8. Summary and Conclusion
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A study was conducted to investigate the effect of soil management practices such as cover crop cultivation, organic mulching and addition of biofertiliser-enriched compost on below ground microbial population in different land use systems of the Restoration Zone of the Kerala part of Nilgiri Biosphere Reserve in the Western Ghats. The experiments were conducted in home garden, coconut plantation, natural forest, plantation forest and in pot culture according to different objectives.

8.1. Effect of cover crops on below ground microbial diversity

In the first objective, *Arachis pintoii*, *Calapagonium mucunoides*, and *Sesbania aculeate*, were planted as cover crops, and a weeded area and non weeded area were maintained as control plots in a coconut plantation. Total microbial population (bacteria, fungi, and actinomycetes) soil fertility indicator group of microorganisms (*Rhizobium*, *Azotobacter* and phosphate solubilising microorganisms) and bio-protectant group of microorganisms (*Trichoderma*, and siderophore and HCN producing bacteria) were enumerated from soil samples taken on 5th, 10th, 15th and 22nd month of plant growth.

The population of bacteria which decreased in all treatments from 5th month to 10th month interval, increased from 15th month onwards in all experimental plots except in weeded area. Comparatively, higher population was observed in cover crop planted area after 15 months growth, and among the cover crops, *Calapagonium* cultivated area showed higher bacterial population. In the case of fungal population, the *Calapagonium* and *Arachis* planted areas showed stability in population from 5th to 22nd month compared with either weeded area or non-weeded area. However, the fungal population status after 22nd month showed no significant difference between experimental plots. The Actinomycetes population in all treatments showed an increase from 5th to 22nd month of experiment except for weeded area. *Arachis pintoii* and *Calapagonium mucunoides* planted area maintained a higher population status throughout the experimental periods.
The population of *Rhizobium* showed an increase from 5th to 10th month in all experimental plots except in weeded area. During 15th month, all the experiment plots showed comparatively lower population of *Rhizobium*, subsequently, on 22nd month, higher number observed only in cover crop planted area. The population of *Azotobacter* showed significant increment from 5th month to 10th month of experiment in all experimental plots except in weeded area. *Calapagonium* and *Sesbania* cultivated area maintained significantly higher population level compared to other experimental plots till the end of experimental period. The population of phosphate solubilising microorganisms showed higher population on 5th month of experiment and maintained almost same status in all experimental plots till the end of experiment period.

The *Trichoderma* sp. maintained population status in all the different period of growth in cover crop planted area. *C. mucunoides* planted area showed higher number of *Trichoderma* sp. *A. pintoii* and *S. aculeata* planted area showed lower number of *Trichoderma* sp. during different period of experiment. But in all intervals, both the non weeded area and weeded area showed few numbers of colonies of *Trichoderma* sp. The results during different sampling intervals of experiment showed that siderophore and HCN producing bacteria consistently present only in *C. mucunoides* planted area.

The cover crop cultivation can develop sustainable soil fertility by maintaining optimum conditions required for the growth and development of the soil fertility related microbial populations. The population dynamics of different groups of soil fertility related microorganisms were very less in weeded area. The *Calpagonium mucunoides* could effectively improve soil fertility related microbial population including bioprotectants.

**8.2. Effect of organic mulching on soil microorganisms**

In the second objective, green mulch of 6 different species individually, along with one mixed species mulch were used in home garden as well as in coconut garden. Population of total microorganisms and enzyme producing microorganisms (amylase, cellulase, phenol oxidase and catalase) were enumerated from soil samples taken during 4th 10th and 18th month of mulch application. Population of soil fertility indicator group of microorganisms and bioprotectants group of microorganisms were enumerated from soil samples taken before mulching and 10th month after mulching.
In home garden, the bacterial population during the different stages of decomposition of various types of mulch were analyzed, which indicated the rise in bacterial population from 4th month of experiment to 10th month of experiment and later it came down during 18th month. The pattern of bacterial population was almost same in all different types of mulches selected for the experiment with comparatively higher population observed in *Ficus asperrima* mulch added plot in all three sampling period. The fungal and actinomycetes populations increased from 4th month to 18th month of mulch decomposition in all different types of mulches. Amylase, cellulase and phenol oxidase enzyme producing microbial population were decreasing from 4th month to 10th month of mulching; but afterwards, on 18th month of mulching, maintained same status of population as in 10th month. But, the catalase enzyme producing microbial population decreased from 4th month to 18th month of mulch decomposition in all different types of mulch added soil. *Eupatorium odoratum*, mixed species and *Ficus asperrima* mulches showed higher population of amylase enzyme producing microbial population during the three sampling periods (4th, 10th, and 18th months). All the different types of mulches showed almost the same range of catalase enzyme producing microbial population.

The bacterial and fungal populations during the different stages of decomposition of various types of mulch were analyzed, which indicates the rise in bacterial population from 4th month of experiment to 10th month of experiment and later it comes down on 18th month. Mixed species, *Ficus asperrima* and *Eupatorium odoratum* mulch added soil showed significantly higher population of bacteria during all three periods of isolations. Actinomycetes population was observed as increasing from 4th month to 18th month of experiment. Among different mulch added area, mixed mulch added area showed significantly higher population of actinomycetes during different periods of isolation. A drastic decline in population of different enzyme producing microorganisms (amylase, cellulase and phenol oxidase) was observed during 4th month to 18th month of experiment. But the population of catalase enzyme producing microorganisms showed a small rise on 18th month after a decline from 4th to 10th month of mulching.

In home garden and coconut plantation, the population of different soil fertility groups of microorganisms (*Rhizobium*, phosphate solubilizing microorganisms and *Azotobacter*) increased significantly in all mulched areas compared to non-mulched
areas. Significant difference ($p < 0.05$) was observed between treatments in populations of all three groups of microorganisms after 10$^{th}$ month of mulching. Generally, higher populations of these groups of organisms were found in mixed mulch-added area in home garden as well as in coconut plantation. The data obtained for microbial parameters from selected mulch added areas both in home garden and coconut plantation were compared with the same species litter fall area in plantation and natural forest. The Nadukani natural forest and Kariem Muriem teak plantation were selected as the sampling plots representing natural and plantation forest, respectively. Significantly higher population of bacteria, fungi and actinomycetes were observed in natural forest where natural leaf fall has formed the mulched area and comparatively higher population of enzyme producing microorganisms were observed in all mulched area of natural and plantation forest, and some of the mulched area of either home garden or coconut plantation.

8.3. Development of a suitable method for enriched compost production

The compost was prepared using mixed weeds as raw material with different types of microbial inoculums such as (I) decomposer microorganisms (DMI) + biofertilisers, and (II) decomposer microorganisms + earth worm + biofertilisers (DMIE), (III) cow dung, (CD), (IV) cow dung + earth worm (CDE), and (V) Without any inoculums. The number of colony forming units (cfu) of microorganisms was enumerated during different periods of composting and compost enrichment. The nutrient status of the finished compost was analyzed and compared.

The carrier based microbial inoculums were prepared by mixing individual broth culture of decomposer microorganisms in its maximum population status (bacteria – $15 \times 10^6$, fungi - $7 \times 10^3$ and Actinomycetes – $2 \times 10^6$) with the inert carrier, talc to obtain a final moisture content of 40%. The number of colony forming units of different groups of microorganisms in the carrier based inoculums was analyzed during different storage period before addition into composting raw materials. After 30 days of storage at room temperature, bacterial populations were observed as $7 \times 10^6$ cells/g, fungal population as $5 \times 10^3$ cells /g and actinomycetes population as $17 \times 10^5$ cells/ gram of carrier based inoculums. Thirty days old carrier based cultures in a concentration of 10 kg/ ton of
weeds were sprayed over intermittent stacks during the compost heap preparation. The number of colony forming units (cfu/g of compost) of bacteria, fungi and actinomycetes were analysed during 12, 23 and 35 days of composting. The results showed that after 12 days, bacteria, fungi and actinomycetes were highest in decomposer microbial inoculum-added composites (compost 1 and compost 2). The compost 5 (without any inoculum) showed lowest cfu/g of compost for bacteria, fungi and actinomycetes.

After 23 days of composting the number of cfu/g of compost for bacteria and actinomycetes was comparatively higher in decomposer microbial inoculum-added composites (DMI and DMIE). But the population status was low compared with those of the previous interval (after 12 days). The number of cfu/g of compost for fungi was almost the same in all treatments and was comparatively higher than that of the previous interval (after 12 days). The compost 5, without any inoculums showed lowest cfu/g for bacteria, fungi and actinomycetes and was also higher than that of the previous sampling results. After 35 days of composting, the number of cfu/g for bacteria was highest in decomposer microbial inoculums added compost (DMI) and was less than the cfu/g found in the sample drawn for previous intervals. The number of cfu/g of compost for fungi was comparatively higher in DMIE compost.

pH of different types of compost was measured during different periods of composting (12, 23 and 35 days). Slightly alkaline pH was observed after 12 days for all heaps except for compost without any inoculum (showed neutral pH). After 35 days of composting, cow dung and microbial inoculum added compost showed a maximum of pH - 7.5 and pH - 8, respectively. The overall temperature range from the beginning to the end of decomposition process of microbial compost, cow dung compost and control (without inoculum) showed almost same pattern of variation except for microbial compost. Microbial compost showed comparatively high temperature after 5th day and an immediate decline afterwards up to 32nd day, other two, cow dung and control, composting processes attained the same low temperature only at 44th and 46th day, respectively. After 12 and 35 days of compost enrichment with biofertilizers, comparatively higher biofertilizer microbial populations were observed both in decomposer microbes added-compost and microbes + earth worm treated-compost except in the case of phosphate solubilising microorganisms and *Trichoderma*. 

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Nutrient status of different types of compost was analyzed after the completion of composting process. N, P, K, C, Cu, Fe, Zn, Mn, Ca and Mg were the parameters considered for analysis. Comparatively higher nutrient status was observed in compost enriched with microbes and earth worm (DMIE); compost added with microbial inoculums (DMI) also showed a better nutrient status. The lowest nutrient status was observed in control (compost 5) prepared without adding any type of inoculum.

8.4. Effect of addition of different compost types in soil fertility

The evaluation of the effect of application of different compost types in soil fertility improvement was done by monitoring the growth parameters of short term vegetable crops viz., cowpea, okra and brinjal as pot culture experiment. The root, shoot and fruit biomass and number of fruits of all the three crops measured after 45 and 90 days of growth showed that a higher value observed by the addition of biofertiliser-enriched DMIE compost. Plants cultivated in biofertilizer enriched DMI compost-added soil showed second highest values for growth parameters at both the period of plant growth. Highest soil nutrient status was observed for the DMIE-compost-applied soil at both the period of evaluation. The nutrient status of DMI compost treated soil also showed improved values for most of soil parameters. In general, higher N, P, K and Mg contents were observed in leaves of plants cultivated in biofertilizer-enriched DMIE compost added pots. The improved soil nutrient status and their effects on annual vegetable crops confirmed that the use of decomposer microbial inoculum or cow dung for improving composting process and the biofertiliser enrichment of such composts can effectively improve the quality of compost further.

8.5. Microbial community analysis of soil micro-aggregates by culture independent method

16S rDNA sequencing was attempted to estimate the comparative density of bacterial community present in soil micro-aggregates of mulched soil. Most of such bacteria were reported to be unculturable using conventional laboratory methods. For the study, DNA was isolated from 48 soil micro-aggregates and quantified using spectrophotometer. The concentration of DNA obtained from individual samples of various land use systems
were statistically analyzed by one way ANOVA. Significant difference in concentration of DNA was observed between treatments in different land use systems. Comparatively higher concentration of DNA was observed in *E. odoratum* mulch added area of home garden and lower at *C. floribunda* mulch added area indicating higher density of microbial community in the former area and lower density in the latter. In general, DNA quantification study showed that large microbial communities resided in soil microaggregates and the microbial density depended on different land use system and management practices. In the present study, using the DNA extracted from soil microaggregates, attempts were also made to identify the bacterial communities present in the soil micro-aggregates through PCR amplification and nucleotide sequencing of 16S rDNA using universal bacterial primers. Out of 48 pooled DNA samples, 34 samples amplified with universal bacterial primer. The PCR amplified DNA from three selected samples was cloned into a plasmid vector (pTZ 57R/T), transformed competent *E. coli* cells and used for amplification of the cloned bacterial 16S rDNA region. The amplified 16S rDNA was forward and reverse sequenced. These sequences were identified as from apparently two new species of uncultured bacteria revealed through comparison with known sequences by BLAST search for sequence similarity.

**Conclusion**

The soil microbial population is one of the most sensitive parameters indicating soil nutrient status. The present study has shown that improvement of soil microbial status and restoration of degraded land, especially converted forest land can be done by adopting different types of soil management practices such as cover crop cultivation, green mulching, organic manure addition, etc. The study also confirms that these types of soil management practices promote wide varieties of soil microbial populations, especially biofertilizers, bioprotectants, and enzyme producing microorganisms. However, measuring sustainable soil fertility by monitoring culturable microbial groups may not give precise values because, soil contains large quantity of unculturable microorganisms also especially those residing in soil microaggregates. Hence, metagenomic approach has to be adopted to identify the unculturable world of microorganisms which may have specific role in soil functions.