3. Effect of leguminous cover crop management system on below ground microbial diversity
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3.1. Introduction

Soil productivity is defined as the rate that a particular land site can accumulate energy in the form of vegetation (Erik, 2006). Although yield of target crop biomass may frequently be the primary focus, meaningful analysis of soil productivity must include an evaluation of the site variables, including soil parameters that influence productivity from time to time throughout the year. The high density cultivation/cover crop between periods of regular crop production or in between trees of plantations is primarily for the purpose of protecting and improving soil (Doran et al., 1994).

Various changes occur in soil properties during the growth period of cover crops. The consumption of soil water and nutrients by the cover crop may create a nutrient deficit for the succeeding main crop, if planted before the soil incorporated all the cover crop. Competition for light, water, and nutrients exists where crops are inter-planted. At the same time, the cover crop promotes the growth of different types of beneficial soil microorganisms. These groups of microorganisms improve soil properties either by providing support for improved nutrient acquisition by plants or by creating disease resistance to plants. If a legume species is used, that will assure special advantage to the above mentioned properties (National Research Council, 2010).

To obtain information on soil nutrient dynamics, detailed soil studies with special reference to microbiological parameters are necessary. For this, each cover crop treatment should be followed by soil evaluation for disease suppression, effects on soil microbial diversity and function, soil microbial communities and their function, and nitrogen fixation and phosphate solubilisation. So, the present study focuses on the assessment of all soil parameters during soil management with different cover crops cultivation. The criteria for selection of different cover crops depend up on the land use system and the type of main crops cultivated in the selected area.

In the present study, five different treatments, with 3 types of cover crop planted area (Arachis pintoi, Calapagonium mucunoides, and Sesbania aculeata), weeded area and non weeded area were compared in respect of total microbial population as well as soil fertility
related microbial population per gram of soil evaluated by plate count method. The selected study area is an agriculture system in the Kerala part of Nilgiri biosphere reserve. There are several reports revealing that most of the agriculture or plantation systems near forest patches were converted-forest lands. According to Rajiv (2002), in Nilgiri biosphere reserve the shola forests have reduced by half (from almost 8,600 ha to about 4,225 ha), the grasslands have come down by a factor of six (from 29,875 ha in 1,849 to about 4,700 ha) during the last one and half century. Since, the restoration of soil fertility is a major concern now-a-days, the present study focuses on the development and dynamics of soil fertility indicator microorganisms during cover crop cultivation.

3.2. Materials and Methods

The cover crops were planted in a coconut plantation which falls under the restorer zone of Kerala part of Nilgiri Biosphere Reserve near Nilambur in Malappuram district (Map 1). The experiment was designed to estimate and compare the microbial populations in different cover crop planted area with the populations in weeded and non-weeded area. Leguminous cover crops, namely *Arachis pintoi*, *Calapagonium mucunoides* and *Sesbania aculeata* were raised in farmer’s field.

3.2.1. Sample collection

Soil samples were collected from the following 5 experimental plots, each of size 30m x 30m during 4 growth periods after planting (sampling at 5 months, 10 months, 15 months and 22 months after cultivation). During each period, three soil samples were collected from each experimental area.

A. Non weeded area - 3 samples - A1 A2 A3
   (Each individual sample is formed after mixing 3 replicates eg: A1=A1_1+A1_2+A1_3)

B. *Arachis pintoi* planted area - 3 samples - B1 B2 B3

C. *Calapagonium mucunoides* planted area - 3 samples - C1 C2 C3

D. *Sesbania aculeata* planted area - 3 samples - D1 D2 D3

E. Weeded area - 3 samples - E1 E2 E3

Total samples - 15 samples
3.2.2. Enumeration of microorganisms by serial dilution plate technique

Ten gm of the soil sample was added to 90 ml of sterile distilled water in a 250 ml of conical flask to get $10^{-1}$ dilution. There were three replicates for each soil sample. The flasks for each sample were shaken uniformly for 30 minutes. 10 ml of the $10^{-1}$ dilution samples were transferred to 90 ml water to get $10^{-2}$ dilution. The process was repeated to get dilution up to $10^{-6}$ for each sample. The flasks were shaken uniformly for 5 minutes after each dilution for each sample. For the isolation of microorganisms pour plate method was used. One ml of the desired dilution was added to a sterile petridish. Sterile medium in bearable temperature was then added to the plate aseptically; rotated the plate carefully for the dispersion of sample uniformly throughout medium. The agar plates were, then, incubated in an inverted position at room temperature. Three replicate plates were inoculated for each sub sample of soil sample (National Standard Methods, 2004).

Nutrient agar, Rose Bengal agar and Starch casein agar were used as the selective media for enumeration of bacteria, fungi and actinomycetes, respectively (Aneja, 2001). After incubation period, the enumeration of total microbial population (bacteria, fungi, and actinomycetes) in the soil sample was done with Quebec colony counter. *Rhizobium*, *Azotobacter* and Phosphate solubilising microorganisms (PSM) present in the soil were enumerated using Yeast extract mannitol agar, Ashby’s glucose agar and Pikovskaya’s Agar, respectively (Subba Rao, 1993). Isolation and quantification of bioprotectants were also done using different media like King’s B medium with Iron chloride and King’s B medium with glycine for enumeration of Siderophore and HCN producing bacteria, respectively; Rose bengal agar medium was used for estimating *Trichoderma* species.

The number of colonies grown on the dilution plates was counted, averaged and multiplied by dilution factor to find out the number of cells per gram of sample:

\[
\text{Number of cells/gm} = \frac{\text{Mean plate count} \times \text{Dilution factor}}{\text{Dry weight of soil}}.
\]
After transforming the values to their corresponding log values, data obtained were statistically analyzed using one way ANOVA.

Map 1. Study area

3.3. Result

3.3.1. Isolation and enumeration of microorganisms

During sampling at 5th month, all the different selected microbial groups showed higher population in all the experimental plots including weeded area. The *Rhizobium* population showed significant difference (p=0.05) between different experimental plots. The population was much higher in cover crop cultivated area than the population in weeded or non weeded area (Table 3.1.). One siderophore and HCN producing colony was isolated from *Calapagonium mucunoides* cultivated area during random selection from $10^5$ dilution plates (14% of colonies selected) and one colony of *Trichoderma*
from $10^3$ dilution plates from both *Calapagonium mucunoides* and *Arachis pintoi* cultivated area (Figures 3.1., 3.2. and 3.3.).

During sampling at 10th month, the population of different groups of microorganisms (bacteria, fungi and actinomycetes) and soil fertility groups (*Rhizobium*, *Azotobacter* and phosphate solubilising microorganisms) were significantly different between experimental plots ($p=0.05$). All the populations were much higher in cover crop planted area than in either weeded or non weeded area (Table 3.2.). Three colonies producing siderophore and HCN were isolated from *C.mucunoides* cultivated area during random selection from $10^{-5}$ dilution plates (17% of colonies selected) and one colony of *Trichoderma* was isolated from each of the *C. mucunoides*, *A. pintoi* and *S. aculeata* cultivated area (Figure 3.1., 3.2. and 3.3.).

During sampling at 15th month, the population of different groups of microorganisms were significantly different between experimental plots ($p=0.05$). Bacteria and fungi were comparatively higher in cover crop planted area. The actinomycetes populations were higher in non-weeded area. There was no significant increment in the populations of *Rhizobium* and *Azotobacter* in cover crop planted area. The populations of PSM were comparatively higher in cover crop planted area. The *Trichoderma* showed higher population in *Calapagonium* planted area (Table 3.3.). Only a single colony of bacteria producing both siderophore and HCN was isolated in cover crop (*C. mucunoides* and *A. pintoi*) planted area and non weeded area during random selection from $10^{-5}$ dilution plates (19% of colonies selected) (Figures 3.1., 3.2. and 3.3.).

Microorganisms including soil fertility indicator groups were significantly different between experimental plots ($p=0.05$) with higher number in cover crop cultivated area after 22nd month of cultivation. *Calapagonium* showed comparatively higher population of bacteria and fungi, and weeded area showed comparatively lower population of microorganisms. *Rhizobium* and *Azotobacter* showed comparatively higher population in cover crop planted area; but phosphate solubilising microorganisms had no significant increase in population status in cover crop cultivated area compared to either
weed area or non weeded area (Table 3.4.). Comparatively higher number of siderophore and HCN producing bacteria were isolated from *Calapagonium* planted area during random selection from 10^{-5} dilution plates (19% of colonies selected). Comparatively higher number of *Trichoderma* sp. was observed in *Calapagonium* planted area. *Trichoderma* sp. was also obtained from *A. pintoi* and *S. aculeata* planted area as well as from non weeded area during 22^{nd} month of this experiment (Figures 3.1., 3.2. and 3.3.).

A reduction in the population of bacteria was observed in all treatments during the growth period from 5^{th} month to 10^{th} month. But the population again increased at 15^{th} and 22^{nd} month. Actinomycetes and fungi also showed a logarithmic increase in populations in other experimental plots except in weeded area. Comparatively higher populations were observed in cover crop planted area after 15 months growth. Among cover crops, *Calapagonium* cultivated area showed higher bacterial and fungal population (Figure 3.4.). In the case of fungal population, the *Calapagonium* and *Arachis* planted area showed more or less similar population size from 5^{th} to 22^{nd} month compared with other experimental plots. The actinomycetes population in all treatments showed increment from 10^{th} to 22^{nd} month except for weeded area (Figure 3.6.).

The population of *Rhizobium* showed an increment from 5^{th} to 10^{th} month in all experimental plots except in weeded area. During 15^{th} month, all the experimental plots showed comparatively lower population of *Rhizobium*. Subsequently, on 22^{nd} month, higher number observed only in cover crop planted area (Figure 3.7.). The population of *Azotobacter* showed significant increment from 5^{th} month to 10^{th} month of experiment in all experimental plots except in weeded area. *Calapagonium* and *Sesbania* cultivated areas maintained significantly higher population level compared to other experimental plot till the end of experiment period (Figure 3.8.). The population of phosphate solubilising microorganisms maintained almost the same status in all experimental plots at 22^{nd} month as at 5^{th} month. The highest population was observed during 10^{th} month but subsequently the population level decreased (Figure 3.9.).
Table 3.1. Population of different groups of microorganisms after 5 months of cover crop growth

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
<th>Rhizobium</th>
<th>Azotobacter</th>
<th>PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non weeded area</td>
<td>6.56 ± 0.31**</td>
<td>4.26 ± 0.38^a</td>
<td>5.48 ± 0.31^a</td>
<td>3.80 ± 0.08^a</td>
<td>2.48 ± 0.31^a</td>
<td>0.77 ± 1.33^a</td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>+6.56 ± 0.19^a</td>
<td>4.46 ± 0.06^a</td>
<td>5.62 ± 0.28^a</td>
<td>4.36 ± 0.11^b</td>
<td>3.25 ± 0.29^a</td>
<td>3.75 ± 0.68^a</td>
</tr>
<tr>
<td><em>Calapagonium mucunoides</em></td>
<td>6.79 ± 0.24^a</td>
<td>4.27 ± 0.26^a</td>
<td>5.57 ± 0.23^a</td>
<td>4.16 ± 0.12^b</td>
<td>3.04 ± 0.39^a</td>
<td>4.11 ± 0.40^a</td>
</tr>
<tr>
<td><em>Sesbania aculeate</em></td>
<td>6.60 ± 0.00^b</td>
<td>4.27 ± 0.12^a</td>
<td>3.53 ± 1.80^a</td>
<td>4.03 ± 0.03^b</td>
<td>3.13 ± 0.73^a</td>
<td>2.01 ± 1.44^a</td>
</tr>
<tr>
<td>Weeded area</td>
<td>6.36 ±0.07^b</td>
<td>4.32 ± 0.29^a</td>
<td>3.23 ± 1.80^a</td>
<td>3.90 ± 0.05^a</td>
<td>3.19 ± 0.20^a</td>
<td>3.72 ± 0.33^a</td>
</tr>
</tbody>
</table>

**Mean ± SE; *Values with the same superscript within a column are not significantly different according to Duncan’s multiple range test (p>0.05). The highest log value of 6.79 is equivalent to 68 X 10^5 cfu/gm soil and the lowest value of 2.01 is equivalent to 9 X 10^2 cfu/gm soil.

Table 3.2. Population of different groups of microorganisms after 10 months of cover crop growth

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
<th>Rhizobium</th>
<th>Azotobacter</th>
<th>PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non weeded area</td>
<td>6.52 ± 0.08**</td>
<td>3.70 ± 0.12^a</td>
<td>5.79 ± 0.19^a</td>
<td>3.70 ± 0.20^b</td>
<td>3.94 ± 0.06^b</td>
<td>3.10 ± 0.17^a</td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>+6.79 ± 0.08^c</td>
<td>3.96 ± 0.20^b</td>
<td>6.19 ± 0.13^b</td>
<td>4.30 ± 0.04^c</td>
<td>4.71 ± 0.08^c</td>
<td>3.97 ± 0.07^c</td>
</tr>
<tr>
<td><em>Calapagonium mucunoides</em></td>
<td>6.84 ± 0.13^c</td>
<td>4.00 ± 0.04^b</td>
<td>6.27 ± 0.10^b</td>
<td>4.40 ± 0.03^c</td>
<td>4.62 ± 0.30^c</td>
<td>3.87 ± 0.23^bc</td>
</tr>
<tr>
<td><em>Sesbania aculeate</em></td>
<td>6.55 ± 0.04^b</td>
<td>3.57 ± 0.10^a</td>
<td>6.39 ± 0.08^b</td>
<td>4.16 ± 0.12^c</td>
<td>3.72 ± 0.32^b</td>
<td>3.73 ± 0.15^b</td>
</tr>
<tr>
<td>Weeded area</td>
<td>5.92 ± 0.23^a</td>
<td>3.27 ± 0.26^a</td>
<td>5.56 ± 0.12^a</td>
<td>3.16 ± 0.28^a</td>
<td>3.10 ± 0.17^a</td>
<td>3.20 ± 0.17^a</td>
</tr>
</tbody>
</table>

**Mean ± SE; *Values with the same superscript within a column are not significantly different according to Duncan’s multiple range test (p>0.05). The highest log value of 6.84 is equivalent to 72 X 10^5 cfu/gm soil and the lowest value of 2.01 is equivalent to 1 X 10^3 cfu/gm soil.
Table 3.3. Population of different groups of microorganisms after 15 months of cover crop growth

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
<th>Rhizobium</th>
<th>Azotobacter</th>
<th>PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non weeded area</td>
<td>5.84 ± 0.05 **</td>
<td>3.82 ± 0.26 b</td>
<td>5.29 ± 0.05 c</td>
<td>4.39 ± 0.06 b</td>
<td>2.88 ± 0.10 b</td>
<td>4.15 ± 0.06 b</td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>5.97 ± 0.06 c</td>
<td>4.26 ± 0.12 b</td>
<td>5.14 ± 0.01 b</td>
<td>4.39 ± 0.15 b</td>
<td>3.79 ± 0.16 d</td>
<td>4.39 ± 0.05 c</td>
</tr>
<tr>
<td><em>Calapagonium mucunoides</em></td>
<td>6.06 ± 0.03 c</td>
<td>4.31 ± 0.15 b</td>
<td>5.17 ± 0.03 c</td>
<td>4.09 ± 0.09 a</td>
<td>3.81 ± 0.05 d</td>
<td>4.40 ± 0.04 c</td>
</tr>
<tr>
<td><em>Sesbania aculeate</em></td>
<td>6.06 ±0.03 c</td>
<td>4.24 ± 0.10 b</td>
<td>5.22 ± 0.04 b</td>
<td>4.10 ± 0.09 a</td>
<td>3.50 ± 0.09 c</td>
<td>4.42 ± 0.01 c</td>
</tr>
<tr>
<td>Weeded area</td>
<td>5.77 ± 0.06 a</td>
<td>3.90 ± 0.08 a</td>
<td>4.75 ± 0.09 a</td>
<td>4.47 ± 0.01 b</td>
<td>1.68 ± 0.20 a</td>
<td>3.20 ± 0.03 a</td>
</tr>
</tbody>
</table>

**Mean ± SE; *Values with the same superscript within a column are not significantly different according to Duncan’s multiple range test (p>0.05). The highest log value of 6.06 is equivalent to 116 X 10^4 cfu/gm soil and the lowest value of 1.68 is equivalent to 0.22 X 10^3 cfu/gm soil.

Table 3.4. Population of different groups of microorganisms after 22 months of cover crop growth

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
<th>Rhizobium</th>
<th>Azotobacter</th>
<th>PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non weeded area</td>
<td>6.37 ±0.09 **</td>
<td>4.24 ±0.37 b</td>
<td>6.05 ±0.06 b</td>
<td>4.80 ±0.03 b</td>
<td>2.98 ±0.15 b</td>
<td>4.00 ±0.04 b</td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>6.62 ±0.05 c</td>
<td>4.67 ±0.06 b</td>
<td>6.14 ±0.08 b</td>
<td>6.14 ±0.05 c</td>
<td>3.62 ±0.15 c</td>
<td>4.37 ±0.04 c</td>
</tr>
<tr>
<td><em>Calapagonium mucunoides</em></td>
<td>6.81 ±0.04 c</td>
<td>4.86 ±0.09 c</td>
<td>6.26 ±0.06 c</td>
<td>6.26 ±0.06 c</td>
<td>3.63 ±0.06 c</td>
<td>4.35 ±0.07 c</td>
</tr>
<tr>
<td><em>Sesbania aculeate</em></td>
<td>6.61 ±0.07 c</td>
<td>4.72 ±0.12 b c</td>
<td>6.18 ±0.13 b c</td>
<td>6.18 ±0.12 c</td>
<td>3.46 ±0.15 c</td>
<td>4.23 ±0.04 c</td>
</tr>
<tr>
<td>Weeded area</td>
<td>6.15 ±0.16 c</td>
<td>4.09 ±0.36 a</td>
<td>5.76 ±0.05 a</td>
<td>4.42 ±0.11 a</td>
<td>1.94 ±1.68 a</td>
<td>3.84 ± 0.06 a</td>
</tr>
</tbody>
</table>

**Mean ± SE; *Values with the same superscript within a column are not significantly different according to Duncan’s multiple range test (p>0.05). The highest log value of 6.81 is equivalent to 65 X 10^3 cfu/gm soil and the lowest value of 1.94 is equivalent to 0.47 X 10^3 cfu/gm soil.
Figure 3.1. Average number of siderophore producing bacterial colonies ($10^5$) in different experiment plots

Figure 3.2. Average number of HCN producing bacterial colonies ($10^5$) in different experiment plots

Figure 3.3. Average number of *Trichoderma* sp ($10^3$) in different experiment plots
Figure 3.4. Pattern of bacterial population during different sampling periods

Figure 3.5. Pattern of fungal population during different sampling periods

Figure 3.6. Pattern of actinomycetes population during different sampling periods
Figure 3.7. Pattern of *Rhizobial* population during different sampling periods

Figure 3.8. Pattern of *Azotobacter* population during different sampling periods

Figure 3.9. Pattern of phosphate solubilising microbial population during different sampling periods
3.4. Discussion

Crop productivity as well as chemical and physical parameters of soil is the major criteria for measuring soil fertility. The biological parameters with due importance to soil fertility related microbial population had not been considered earlier as a major indicator of soil productivity. A fertile soil is one that contains an adequate reserve of nutrients available to the plant, or alternatively, a microbial population that release nutrients, making them available to the plant and, thus, allowing a good plant development (Estudio, 2006). Thus, the present study is focusing on the population of soil fertility related microbial populations with respect different soil management practices.

Cover crop cultivation is considered as one of the most accepted traditional method of soil management practice, because the crop usually improved soil fertility through supporting growth and multiplication of fertility enhancing soil microbial population. The microbiological parameters were observed as the sensitive indicators to monitor the soil fertility improvement in a short period of time. In the present study, the bacterial population showed increment from 10th month of cover crop cultivation in all the experiment plots. The total number of microorganisms, especially bacteria, was related to the developmental phase of the legume plants and was greatest at the blooming phase which is the period of the highest photosynthetic activity of these plants. The population of microorganisms in the soil during the vegetative period depends up on the crop species (Sawicka et al., 1998). In the present study, non weeded area also showed almost the same range of bacterial population along with the cover crop planted area. All types of vegetation including weeds influence the general microbial populations in soil (Boyetchko, 1996).

The fungal population which significantly increased in cover crop planted area up to 10th month of the experiment did not show significant difference at the end of the experiment (22nd month). Manter (2007) reported that the cover crops increased the soil fungal biomass as compared to fallow land but decrease in population was observed towards the end of vegetative phase because of antagonism among different groups of microorganisms which results in reduction of the overall strength of population. All the experimental plots except weeded area showed significantly higher population of
Actinomycetes at 22\textsuperscript{nd} month of experiment because of the availability for decomposition of dead tissue of the cover crops and weeds which functions as mulch. Actinomycetes are commonly identified as one the main groups responsible for organic matter conversion during latter stages of decomposition according to Chopra (2004).

The \textit{Rhizobial} strength in soil can be utilized for direct measurement of the efficiency of soil to promote nitrogen fixation. The population of \textit{Rhizobium} depends upon not only the plant demand for soil nitrogen (Marianne Sarrantonio, 2007) but also the type of plant cultivated and age of the cultivated plant (Singleton and Tavares, 1986). Comparatively, higher \textit{Rhizobial} population is reported in soil cultivated with legumes (Andrade \textit{et al.}, 2002). In the present study, significantly higher population of \textit{Rhizobium} was observed during different stages of experiments in cover crop planted area, compared with either weeded or non weeded area. The higher population is due to viable bacteroids released into soil from legume nodules. \textit{Rhizobia} originating from persistent infection threads in senescing nodules are the source of viable cells required for perpetuation of the \textit{Rhizobium} sp. population in the soil (Paau \textit{et al.}, 1980).

The cover crops planted in different experimental plots function as green mulch and its decomposition progressed well during 22\textsuperscript{nd} month of experiment. The highest \textit{Rhizobial} population during 22\textsuperscript{nd} month of experiment in cover crop planted area might be due to the release of bacteroids from degraded legume nodules. The increased organic content in the experiment plots due to green mulch would promote the growth and multiplication \textit{Rhizobium} in the soil. According to Rebecca \textit{et al.} (2006) and Grossman \textit{et al.} (2011) organic manure application in soil increased \textit{Rhizobial} population and diversity. Under nitrogen-depleted conditions, \textit{Rhizobial} population existing in soil was able to induce symbiotic nodules on the roots of leguminous plants where bacteroids convert atmospheric nitrogen to ammonia. But the presence of exogenous nitrogen source inhibited the development and the functioning of the bacterium-plant symbiosis (Dusha, 2002).

The population of \textit{Azotobacter} showed significantly higher population at 10\textsuperscript{th} month of experiment in all experimental plots except in weeded area and, subsequently, significant reduction observed in population during rest of the sampling period in all experiments. \textit{Calapagonium} and \textit{Arachis} planted plot showed comparatively higher population status during the 22\textsuperscript{nd} month sampling. \textit{Sesbania} planted area showed
significantly more number of cells/gram of soil than either weeded area or non weeded area during the same interval. Martyniuk (2003) reported that significant correlation between numbers of *Azotobacter* sp. and the organic C and total N content in soils indicated that soil fertility was also an important factor influencing colonization of soils by *Azotobacter*. *Azotobacter* sp. were observed more frequently and in higher numbers, in cultivated soils than in uncultivated (sodded) soils, suggesting that agricultural practices might create environmental conditions more favorable for the development and survival of *Azotobacter* in soil (Martyniuk, 2003). Since, the population of *Azotobacter* depended on the total soil fertility status such as organic matter, minerals, etc., the results obtained during the present study confirmed the efficiency of cover crop in improving soil fertility. The results also confirmed the importance of *Azotobacter* cell count for monitoring soil fertility.

Greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and cannot be utilized by the plants (Vassileva *et al*., 1998). During the present study the population of phosphate solubilising microorganisms increased in all treatments up to 10th month of experiment, then reduced to a lower range during 15th and 22nd month of sampling. However, in all the soil samples collected at different intervals of experiment, comparatively higher population was observed in cover crop planted area than either weeded area or non weeded area. Yahya and Azawi (1998) reported the highest population of phosphate solubilizing bacteria in agricultural and range land soils. Kim *et al*., (1989) indicated that the population of phosphate solubilizing bacteria depended on cultural activities and different soil properties (physical and chemical properties, organic matter, and soil phosphorus content). In the present study, improved soil properties created by the cover crop growth could be the factors responsible for the higher number of cells/gram of PSM during the 10th month of cover crop growth. Mikanova *et al*. (2002) reported that phosphate availability could regulate mineral phosphate solubilization by some species of microorganisms. The significant reduction in phosphate solubilizing microorganisms after 10th month of cover crop growth might be due to depletion of P caused by the microbial solubilisation of available P and its uptaking by the growing plant. The present experiment also indicated that the count of phosphate solubilizing microorganisms can be a measure for the probable quantity of available phosphate in soil.
Generally, the bio-protectant groups of microorganisms are present more in fertile soil.

In the present study, the soil population of *Trichoderma* sp. was observed consistently at all stages of the growth of the cover crop. *C. mucunoides* planted area showed a slight increase in number of *Trichoderma* sp. *A. pintoi* and *S. aculeata* planted area showed lesser number of *Trichoderma* sp. during different intervals of soil sampling. But in all intervals, both the non weeded area and weeded area showed few numbers of colonies of *Trichoderma* sp. This may be probably because of the absence of organic matter addition in the weeded and non weeded area. Since, organic soil management can improve the *Trichoderma* population in soil, the *Trichodermal* population can be considered as a measure for soil fertility (Thuranira *et al.*, 2009).

The results of the present field experiment during different stages of experiment showed that siderophore and HCN producing bacteria were consistently present only in *C. mucunoides* planted area. Numerous environmental factors modulate siderophore synthesis, including pH, the level and form of iron ions, the presence of other trace elements and supply of carbon, nitrogen, and phosphorus (Duffy and Défago, 1999). The bacterial growth as well as siderophore production is stimulated by (NH$_4$)$_2$SO$_4$ and amino acids; however, optimum siderophore yield is obtained with higher nitrogen content (Sayyed *et al.*, 2005). It may be concluded that the change in soil nutrient status will affect the population level of siderophore producing bacteria in soil. This group of bacteria also could promote the growth of cultivated crop in soil. The rhizobacteria which are able to produce siderophores *in vitro* increases early soybean growth in non-sterile soil (Cattelan, 1999). The level of HCN produced in root-free soil by *P. putida* and *A. delafieldii* generally increased with higher amounts of supplemental glycine, with *P. putida* typically generating more HCN (8–38 μM) at a given glycine level (Owen and Zdor, 2001). Castric (1977) presented evidence that other amino acids besides glycine can also stimulate HCN production. Generally, presences of higher amount of available amino acids are expected in soil. The overall observation from different cover crop planted area, weeded area and non weeded area during various stages of experiment confirmed that the microbial populations with special preference to soil fertility related groups observed in the present study could be a measure of sustainable soil fertility in cover crop based soil improvement.
Plate 3.2: Quantification and identification of soil bioprotectant groups of microorganisms; 
a. Siderophore producing bacteria, b. Control, c. HCN producing bacteria, d. Trichoderma sp