties of soil

The soil in the three study sites was classified as sandy loam with good depth except on eroded hill slopes. Soils were slightly acidic across the three study sites (Table 2). The variation in pH of soil collected from the slash-and-burn field and monoculture forest was insignificant, whereas pH of the soil collected from undisturbed forest was lower than the slash-and-burn field. With regard to the organic matter content, the soil under monoculture forest had the lowest content. Monoculture forest also showed the lowest
Figure 6. Study sites showing undisturbed forest and slash-and-burn field.

Figure 7. Close view of slash-and-burn field and adjacent undisturbed forest.
Figure 8. Study site showing monoculture forest (teak plantation).

Figure 9. Rhizospheric soil sampling from the study site.
total N and available K content whereas the undisturbed forest soil had the highest. In contrast, available P content was highest in soil under monoculture forest and slash-and-burn field as compared to undisturbed forest.

Table 2. Physicochemical properties of soils collected from the three study sites.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Texture</th>
<th>pH</th>
<th>Total N</th>
<th>Total C</th>
<th>Total P</th>
<th>Total K</th>
<th>Avail K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undisturbed forest</td>
<td>Sandy loam</td>
<td>6.2</td>
<td>1.5</td>
<td>25.1</td>
<td>8.2</td>
<td>6.6</td>
<td>122.5</td>
</tr>
<tr>
<td>Slash-and-burn field</td>
<td>Sandy loam</td>
<td>6.5</td>
<td>1.2</td>
<td>16.4</td>
<td>11.3</td>
<td>12</td>
<td>130</td>
</tr>
<tr>
<td>Monoculture forest</td>
<td>Sandy loam</td>
<td>6.6</td>
<td>0.4</td>
<td>9.4</td>
<td>12.1</td>
<td>13.3</td>
<td>92.1</td>
</tr>
</tbody>
</table>
4.2 Results of first year study

During the first year of study (June 2009 to January 2010) soil was sampled in rainy season (June and September) and dry season (November and January). Further, to confirm the findings of the first year and to establish a possible trend, AMF diversity studies were repeated in subsequent year (June 2010 to January 2011) and soil was again sampled in rainy season (June and September) and dry season (November and January). The first year result, which is the average of the total sampling carried out between June 2009 and January 2010 is discussed below.

4.2.1 AM fungal composition

A total of 21 AM fungal taxa were identified morphologically from soil samples collected at three study sites during the first year sampling period. A list of morphotypes found along with their frequency of occurrence, spore density and relative abundance is presented in Table 3. There were large numbers of unidentified morphotypes. The identification of these morphotypes could not be carried out because of unavailability of sufficient number of individual spores. The unidentified morphotypes were distinguished from one another on the basis of characteristics of spore wall, hyphal attachment and spore size. Of the total 21 AM fungal taxa, only nine (42.8 %) could be identified at the species level. The remaining 12 (57.2 %) were identified at the generic level. Fourteen of them belonged to ‘species forming glomoid spores’ comprised of *Glomus* sensu lato, *Rhizophagus* and *Claroideoglomus*; five belonged to ‘species forming acaulosporoid spores’ comprised of *Acaulospora*; and one each to *Gigaspora* and *Ambispora*. Out of the total 21 AM fungal taxa isolated, 19 were found in UF, 18 in SBF and only 8 in MF. Some AM fungal species were apparently more or less restricted to specific study sites. Three species were found only in UF, 1 only in SBF and there was none specific to MF. The most notable ‘specialists’ AMF was *Glomus* sp. 6 which was found exclusively in slash-and-burn field. Some others like *Glomus* sp. 2, *Glomus* sp. 4 and *Gigaspora* sp. 1 occurred exclusively in undisturbed forest. Other species like *G. botryoides*, *Glomus* sp. 1 and *A. mellea* were more numerous in the UF than in SBF.

To evaluate the diversity of AMF in all the three study sites various diversity indexes were calculated (Table 4). The Shannon-Wiener index of diversity \( (H) \) was almost
similar in the UF (2.28) and SBF (2.25), but was significantly lower in MF (1.64). The Sorenson’s similarity coefficient ($C_s$) of AM fungal community composition was highest between UF and SBF (0.81) than between UF and MF (0.51). Similarly, Simpson’s diversity index ($D$) was almost same in UF (0.86) and SBF (0.88), but was again lower in MF (0.76) (Table 4).

4.2.2 Frequency and relative abundance of AMF

'Species forming glomoid' spores were dominant in all the three sites followed by acaulosporoid spores (Figure 10 and Table 3). All the three study sites regardless of the vegetation types and degree of disturbances harboured several common species. Such AMF ‘generalists’ were *R. manihotis*, *C. claroideum*, *G. botryoides*, *G. aureum*, *Glomus* sp. 1, *A. mellea* and *Acaulospora* sp. 1 (Figure 13). Among the total seven AM fungal species occurring in all the three sites, five species i.e., *C. claroideum*, *G. botryoides*, *Glomus* sp. 1, *A. mellea* and *Acaulospora* sp. 1 had lower frequency and spore density in monoculture forest. Two ‘generalists’ AM fungal species (*R. manihotis* and *Acaulospora* sp. 1), however, showed the highest frequency in all the study sites. The frequency of *R. manihotis* was 82.7 %, 65.6 % and 60.9 % in UF, SBF and MF respectively. Similarly, *Acaulospora* sp. 1 showed frequency of 71.8 %, 74.9 % and 59.3 % in UF, SBF and MF respectively.

The relative abundance of dominant ‘species forming glomoid spores’ was highest in UF (70.9 %) and lowest in MF (65.7 %). In SBF, it was in between UF and MF. Acaulosporoid spores showed the highest relative abundance in MF (34.3 %) compared to SBF (29.9 %) and UF (28 %). The dominant species, *R. manihotis*, had the highest relative abundance in all the three sites, and it was found highest in MF (32.8 %) and lowest in SBF (23.2 %).

A Pearson product-moment correlation coefficient was computed to assess the relationship between frequency and relative abundance of AMF. A scatterplot summarizes the positive correlation between the two variables i.e. frequency and relative abundance [$r = 0.969, n = 21, p = 0.000$] (Figure 11). In order to understand how disturbance affected sporulation of AMF communities, relative abundance of the five most prolific sporulators was compared (Figure 12). *R. manihotis* and *Acaulospora* sp.1 sporulated more vigorously in all the study sites. These two AM
fungal species accounted for 44.4 % to 65.9 % of sporulation in all study sites. In UF, two species of *Rhizophagus* alone contributed for 38.3 % of total spores counted, whereas in SBF and MF, the three most abundant sporulating species accounted for 54.4 % and 76 % respectively of the total spores.

### 4.2.3 Spore density of AMF

The total spore densities in soil of different sites are shown in Figure 10. The total spore density of AM fungi among the three study sites, i.e. UF (873.7 spores per 100g soil), SBF (376.25 spores per 100g soil) and MF (121.75 spores per 100g soil) varied from each other. It decreased by 56.9 % and 86 % respectively in SBF and MF when compared to UF. The undisturbed forest harboured the highest spore density of dominant ‘species forming glomoid spores’ (615.7 spores per 100g soil). The density of these spores was less in SBF (265.5 spores per 100g soil) and least in MF (79.5 spores per 100g soil). The species forming acaulosporoid spores also exhibited similar trend. The UF harboured 250.2 spores per 100g soil followed by SBF (107.5 spores per 100g soil) and MF (42.2 spores per 100g soil). Spore abundance of particular AM genus or species varied among the three study sites. At the generic level, the abundance of *Rhizophagus*, *Glomus* and *Acaulospora* spores was minimum in monoculture forest (Table 5). Of the total six genera, *Glomus* had the highest species number (11), of which 9 were found both in UF and SBF. However, there was a distinct decrease in species number of *Glomus* in MF which was reduced to only four.

ANOVA descriptive statistics showed that the mean spore density was found highest in undisturbed forest and was reduced in monoculture forest (Table 6 and Figure 14). The homogeneity of variances was assessed using Levene’s statistic test (Table 7). The Levene’s statistic was significant at 0.05 level, which confirms that the obtained differences in the sample variances are unlikely to have occurred based on random sampling from a population with equal variances. Therefore, the null hypothesis that the study sites have equal variances was rejected.

The disturbance in the study sites significantly affected the spore densities $[F(2,60)=5.11, p=0.009]$ (Table 8).
Table 3. Frequency, spore density and relative abundance of AM fungal species isolated from the undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF).

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
<th>Spore Density</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. minorotis</td>
<td>82.7</td>
<td>61.7</td>
<td>216.7</td>
</tr>
<tr>
<td>R. fasciculatus</td>
<td>62.4</td>
<td>15.6</td>
<td>0</td>
</tr>
<tr>
<td>C. claroideum</td>
<td>14.1</td>
<td>6.25</td>
<td>36.95</td>
</tr>
<tr>
<td>G. coeruleum</td>
<td>34.3</td>
<td>17.1</td>
<td>0</td>
</tr>
<tr>
<td>G. botryosum</td>
<td>49.9</td>
<td>31.2</td>
<td>10.9</td>
</tr>
<tr>
<td>G. aureum</td>
<td>4.67</td>
<td>7.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Glomus sp. 1</td>
<td>24.9</td>
<td>4.67</td>
<td>1.55</td>
</tr>
<tr>
<td>Glomus sp. 2</td>
<td>15.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glomus sp. 3</td>
<td>10.9</td>
<td>4.67</td>
<td>0</td>
</tr>
<tr>
<td>Glomus sp. 4</td>
<td>4.67</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glomus sp. 5</td>
<td>9.37</td>
<td>1.55</td>
<td>0</td>
</tr>
<tr>
<td>Glomus sp. 6</td>
<td>0</td>
<td>9.37</td>
<td>0</td>
</tr>
<tr>
<td>Glomus sp. 7</td>
<td>6.22</td>
<td>12.4</td>
<td>0</td>
</tr>
<tr>
<td>Glomus sp. 8</td>
<td>0</td>
<td>3.12</td>
<td>7.8</td>
</tr>
<tr>
<td>Acaulospora mellea</td>
<td>17.1</td>
<td>12.5</td>
<td>3.12</td>
</tr>
<tr>
<td>A. spinosa</td>
<td>12.4</td>
<td>7.8</td>
<td>0</td>
</tr>
<tr>
<td>A. rehmiit</td>
<td>6.22</td>
<td>1.55</td>
<td>0</td>
</tr>
<tr>
<td>Acaulospora sp. 1</td>
<td>71.85</td>
<td>74.97</td>
<td>59.32</td>
</tr>
<tr>
<td>Acaulospora sp. 2</td>
<td>7.8</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Ambispora sp. 1</td>
<td>7.8</td>
<td>6.22</td>
<td>0</td>
</tr>
<tr>
<td>Gigaspora sp. 1</td>
<td>4.67</td>
<td>0</td>
<td>1.25</td>
</tr>
<tr>
<td>Total</td>
<td>873.7</td>
<td>376.2</td>
<td>121.7</td>
</tr>
</tbody>
</table>
Table 4. Diversity indices of AMF in different study sites (UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest. Different letters (row) indicate differences at 0.05 probability level.

<table>
<thead>
<tr>
<th>Diversity Indices</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon-Wiener (H')</td>
<td>2.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Simpson's diversity (D)</td>
<td>0.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.76&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sorenson's Coefficients (Cs)</td>
<td>0.81(UF &amp; SBF)</td>
<td>0.61(SBF &amp; MF)</td>
<td>0.51(UF &amp; MF)</td>
</tr>
</tbody>
</table>

Figure 10. Spore density of total AMF genera identified from the three study sites (Error bars at 5 % value). UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest.
Table 5. Species number, spore density and relative abundance of the total AMF genera identified from the three study sites. UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest.

<table>
<thead>
<tr>
<th>AMF genus</th>
<th>Species number</th>
<th>Spore density</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>SBF</td>
<td>MF</td>
</tr>
<tr>
<td>Rhizophagus</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Claroideoglomus</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glomus</td>
<td>9</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Acaulospora</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Ambispora</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gigaspora</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>18</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 11. Correlation between frequency (%) and relative abundance (%).
Figure 12. Relative abundance (% of the total spores) of five most prolific AMF sporulators of the three study sites.
Table 6. Descriptive statistics of the AM fungal spore density of the three study sites.

<table>
<thead>
<tr>
<th>Study Sites</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95 % Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undisturbed Forest</td>
<td>21</td>
<td>41.6071</td>
<td>58.74992</td>
<td>12.8202</td>
<td>14.8645 68.3498</td>
<td>.00</td>
<td>216.75</td>
</tr>
<tr>
<td>Slash-and-Burn Field</td>
<td>21</td>
<td>17.9167</td>
<td>22.15603</td>
<td>4.83484</td>
<td>7.8314 28.0020</td>
<td>.00</td>
<td>81.75</td>
</tr>
<tr>
<td>Monoculture Forest</td>
<td>21</td>
<td>5.7976</td>
<td>11.94352</td>
<td>2.60629</td>
<td>.3610 11.2342</td>
<td>.00</td>
<td>41.00</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>21.7738</td>
<td>39.27464</td>
<td>4.94814</td>
<td>11.8826 31.6650</td>
<td>.00</td>
<td>216.75</td>
</tr>
</tbody>
</table>

Table 7. Levene’s test of homogeneity of variances. (df, degrees of freedom; Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>Levene Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.973</td>
<td>2</td>
<td>60</td>
<td>.000</td>
</tr>
</tbody>
</table>

Table 8. ANOVA showing the F-value (df, degrees of freedom; Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>13933.024</td>
<td>2</td>
<td>6966.512</td>
<td>5.116</td>
</tr>
<tr>
<td>Within Groups</td>
<td>81701.815</td>
<td>60</td>
<td>1361.697</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>95634.839</td>
<td>62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 13. Spore density of selected AMF ‘generalist’ species. Data averages of the first year sampling (Error bars at 5% value). UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF-Monoculture Forest.
Since Levene's test rejected the assumption of equal variances, therefore the findings of ANOVA were further confirmed by Welch and Brown-Forsythe robust test of equality of means. These statistics were preferred over others, as these tests do not assume homogeneity of variances. The F-ratio statistic obtained was significant at 0.05 level for both test (Table 9), therefore the null hypothesis that the study sites have equal means was rejected. The means were further examined in order to determine the nature of effect of disturbances on AM fungal mean spore population. ANOVA post hoc analysis using Games-Howell test was carried out, wherein multiple comparisons were made to examine possible differences between the study sites. The mean spore density difference was insignificant between undisturbed forest and slash-and-burn field but was significant between undisturbed forest and monoculture forest and slash-and-burn field and monoculture forest at the 0.05 level (Table 10).

**Table 9.** Welch and Brown-Forsythe robust test of equality of means (Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th></th>
<th>Welch</th>
<th>Brown-Forsythe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-ratio</td>
<td>F-ratio</td>
</tr>
<tr>
<td></td>
<td>5.548</td>
<td>5.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>33.080</td>
<td>27.415</td>
</tr>
<tr>
<td></td>
<td>.008</td>
<td>.013</td>
</tr>
</tbody>
</table>

**Table 10.** ANOVA post hoc multiple comparison of mean spore density by Games-Howell test (Std. Error, Standard Error; Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>(I) Disturbed and Undisturbed Forests</th>
<th>(J) Disturbed and Undisturbed Forests</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>undisturbed forest</td>
<td>slash-and-burn field</td>
<td>23.69048</td>
<td>13.70166</td>
<td>.214</td>
<td>-10.3903 - 57.7713</td>
</tr>
<tr>
<td></td>
<td>monoculture forest</td>
<td>35.80952*</td>
<td>13.08252</td>
<td>.031</td>
<td>2.9077 - 68.7113</td>
</tr>
<tr>
<td>slash-and-burn field</td>
<td>undisturbed forest</td>
<td>-23.69048</td>
<td>13.70166</td>
<td>.214</td>
<td>-57.7713 - 10.3903</td>
</tr>
<tr>
<td></td>
<td>monoculture forest</td>
<td>12.11905</td>
<td>5.49258</td>
<td>.086</td>
<td>-1.4054 - 25.6435</td>
</tr>
<tr>
<td>monoculture forest</td>
<td>undisturbed forest</td>
<td>-35.80952*</td>
<td>13.08252</td>
<td>.031</td>
<td>-68.7113 - 2.9077</td>
</tr>
<tr>
<td></td>
<td>slash-and-burn field</td>
<td>-12.11905</td>
<td>5.49258</td>
<td>.086</td>
<td>-25.6435 - 1.4054</td>
</tr>
</tbody>
</table>
4.2.4 Similarity of AM fungal composition in different study sites

A hierarchical cluster analysis (HCA) based on the similarity in AM fungal spore population among study sites showed that spore population in undisturbed forest resembled to slash-and-burn field as compared to monoculture forest (Figure 15).

Figure 14. Mean AM fungal spore density per 100g soil in undisturbed forest, slash-and-burn field and monoculture forest (CI, confidence interval).

Figure 15. Dendogram based on the similarity of AM fungal spore population across three study sites.
HCA also showed that AM fungal communities of the three study sites were clearly segregated into four distinct clusters based on their sporulation ability i.e. very high, high, medium and low sporulators. Each cluster was composed of different fungal species (Figure 16). The first cluster (from bottom upwards), comprised of *R. manihotis* and *Acaulospora* sp. 1, were the most prolific sporulators during the entire first year sampling period and their population ranged from 38.5 to 216.7 spores/100g soil. Closely related to these species were *G. botryoides* and *R. fasciculatus* which had high spore formation ability with spore number ranging from 2.7 to 95 spores/100g soil. The third cluster, comprised of *A. mellea*, *G. fuegianum*, *Glomus* sp. 1 and *Glomus* sp. 2, had medium spore formation ability, with spore number ranging between 1.2 and 57.2 spores/100g soil. The fourth cluster, comprised of seemingly diverse thirteen AM fungal species belonging to five different genera, had low to very low spore production ability ranging from 1.2 to 36.5 spores/100g soil.

![Dendrogram showing similarity and relatedness of AM fungal species of three study sites based on sporulation pattern.](image)

**Figure 16.** Dendrogram showing similarity and relatedness of AM fungal species of three study sites based on sporulation pattern.
Multivariate analyses were conducted on the whole dataset using the spore density data of each AMF morphotype as affected by environmental factors. Correspondence analysis (CA) revealed that environmental factors (soil chemical properties) and management type significantly affected the AM fungal composition (Figure 17). Soil chemical properties viz. organic C and available N were positively correlated. The graph also indicates the virtual distribution pattern of AM fungal species in different study sites. In general, many species notably *Rhizophagus manihotis*, *R. fasciculatus*, *Glomus botryoides*, *Acaulospora rehmi*, *Acaulospora* sp. 1 etc., were most abundant in UF. On the contrary, species like *Claroideoglomus claroideum*, *Glomus* sp. 6 and *Glomus* sp. 7 were common in SBF. Some species like *G. aureum* and *Glomus* sp. 8 were isolated more in MF.
Figure 17. Ordination diagram displaying the first two ordination axes of correspondence analysis (CA) summarizing effects of environmental variables (soil chemical properties—pH, available N and organic C) and management type on AM fungal communities. Vectors representing soil chemical properties are shown as solid lines and management type as inverted triangles. Size and orientation of the vectors represent correlation among them and with the axis. The smaller the angle between the vectors and longer the vectors, the more the variables represented by the vectors are correlated. AMF are shown as upright triangles. R man, Rhizophagus manihotis; R fas, Rhizophagus fasciculatus; C cla, Claroideoglomus claroideum; G fue, Glomus fuegianum; G bot, Glomus botryoides; G aur, Glomus aureum; G sp1, Glomus sp. 1; G sp2, Glomus sp. 2; G sp3, Glomus sp. 3; G sp4, Glomus sp. 4; G sp5, Glomus sp. 5; G sp6, Glomus sp. 6; G sp7, Glomus sp. 7; G sp8, Glomus sp. 8; A mel, Acaulospora mellea; A spi, Acaulospora spinosa; A reh, Acaulospora rehmi; A sp1, Acaulospora sp. 1; A sp2, Acaulospora sp. 2; Am sp1, Ambispora sp. 1; Gi sp1, Gigaspora sp. 1; UF, Undisturbed Forest; SBF, Slash-and-Burn Field; MF, Monoculture Forest.
4.3 Results of second year study

The second year result, which is the average of the total sampling carried out between June 2010 and January 2011 is discussed below.

4.3.1 AM fungal composition

A total of 35 AM fungal taxa (Figure 18) were identified morphologically from soil samples collected at three different sites during the second year sampling period. A list of morphotypes found along with their frequency of occurrence, spore density and relative abundance is presented in Table 11. Of the total 35 AM fungal taxa, only fifteen (42.9 %) could be identified at the species level. The remaining twenty (57.1 %) were identified at the generic level. Twenty one of them belonged to ‘species forming glomoid spores’ comprised of *Glomus* sensu lato, *Rhizophagus*, *Claroideoglomus* and *Septoglomus*; seven belonged to ‘species forming acaulosporoid spores’ comprised of *Acaulospora*; two each to *Scutellospora*, *Entrophospora* and *Gigaspora* spp.; and one to *Ambispora* spp. Out of the total 35 AM fungal taxa isolated, 32 were found in UF, 26 in SBF and only 8 in MF. Some AM fungal species were apparently more or less restricted to specific study sites. Eight species were found only in UF, 3 only in SBF and there was none specific to MF. The most notable were *Glomus mosseae*, *Septoglomus constrictum* and *Scutellospora* sp. 2 which were found exclusively in slash-and-burn field. *G. pustulatum*, *Glomus* sp. 4, *Glomus* sp. 9, *Glomus* sp. 10, *A. rehmi*, *A. foveata*, *Entrophospora* sp. 2 and *Gigaspora* sp. 1 occurred exclusively in undisturbed forest. Other species like *R. manihotis*, *G. botryoides*, *Glomus* sp. 1, *A. mellea* and *Acaulospora* sp. 1 were more numerous in the undisturbed forest than in slash-and-burn field.

To evaluate the diversity of AMF in all the three study sites various diversity indexes were calculated (Table 12). The Shannon-Wiener index of diversity (*H*) was almost similar in the UF (2.69) and SBF (2.87), but was significantly lower in MF (1.63). The Sorenson’s similarity coefficient (*Cs*) of AM fungal community composition was highest between UF and SBF (0.79) than between UF and MF (0.44). Similarly, Simpson’s diversity index (*D*) was almost same in UF (0.89) and SBF (0.91), but was again lower in MF (0.77) (Table 12).
4.3.2 Frequency and relative abundance of AMF

'Species forming glomoid' spores were dominant in all the three sites followed by acaulosporoid spores (Figure 19 and Table 11). All the three study sites regardless of the vegetation types and degree of disturbances harboured several common species. Such AMF 'generalists' were *R. manihotis*, *C. claroideum*, *G. botryoides*, *G. aureum*, *Glomus* sp. 1, *Glomus* sp. 8, *A. mellea* and *Acaulospora* sp. 1 (Figure 20). Among the total eight AM fungal species occurring in all the three sites, four species i.e., *C. claroideum*, *Glomus* sp. 1, *A. mellea* and *Acaulospora* sp. 1 had lower frequency and spore density in monoculture forest. Two 'generalists' AM fungal species, *R. manihotis* and *Acaulospora* sp. 1, however, showed the highest frequency in all the sites. The frequency of *R. manihotis* was 81.2 %, 72.8 % and 68.7 % in UF, SBF and MF respectively. Similarly, *Acaulospora* sp. 1 showed frequency of 79.6 %, 73.4 % and 49.9 % in UF, SBF and MF respectively.

The relative abundance of dominant 'species forming glomoid spores' was highest in UF (71.6 %) and lowest in MF (58.3 %). It was in between UF and MF in SBF. Acaulosporoid spores showed the highest relative abundance in MF (41.6 %) than in SBF (28.5 %) and UF (21.9 %). The dominant species, *R. manihotis*, had the highest relative abundance in all the three sites, and it was found highest in MF (31.8 %) compared to UF (24 %) and SBF (21.9 %).

A Pearson product-moment correlation coefficient was computed to assess the relationship between frequency and relative abundance of AMF. A scatterplot summarizes the positive correlation between the two variables i.e. frequency and relative abundance \[ r = 0.930, n = 35, p = 0.000 \] (Figure 21). In order to understand how disturbance affected sporulation of AMF communities, relative abundance of the five most prolific sporulators was compared. *R. manihotis* and *Acaulospora* sp. 1 sporulated more vigorously in all the study sites (Figure 22). These two AM fungal species accounted for 37.7 % to 59.3 % of sporulation in all study sites. In the undisturbed forest, two species of *Rhizophagus* alone contributed 31.8 % of total spores counted, whereas in SBF and MF, the three most abundant sporulating species accounted for 44.4 % and 77.9 % respectively of the total spores.
4.3.3 Spore density of AMF

The total spore densities in soil of different sites are shown in Figure 19. The total spore density of AMF among the three study sites i.e. UF (876.2 spores per 100g soil), SBF (628.7 spores per 100g soil) and MF (129.7 spores per 100g soil) varied from each other. It decreased by 28.2 % and 85.2 % respectively in SBF and MF when compared to UF. The undisturbed forest harboured the highest density of dominant ‘species forming glomoid spores’ (605.5 spores per 100g soil). The density of these spores was less in SBF (403 spores per 100g soil) and least in MF (76.7 spores per 100g soil). The ‘species forming acaulosporoid spores’ also exhibited similar trend. The UF harboured 210.2 spores per 100g soil, followed by SBF (190 spores per 100g soil) and MF (53 spores per 100g soil). Spore abundance of particular AM fungal genus or species varied among the three study sites. At the generic level, the abundance of *Rhizophagus*, *Glomus* and *Acaulospora* spores was minimum in monoculture forest (Table 13). Of the total nine genera, *Glomus* had the highest species number (17), of which 16 and 12 were found in UF and SBF respectively. However, there was a distinct decrease in species number of *Glomus* in MF, and it was reduced to only five.

ANOVA descriptive statistics showed that the mean spore density was found highest in undisturbed forest and was reduced in monoculture forest (Table 14 and Figure 23). The homogeneity of variances was assessed using Levene’s statistic test (Table 15). The Levene’s statistic was significant at 0.05 level, which confirms that the obtained differences in the sample variances are unlikely to have occurred based on random sampling from a population with equal variances. Therefore, the null hypothesis that the study sites have equal variances was rejected.

The disturbance in the study sites significantly affected the spore densities \[F(2,102)=4.99, p=0.009\] (Table 16).
Table 11. Frequency, spore density and relative abundance of AM fungal species isolated in the undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF).

<table>
<thead>
<tr>
<th>AMF Species</th>
<th>Frequency (%)</th>
<th>Spore density</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>SBF</td>
<td>MF</td>
</tr>
<tr>
<td><em>Rhizophagus manihotis</em></td>
<td>81.2</td>
<td>72.8</td>
<td>68.7</td>
</tr>
<tr>
<td><em>R. fasciculatus</em></td>
<td>51.5</td>
<td>17.2</td>
<td>0</td>
</tr>
<tr>
<td><em>Claronodeoglomus clarogenum</em></td>
<td>17.2</td>
<td>26.6</td>
<td>1.55</td>
</tr>
<tr>
<td><em>Glomus aggregatum</em></td>
<td>3.1</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>0</td>
<td>7.8</td>
<td>0</td>
</tr>
<tr>
<td><em>G. pustulatum</em></td>
<td>6.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>G. claroideum</em></td>
<td>7.8</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td><em>G. aggregatum</em></td>
<td>34.4</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td><em>G. botryosides</em></td>
<td>34.32</td>
<td>17.2</td>
<td>29.67</td>
</tr>
<tr>
<td><em>G. aureum</em></td>
<td>7.8</td>
<td>6.2</td>
<td>1.55</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 1</td>
<td>29.67</td>
<td>12.5</td>
<td>1.55</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 2</td>
<td>15.6</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 3</td>
<td>4.675</td>
<td>0</td>
<td>1.55</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 4</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 5</td>
<td>14.05</td>
<td>10.9</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 6</td>
<td>3.125</td>
<td>20.3</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 7</td>
<td>28.12</td>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 8</td>
<td>1.55</td>
<td>10.9</td>
<td>3.125</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 9</td>
<td>23.42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp.</em> 10</td>
<td>14.05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Septoglomus constrictum</em></td>
<td>0</td>
<td>10.9</td>
<td>0</td>
</tr>
<tr>
<td><em>Acaulospora mellea</em></td>
<td>35.92</td>
<td>15.6</td>
<td>12.5</td>
</tr>
<tr>
<td><em>A. spinosa</em></td>
<td>9.35</td>
<td>4.7</td>
<td>0</td>
</tr>
<tr>
<td><em>A. rehmi</em></td>
<td>15.62</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>A. foveata</em></td>
<td>6.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Acaulospora sp.</em> 1</td>
<td>79.65</td>
<td>73.4</td>
<td>49.97</td>
</tr>
<tr>
<td><em>Acaulospora sp.</em> 2</td>
<td>4.675</td>
<td>18.7</td>
<td>0</td>
</tr>
<tr>
<td><em>Acaulospora sp.</em> 3</td>
<td>4.675</td>
<td>18.7</td>
<td>0</td>
</tr>
<tr>
<td><em>Scutellospora sp.</em> 1</td>
<td>21.85</td>
<td>15.6</td>
<td>0</td>
</tr>
<tr>
<td><em>Scutellospora sp.</em> 2</td>
<td>0</td>
<td>9.4</td>
<td>0</td>
</tr>
<tr>
<td><em>Entrophospora sp.</em> 1</td>
<td>10.92</td>
<td>9.4</td>
<td>0</td>
</tr>
<tr>
<td><em>Entrophospora sp.</em> 2</td>
<td>4.675</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ambispora sp.</em> 1</td>
<td>6.2</td>
<td>10.9</td>
<td>0</td>
</tr>
<tr>
<td><em>Gigaspora sp.</em> 1</td>
<td>3.125</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td><em>Gigaspora sp.</em> 2</td>
<td>10.92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>876.25</td>
<td>628.75</td>
<td>129.75</td>
</tr>
</tbody>
</table>
Table 12. Diversity indices of AMF in different study sites (UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest. Different letters (row) indicate differences at 0.05 probability level.

<table>
<thead>
<tr>
<th>Diversity Indices</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon-Wiener ($H'$)</td>
<td>2.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Simpson's diversity ($D$)</td>
<td>0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.77&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sorenson's Coefficients ($C_s$)</td>
<td>0.79 (UF &amp; SBF)</td>
<td>0.45 (SBF &amp; MF)</td>
<td>0.44 (UF &amp; MF)</td>
</tr>
</tbody>
</table>

Figure 19. Spore density of total AMF genera identified from the three study sites (Error bars at 5% value). UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest.
Figure 20. Spore density of selected AMF 'generalist' species. Data averages of the second year sampling (Error bars at 5 %). UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF-Monoculture Forest.
Table 13. Species number, spore density and relative abundance of the total AMF genera identified from the three study sites. UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest.

<table>
<thead>
<tr>
<th>AMF genus</th>
<th>Species number UF</th>
<th>Species number SBF</th>
<th>Species number MF</th>
<th>Spore density UF</th>
<th>Spore density SBF</th>
<th>Spore density MF</th>
<th>Relative abundance (%) UF</th>
<th>Relative abundance (%) SBF</th>
<th>Relative abundance (%) MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizophagus</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>257.2</td>
<td>160.7</td>
<td>40.5</td>
<td>26.9</td>
<td>31.8</td>
<td>31.8</td>
</tr>
<tr>
<td>Claroideoglomus</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>31.7</td>
<td>15.2</td>
<td>2</td>
<td>5.5</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Glomus</td>
<td>16</td>
<td>12</td>
<td>5</td>
<td>316.5</td>
<td>216</td>
<td>34.2</td>
<td>34.2</td>
<td>31.1</td>
<td>25.2</td>
</tr>
<tr>
<td>Septoglomus</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>3.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acaulospora</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>210.2</td>
<td>190</td>
<td>53</td>
<td>21.9</td>
<td>28.5</td>
<td>41.6</td>
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<tr>
<td>Scutellospora</td>
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<td>2</td>
<td>0</td>
<td>20.2</td>
<td>15</td>
<td>0</td>
<td>2.5</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>Entrophospora</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>13.2</td>
<td>10.2</td>
<td>0</td>
<td>0.9</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>Ambispora</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>15</td>
<td>9.2</td>
<td>0</td>
<td>2</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>Gigaspora</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>1.2</td>
<td>0</td>
<td>0.9</td>
<td>0.3</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 21. Correlation between frequency (%) and Relative abundance (%).
Figure 22. Relative abundance (% of the total spores) of five most prolific AMF sporulators of the three study sites.
**Table 14.** Descriptive Statistics of the AM fungal spore density of the three study sites.

<table>
<thead>
<tr>
<th>Study Sites</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td>Minimum</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>Undisturbed Forest</td>
<td>35</td>
<td>25.0357</td>
<td>40.74550</td>
<td>6.88725</td>
<td>11.0391</td>
<td>39.0323</td>
</tr>
<tr>
<td>Slash-and-Burn Field</td>
<td>35</td>
<td>17.9643</td>
<td>26.84378</td>
<td>4.53743</td>
<td>8.7431</td>
<td>27.1854</td>
</tr>
<tr>
<td>Monoculture Forest</td>
<td>35</td>
<td>3.7071</td>
<td>10.06093</td>
<td>1.70061</td>
<td>.2511</td>
<td>7.1632</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>15.5690</td>
<td>29.84742</td>
<td>2.91281</td>
<td>9.7928</td>
<td>21.3453</td>
</tr>
</tbody>
</table>

**Table 15.** Levene’s test of homogeneity of variances. (df, degrees of freedom; Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>Levene Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.158</td>
<td>2</td>
<td>102</td>
<td>.003</td>
</tr>
</tbody>
</table>

**Table 16.** ANOVA showing the F-value (df, degrees of freedom; Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>8262.090</td>
<td>2</td>
<td>4131.045</td>
<td>4.993</td>
</tr>
<tr>
<td>Within Groups</td>
<td>84388.221</td>
<td>102</td>
<td>827.336</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>92650.312</td>
<td>104</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Since Levene’s test rejected the assumption of equal variances, therefore the findings of ANOVA were further confirmed by Welch and Brown-Forsythe robust test of equality of means. These statistics were preferred over others, as these tests do not assume homogeneity of variances. The F-ratio statistic obtained was significant at 0.05 level for both test (Table 17), therefore the null hypothesis that the study sites have equal means was rejected. The means were further examined in order to determine the nature of effect of disturbances on AM fungal mean spore population. ANOVA post hoc analysis using Games-Howell test was carried out, wherein multiple comparisons were made to examine possible differences between the study sites. The mean spore density difference was insignificant between undisturbed forest and slash-and-burn field but was significant between undisturbed forest and monoculture forest and slash-and-burn field and monoculture forest at the 0.05 level (Table 18).

Table 17. Welch and Brown-Forsythe robust test of equality of means (Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
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<tr>
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<td>2</td>
<td>53.052</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>4.993</td>
<td>2</td>
<td>63.746</td>
</tr>
</tbody>
</table>

Table 18. ANOVA post hoc multiple comparison of mean spore density by Games-Howell test (Std. Error, Standard Error; Sig., Significance; *, the mean difference is significant at the 0.05 level).

<table>
<thead>
<tr>
<th>(I) Disturbed and Undisturbed Forests</th>
<th>(J) Disturbed and Undisturbed Forests</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95 % Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>slash-and-burn field</td>
<td>7.07143</td>
<td>8.24757</td>
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<td>-12.7592</td>
</tr>
<tr>
<td></td>
<td>monoculture forest</td>
<td>21.32857*</td>
<td>7.09410</td>
<td>.013</td>
<td>4.0297</td>
</tr>
<tr>
<td>slash-and-burn field</td>
<td>undisturbed forest</td>
<td>-7.07143</td>
<td>8.24757</td>
<td>.669</td>
<td>-26.9020</td>
</tr>
<tr>
<td></td>
<td>monoculture forest</td>
<td>14.25714*</td>
<td>4.84565</td>
<td>.014</td>
<td>2.4982</td>
</tr>
<tr>
<td>monoculture forest</td>
<td>undisturbed forest</td>
<td>-21.32857*</td>
<td>7.09410</td>
<td>.013</td>
<td>-38.6275</td>
</tr>
<tr>
<td></td>
<td>slash-and-burn field</td>
<td>-14.25714*</td>
<td>4.84565</td>
<td>.014</td>
<td>-26.0161</td>
</tr>
</tbody>
</table>
Figure 23. Mean AM fungal spore density per 100 g soil in undisturbed forest, slash-and-burn field and monoculture forest (CI, confidence interval).

4.3.4 Similarity of AM fungal composition in different study sites

A hierarchical cluster analysis (HCA) based on the similarity in AM fungal spore population among study sites showed that spore population in undisturbed forest resembled to slash-and-burn field as compared to monoculture forest (Figure 24).

Figure 24. Dendogram based on the similarity of AM fungal spore population across three study sites.
Hierarchical cluster analysis also showed that AM fungal communities of the three study sites were clearly segregated into three distinct clusters based on their sporulation ability viz. very high, high and low sporulators. Each cluster was composed of different fungal species (Figure 25). The first cluster (from bottom upwards) comprised of *R. manihotis* and *Acaulospora* sp. 1 were the most prolific

**Figure 25.** Dendogram showing similarity and relatedness of AM fungal species across the study sites based on sporulation pattern.
sporulators during the entire second year of sampling, and their population ranged from 37.7 to 196 spores/100g soil. Closely related to these species were *G. botryoides*, *G. fuegianum*, *Glomus* sp. 1 and *R. fasciculatus* which had high spore formation ability with spore number ranging from 1 to 79.2 spores/100g soil. The third cluster, comprised of seemingly diverse twenty nine AM fungal species belonging to eight different genera, had low to very low spore production ability ranging from 1.25 to 40.5 spores/100g soil.

Multivariate analyses were conducted on the entire dataset using the spore density data of each AMF morphotype as affected by environmental factors. Correspondence analysis (CA) clearly revealed that environmental factors (soil chemical properties) and management type significantly affected the AM fungal composition (Figure 26). Soil chemical properties viz. organic C and available N were positively correlated. The graph indicates the virtual distribution of AM fungal species in different study sites. In general, most of the fungal species were found abundant in undisturbed forest. In contrast, species like *G. mosseae*, *G. aggregatum*, *S. constrictum*, *Acaulospora* sp. 2, *Acaulospora* sp. 3 and *Scutellospora* sp. 2 were common in slash-and-burn field. In particular, *S. constrictum* was isolated only from slash-and-burn field.

During both first and second years of sampling, monoculture forest showed reduced species richness, in particular, species of Gigasporaceae, Scutellosporaceae, Entrophosporaceae were completely absent. Some species of *Glomus* such as *G. aggregatum*, *G. mosseae*, *G. pustulatum*, *G. clavisporum* and *G. fuegianum* were also absent in MF (Table 19).
Figure 26. Ordination diagram displaying the first two ordination axes of correspondence analysis (CA) summarizing effects of environmental variables (soil chemical properties - pH, available N and organic C) and management type on AM fungal communities. Vectors representing soil chemical properties are shown as solid lines and management type as inverted triangles. Size and orientation of the vectors represent correlation among them and with the axis. The smaller the angle between the vectors and longer the vectors, the more the variables represented by the vectors are correlated. AMF are shown as upright triangles. Abr. R man, R manihotis; R fas, R fasciculatus; C cla, C claroideum; G agg, G aggregatum; G mos, G mosseae; G pus, G pustulatum; G cla, G clavisporum; G fue, G fuegianum; G bot, G botryoides; G aur, G aureum; G sp. 1-10, Glomus sp. 1-10; S con, S constrictum; A mel, A mellea; A spi, A spinosa; A reh, A rehmi; A sp. 1-2, Acaulospora sp. 1-2; S sp. 1-2, Scutellospora sp. 1-2; E sp. 1-2, Entrophospora sp. 1-2; S sp. 1-2, SBF, Slash-and-Burn Field; MF, Monoculture Forest.
Table 19. AM fungal species detected from the three study sites during first and second years of sampling. UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<td>x</td>
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<td>x</td>
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<td>x</td>
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<td><em>R. fasciculatus</em></td>
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<td>x</td>
<td>x</td>
<td>x</td>
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<td></td>
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<td>x</td>
<td>x</td>
<td></td>
</tr>
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</tr>
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<td>G. pastulatum</td>
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</tr>
<tr>
<td>G. clavisporum</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Total AMF species 19 18 8 32 26 9

x, species is present
4.4 Seasonal dynamics of AM fungal spore diversity

Investigations on the influence of seasons on AM fungal communities were carried out over two sampling periods (2009/2010 and 2010/2011). Soil was sampled in June 2009 and 2010 (start of rainy season), September 2009 and 2010 (end of and rainy season), November 2009 and 2010 (start of dry season) and January 2010 and 2011 (middle of dry season). The effect of seasonal changes on AM fungal diversity was analyzed.

A list of morphotypes found in the first sampling period (June '09, Sep '09, Nov '09 and Jan '10) and second sampling period (June '10, Sep '10, Nov '10 and Jan '11) along with their frequency of occurrence, spore density and relative abundance is presented in Table 20, 21, 22, 23, 24, 25, 26 and 27. Seasonal variation in the compositions of the AMF communities was evaluated by comparing the relative abundance of spore of each AMF morphotype. The proportional distribution of each AMF morphotype in the three study sites in different seasons during first and second years of sampling is given in Figure 27 and 28. The species richness of AMF was affected by seasons. The richness of AMF was highest in November and January and lowest in June and September in both undisturbed and slash-and-burn field soils. Seasonal changes, however, had no marked effect on AM fungal richness in monoculture forest. In the subsequent year, a similar trend in AM fungal species richness was observed in both undisturbed forest and slash-and-burn field (Figure 28).
Table 20. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in June 2009.

<table>
<thead>
<tr>
<th>Species</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizophagus mamhotis</em></td>
<td>812</td>
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<td>75</td>
<td>212</td>
<td>56</td>
<td>48</td>
<td>241</td>
<td>322</td>
<td>466</td>
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<td><em>R. fasciculatus</em></td>
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<td>0</td>
<td>66</td>
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<td>0</td>
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<td>37.5</td>
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<td>15</td>
<td>0</td>
<td>48</td>
<td>8.6</td>
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<td>0</td>
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<td>10.3</td>
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<tr>
<td><em>G. botryoides</em></td>
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<td>51</td>
<td>0</td>
<td>80</td>
<td>18</td>
<td>0</td>
<td>91</td>
<td>10.3</td>
<td>0</td>
</tr>
<tr>
<td><em>G. aureum</em></td>
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<td>13</td>
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<td>81</td>
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<td>22.4</td>
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<td>0.2</td>
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<tr>
<td>Total</td>
<td>879</td>
<td>174</td>
<td>103</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

* 0 indicates absence
Table 21. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in September 2009.

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
<th>Spore density</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>SBF</td>
<td>MF</td>
</tr>
<tr>
<td><em>Rhzopogon mammotus</em></td>
<td>68.7</td>
<td>56.2</td>
<td>37.5</td>
</tr>
<tr>
<td><em>R. fasciculatus</em></td>
<td>62.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Clarideoglomus claroideum</em></td>
<td>18.7</td>
<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Glomus fijeanum</em></td>
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<td>187</td>
<td>0</td>
</tr>
<tr>
<td><em>G. botryoides</em></td>
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<td>0</td>
<td>187</td>
</tr>
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<td><em>G. aureum</em></td>
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<td>0</td>
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<tr>
<td><em>A. rehmi</em></td>
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</tr>
<tr>
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<td>62</td>
<td>0</td>
</tr>
<tr>
<td><em>Ambispora sp 1</em></td>
<td>62</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td><em>Gigaspora sp 1</em></td>
<td>125</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>519</td>
<td>192</td>
<td>65</td>
</tr>
</tbody>
</table>

*0 indicates absence
Table 22. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in November 2009.

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
<th>Spore density</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>SBF</td>
<td>MF</td>
</tr>
<tr>
<td><em>R. manihotis</em></td>
<td>87.5</td>
<td>75</td>
<td>68.7</td>
</tr>
<tr>
<td><em>R. fasciculatus</em></td>
<td>75</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td><em>Claronidoglomus claronidum</em></td>
<td>0</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus fucales</em></td>
<td>31.2</td>
<td>0</td>
<td>23</td>
</tr>
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<td><em>G. botryoides</em></td>
<td>68.7</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td><em>G. aureum</em></td>
<td>0</td>
<td>187</td>
<td>46</td>
</tr>
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<td>43.7</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp 2</em></td>
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<td>43</td>
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<td>17</td>
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<td>12</td>
</tr>
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<td><em>Glomus sp 7</em></td>
<td>62</td>
<td>187</td>
<td>8</td>
</tr>
<tr>
<td><em>Glomus sp 8</em></td>
<td>0</td>
<td>12.5</td>
<td>18.7</td>
</tr>
<tr>
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<td>5</td>
</tr>
<tr>
<td><em>A. spinosa</em></td>
<td>18.7</td>
<td>12.5</td>
<td>10</td>
</tr>
<tr>
<td><em>A. rehmi</em></td>
<td>62</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
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<td>34</td>
</tr>
<tr>
<td><em>Acaulospora sp 2</em></td>
<td>31.2</td>
<td>62</td>
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<td>0</td>
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<tr>
<td>Total</td>
<td>778</td>
<td>434</td>
<td>110</td>
</tr>
</tbody>
</table>

* 0 indicates absence
Table 23. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in January 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>Relative Frequency (%)</th>
<th>Spore Density</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>SBF</td>
<td>MF</td>
</tr>
<tr>
<td><em>Rhusophagmus manihotis</em></td>
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<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td><em>Rhizophagus fasciculus</em></td>
<td>68.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Claroideoglomus claroideum</em></td>
<td>0</td>
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<td>12.5</td>
</tr>
<tr>
<td><em>Glomus fueganum</em></td>
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<td>31.2</td>
<td>0</td>
</tr>
<tr>
<td><em>G. botryodisc</em></td>
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</tr>
<tr>
<td><em>G. aureus</em></td>
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<td>25</td>
</tr>
<tr>
<td><em>Glomus sp 1</em></td>
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</tr>
<tr>
<td><em>Glomus sp 2</em></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp 3</em></td>
<td>31.2</td>
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</tr>
<tr>
<td><em>Glomus sp 4</em></td>
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<td>0</td>
</tr>
<tr>
<td><em>Glomus sp 5</em></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp 6</em></td>
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<td>25</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp 7</em></td>
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<tr>
<td><em>Glomus sp 8</em></td>
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<td>0</td>
<td>12.5</td>
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<tr>
<td><em>Acaulospora melletea</em></td>
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</tr>
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<td><em>A. spinosa</em></td>
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<td><em>A. rhenii</em></td>
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<td>0</td>
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</tr>
<tr>
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<td>705</td>
<td>209</td>
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</tbody>
</table>

* 0 indicates absence
Table 24. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in June 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
<th>UF</th>
<th>SBF</th>
<th>MF</th>
<th>Relative Abundance (%)</th>
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</thead>
<tbody>
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<td>Rhizophagus manihotis</td>
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<td>R. fasciculatus</td>
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<td>84</td>
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<td>0</td>
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<td>G. mosseae</td>
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<tr>
<td>Glomus sp 4</td>
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</tr>
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<td>0</td>
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</tr>
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</tr>
<tr>
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<td>6</td>
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<td>54</td>
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<td>87.5</td>
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<td>143</td>
<td>91</td>
<td>25</td>
<td>19.3</td>
<td>21.8</td>
<td>27.5</td>
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</tr>
<tr>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Acaulospora sp 3</td>
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</tr>
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</tr>
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<td>1</td>
<td>12</td>
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</tr>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* 0 indicates absence
Table 25. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in September 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
<th>Spore Density</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>SBF</td>
<td>MF</td>
</tr>
<tr>
<td><em>Rhizophagus manihotis</em></td>
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<td>50</td>
<td>562</td>
</tr>
<tr>
<td><em>R. fasciculatus</em></td>
<td>562</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td><em>Claroideoglomus claroides</em></td>
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<td>0</td>
</tr>
<tr>
<td><em>Glomus aggregatum</em></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>G. postulantum</em></td>
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<td>0</td>
<td>0</td>
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<tr>
<td><em>G. claroideum</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>G. botryosum</em></td>
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<td>31</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>21</td>
</tr>
<tr>
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<td>0</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td><em>Glomus sp 2</em></td>
<td>0</td>
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*0 indicates absence.*
Table 26. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in November 2010.

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<th>Spore Density</th>
<th>Relative Abundance (%)</th>
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*0 indicates absence*
Table 27. Frequency, spore density and relative abundance of AM fungal species isolated from undisturbed forest (UF), slash-and-burn field (SBF) and monoculture forest (MF) in January 2011.

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Entrophospora</em></td>
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<td>7</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>0</td>
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</tr>
<tr>
<td><em>Ambispora</em></td>
<td>62</td>
<td>18</td>
<td>7</td>
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<td>10</td>
<td>5</td>
<td>0</td>
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<td></td>
<td>0.4</td>
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<tr>
<td><em>Gigaspora</em></td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td><em>Gigaspora</em></td>
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<td>0</td>
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<td>1528</td>
<td>1145</td>
<td>153</td>
<td>100</td>
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<td>100</td>
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</tbody>
</table>

* 0 indicates absence
Figure 27. Proportional distribution of AMF in the three study sites in different seasons during the first year of sampling. The y axis indicates the proportion of relative abundance of each AMF morphotype. Total spore number in each sampling season = 100%. The bar widths are proportional to the relative abundance (percentage) of studied AMF morphotype. The richness of AMF in each study sites at different season is indicated on the tops of the respective bars. Abbreviations: R man, R manihotis; R fas, R fasciculatus; C cla, C clarodeum; G fue, G fuegianum; G bot, G botryoides; G aur, G aureum; G sp. 1-8, Glomus sp. 1-8; A mel, A mellea; A spi, A spinosa; A reh, A rehmi; A sp. 1-2, Acaulospora sp. 1-2; Am sp. 1, Ambispora sp. 1; Gi sp. 1, Gigaspora sp. 1; UF, Undisturbed Forest; SBF, Slash-and-Burn Field; MF, Monoculture Forest.
Figure 28. Proportional distribution of AMF in the three study sites in different seasons during the second year of sampling. The y axis indicates the proportion of relative abundance of each AMF morphotype. The bar widths are proportional to the relative abundance (percentage) of studied AMF morphotype. The richness of AMF in each study sites at different season is indicated on the tops of the respective bars. Abbreviations: R man, R manihotis; R fas, R fasciculatus; C cla, C claroideum; G agg, G aggregatum; G mos, G mosseae; G pus, G pustulatum; G cla, G clavisporum; G fue, G fuegianum; G bot, G botryoides; G aur, G aureum; G sp. 1-10, Glomus sp. 1-10; S con, S constrictum; A mel, A melleae; A spi, A spinosa; A reh, A rehmi; A sp. 1-2, Acaulospora sp. 1-2; S sp. 1-2, Scutellospora sp. 1-2; E sp. 1-2, Entrophospora sp. 1-2; Am sp. 1, Ambispora sp. 1; Gi sp. 1, Gigaspora sp. 1; UF, Undisturbed Forest; SBF, Slash-and-Burn Field; MF, Monoculture Forest.
A significant variation in AM fungal spore density was observed according to the seasons. Total number of spore ranged from 65 to 1319 spores/100g soil and 91 to 1528 spores/100g soil respectively in the first and second years of sampling. The maximum number of AM fungal spores in undisturbed forest, slash-and-burn field and monoculture forest was isolated in dry season (January 2010 and 2011) and the number decreased considerably in wet season (September 2009 and 2010). The number of spores decreased by 65.2 % and 71.7 % in the wet season as compared to the dry season during first and second years of study period respectively (Figure 29).

**Figure 29.** Seasonal dynamics of AMF spore density in the three study sites during first and second years of sampling.
Multivariate analyses were conducted on the entire dataset. Ordination analysis revealed that seasons significantly affected the AM fungal spore density, species richness and Shannon-Wiener diversity index. Ordination diagram was plotted to indicate the patterns of variation in AM fungal species composition across the seasons in both the years of study (Figure 30 and 31). Results of redundancy analysis (RDA) are shown using the spore density of each AMF morphotype as affected by season. RDA revealed higher AMF scores in dry and winter season (November and January) and lower AMF scores in wet and summer season (June and September) during both first and second years of study.

The relative seasonal abundance of individual AM fungal species was analyzed by pie class chart for both first and second years of sampling (Figure 32 and 33). The pie class chart of the first year study revealed greater relative abundance of most of the fungal species in dry season (November '09 and January '10). AM fungal species which were exclusively found in dry seasons were *Glomus* sp. 3, *Glomus* sp. 4, *Glomus* sp. 5, and *Glomus* sp. 8. Some notable exceptions were *Gigaspora* sp. 1, *G. botryoides*, *A. mellea*, and *Ambispora* sp. 1 which were found distributed both in wet and dry seasons. The pie class chart of the second year study also revealed greater relative abundance of most of the AM fungal species in dry season (November '10 and January '11).
Figure 30. Ordination diagram displaying the first two ordination axes of a redundancy analysis summarizing effects of the seasons on AM fungal communities during first year sampling. Vectors representing AMF are shown as solid lines. Size and orientation of the vectors represent correlation among them and with the axis. The smaller the angle between the vectors and longer the vectors, the more the variables represented by the vectors are correlated. R man, R manihotis; R fas, R fasciculatus; C cla, C claroideum; G fue, G fuegianum; G bot, G botryoides; G aur, G aureum; G sp. 1-8, Glomus sp. 1-8; A mel, A mellea; A spi, A spinosa; A reh, A rehmi; A sp. 1-2, Acaulospora sp. 1-2; Am sp. 1, Ambispora sp. 1; Gi sp. 1, Gigaspora sp. 1
Figure 31. Ordination diagram displaying the first two ordination axes of a redundancy analysis summarizing effects of the seasons on AM fungal communities during second year sampling. Vectors representing AMF are shown as solid lines. Size and orientation of the vectors represent correlation among them and with the axis. The smaller the angle between the vectors and longer the vectors, the more the variables represented by the vectors are correlated. R man, R manihotis; R fas, R fasciculatus; C cla, C claroideum; G agg, G aggregatum; G mos, G mosseae; G pus, G pustulatum; G cla, G clavisporum; G fue, G fuegianum; G bot, G botryoides; G aur, G aureum; G sp. 1-10, Glomus sp. 1-10; S con, S constrictum; A mel, A mellea; A spi, A spinosa; A reh, A rehmi; A sp. 1-2, Acaulospora sp. 1-2; S sp. 1-2, Scutellospora sp. 1-2; E sp. 1-2, Entrophospora sp. 1-2; Am sp. 1, Ambispora sp. 1; Gi sp. 1, Gigaspora sp. 1
Figure 32. Pie class chart showing the relative seasonal abundance of individual AM fungal species during first year sampling. R man, R manihotis; R fas, R fasciculatus; C cla, C claroideum; G fue, G fuegianum; G bot, G botryoides; G aur, G aureum; G sp. 1-8, Glomus sp. 1-8; A mel, A mellea; A spi, A spinosa; A reh, A rehmi; A sp. 1-2, Acaulospora sp. 1-2; Am sp. 1, Ambispora sp. 1; Gi sp. 1, Gigaspora sp. 1
Figure 33. Pie class chart showing the relative seasonal abundance of individual AM fungal species during second year sampling. *R* man, *R* manihotis; *R* fas, *R* fasciculatus; *C* cla, *C* clarioideum; *G* agg, *G* aggregatum; *G* mos, *G* mosseae; *G* pus, *G* pustulatum; *G* cla, *G* clavisporum; *G* fue, *G* fuegianum; *G* bot, *G* botryoides; *G* aur, *G* aureum; *G* sp. 1-10, *Glomus* sp. 1-10; *S* con, *S* constrictum; *A* mel, *A* mellea; *A* spi, *A* spinosa; *A* reh, *A* rehmi; *A* sp. 1-2, *Acaulospora* sp. 1-2; *S* sp. 1-2, *Scutellospora* sp. 1-2; *E* sp. 1-2, *Entrophospora* sp. 1-2; *Am* sp. 1, *Ambispora* sp. 1; *Gi* sp. 1, *Gigaspora* sp. 1
Temporal patterns of diversity were examined by diversity indices to determine whether the evenness of AM fungal assemblages increases from wet to dry season. The highest value of AM fungal biodiversity, measured as Shannon-Wiener index ($H'$) of diversity, was found in dry seasons (November and January) in first and second years of sampling (Figure 34 and 35). Bigger circles (blue) depict higher Shannon-Wiener index value and smaller circles (green) depict lower Shannon-Wiener index value.

**Figure 34.** Seasonal variation in Shannon-Wiener index ($H'$) of diversity in the first year of sampling period.
4.4.1 Single spore and trap culture

Single spore and trap culture was successfully carried out with the help of *Allium cepa* as host plant (Figure 36) Permanent slides were prepared from freshly produced spores.
Figure 36. Single spore culture with *Allium cepa* as host plant.
4.5 Seasonality of arbuscular mycorrhizal colonization

To understand the seasonality of arbuscular mycorrhizal (AM) colonization, three indicator plant species growing in all the three study sites were surveyed during the first year of sampling period. The surveyed plants were colonized by AMF as evidenced by the formation of typical AM structures. For all the three plants surveyed, AM colonization rates were higher in rainy season (June ’09 and ‘10) and lower in dry season (Jan ’10 and ‘11). The rate and frequency of AM colonization is given in Table 28. During the first year, average colonization rates were higher in undisturbed forest (38.4 %) and slash-and-burn field (34.4 %) as compared to lower colonization rate in monoculture forest (18.7 %). Among the three plant species, the average colonization rates were found higher in Costus (33.4 %) and Ixora (30.7 %) as compared to Mellastoma (27.3 %). AM colonization of Costus was much higher in UF (51.2 %) and SBF (42.8 %) compared to MF (21.8 %). A similar pattern in AM colonization was observed during the second year (Table 28).

A Pearson product-moment correlation coefficient was computed to assess the relationship between frequency of colonization and % root length colonization. A scatterplot summarizes the positive but weak correlation between the two variables i.e. % root length colonization and frequency ($r = 0.711, n = 18, p = 0.001$) (Figure 37).

Table 28. Root length colonization rate (CR) and frequency (F) of AMF in the rhizosphere of three plant species of different study sites in different seasons.

| Plant | June '09 | | | | Jan '10 | | | | | | June '10 | | | | Jan '11 |
|-------|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| CR    | UF      | SBF | MF | UF | SBF | MF | UF | SBF | MF | UF | SBF | MF | UF | SBF | MF |
| Costus| 51.2    | 42.8 | 21.8 | 36.8 | 27.8 | 20.2 | 58.2 | 52.4 | 19.4 | 32.3 | 31.6 | 13.2 |
|       | 80      | 84 | 70 | 74 | 76 | 66 | 74 | 80 | 76 | 68 | 70 | 54 |
| Mellastoma | 38.4 | 35.8 | 16.4 | 30.2 | 30 | 13.2 | 32.6 | 41.5 | 14.4 | 24.5 | 27.2 | 16.6 |
|       | 84 | 68 | 66 | 78 | 80 | 54 | 70 | 80 | 76 | 70 | 66 | 50 |
| Ixora | 44.2 | 42.4 | 22.2 | 29.6 | 27.4 | 18.4 | 52.8 | 47.3 | 18.4 | 33.5 | 26.4 | 21.2 |
|       | 70 | 76 | 64 | 66 | 74 | 66 | 80 | 74 | 70 | 76 | 68 | 66 |

UF- Undisturbed Forest; SBF- Slash-and-Burn Field; MF- Monoculture Forest.
Figure 37. Correlation between frequency of colonization and root length colonization (%).
4.6 Molecular studies

DNA was successfully obtained from different plant roots of the three study sites. The presence of DNA was confirmed in 1 % agarose gel immediately after the CTAB method. Figure 38 shows the result of the electrophoresis run for the DNA obtained from three samples by CTAB method. Nested PCR with universal eukaryotic primers (NS1 and NS4) and AMF specific primers (AML1 and AML2) specifically amplified the small subunit rDNA (SSU rDNA). Figure 39 shows the amplified SSU rDNA obtained in 1 % agarose gel after the second PCR amplification. It is observed that for the amplified product of the root sample R1 (undisturbed forest) and root sample R2 (slash-and-burn field), the band had an approximate size of 175 bp, while for third root sample R3 (monoculture forest), the size of the band was approximately 200 bp (faint band).

Figure 38. Plant root DNA (containing mycorrhizal DNA) obtained by CTAB method. S1, sample 1; S2, sample 2; S3, sample 3.
Figure 39. Amplified SSU rDNA obtained in 1 % agarose gel after the second PCR amplification. Abbreviations R1 Root sample 1, R2 Root sample 2, R3 Root sample 3, DNA ladder, 100 bp ladder.
Enzymes Hinf I and Alu I were effective in the restriction digestion of the amplified SSU rDNA, resulting in patterns of two to seven fragments that allowed effective comparison among the three samples. Figure 40 shows the result of restriction digestion of the nested PCR product with Hinf I and Alu I.

Figure 40. Restriction products of the amplified SSU rDNA region of arbuscular mycorrhizal fungi with enzymes Hinf I and Alu I. S1- sample 1; S2- sample 2; S3- sample 3; DNA ladder- 100 bp ladder.
The approximate restriction fragment lengths of the AM fungal species were calculated by using the molecular weight marker (Table 29). From the described morphotypes, 22 RFLP patterns (Figure 40) were obtained for all PCR products; 5 RFLP patterns from undisturbed forest, 5 from slash-and-burn field and 2 from monoculture forest by restriction digestion with Hinf I enzyme and 4 RFLP patterns from undisturbed forest, 4 from slash-and-burn field and 2 from monoculture forest by restriction digestion with Alu I enzyme. The restriction fragment (RF) bands with an approximate size of 75, 100, 175, 200 bp found in UF were also found in SBF. Thus, greater similarity in RF bands was found between UF and SBF.

Table 29. Apparent lengths of restriction fragments of PCR products from samples of three study sites

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fragment lengths (bp)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hinf I</td>
</tr>
<tr>
<td>Sample 1 (UF)</td>
<td>(75), (100), 175, (200), (275)</td>
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<tr>
<td>Sample 2 (SBF)</td>
<td>(75), (100), 175, 200, (275)</td>
</tr>
<tr>
<td>Sample 3 (MF)</td>
<td>25, 50</td>
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</tbody>
</table>

*PCR was performed with primers NS1/NS4 and AML1/AML2. Numbers in parentheses represent faint bands.