ROLE OF SOIL MICROORGANISMS IN C AND P DYNAMICS DURING RECOVERY OF DEGRADED TERRESTRIAL ECOSYSTEMS OF CHERRAPUNJEE PLATEAU

(ABSTRACT)

BY

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Soil microorganisms mediate many critical ecosystem processes and functions that determine nutrient availability to regenerating vegetation during the recovery of degraded ecosystems following disturbance events. Our understanding on how microbial communities are shaped due to changed environmental conditions during the recovery period following disturbance events, and how the structure and function of soil microbial communities influence rates of key ecosystem processes is limited. Considering the importance of P and C cycles in recovery of degraded ecosystems and relatively less knowledge on the role of microbial communities in regulating their dynamics particularly during post–disturbance recovery period, the present study was undertaken in Cherrapunjee plateau (Latitude 25° 16.49' N and Longitude 091° 43.59' E; altitudinal range 1480–1690 m a.s.l) of Meghalaya, Northeast India. The two natural ecosystems of Cherrapunjee viz., forests and grasslands are under constant anthropogenic pressure and highly degraded. Despite all the disturbances, the ecosystems of Cherrapunjee exhibit a high degree of resilience. This resiliency of Cherrapunjee landscape offered an interesting case study to understand the underlying processes and mechanisms, particularly the role of microbial communities that result in the ecosystem stability. The objectives of the present study were: (i) to study the changes in soil and vegetation characteristics during recovery process; (ii) to elucidate the role of microorganisms in C and P dynamics during recovery period; and (iii) to identify a few P solubilising microbial strains based on their relative efficiency during different years of recovery.

The present study was conducted in two natural ecosystems of Cherrapunjee viz., forest and grassland. The forest ecosystem was disturbed due to deforestation, and the grassland ecosystem was disturbed due to mining related activities such as dumping of coal and movement of trucks. The study followed a chronosequence approach to understand the recovery pattern of these ecosystems. Two replicate stands for each recovery age category and undisturbed forest/grassland in each ecosystem were selected at similar elevation within 2.7 km radius in Sohrarim and Laitryngew villages of Cherrapunjee plateau. The four categories of stands in forest ecosystem were: i) old–growth undisturbed forest stands (UF), and a chronosequence of three clearcut and regrowing stands viz., ii) freshly clear–cut forest representing the post–disturbance stands (CF1), iii) 5–year old recovering forest stands (RF5) and iv) 10–year old recovering forest stands (RF10). The four categories of stands in grassland ecosystem were: i) undisturbed grassland stands (UG), and a chronosequence of three mining affected and recovering stands viz., ii) mining affected grassland stands (MG), iii) 15–year old recovering grassland stands (RG15) and iv) 50–year old recovering grassland stands (RG50). All the stands were once the part of the undisturbed forest/grassland ecosystems having similar vegetation and soil characteristics.

The findings of the study may be summarized as follows:

Change in soil characteristics during the recovery of ecosystems

- In the forest ecosystem, the soil of CF1 stands, i.e. immediately after deforestation had the highest clay content (16.4%). As recovery progressed, clay content reduced significantly ($p<0.00$). There was a significant variation ($p<0.01$) in the proportion of sand and clay fractions in soils of CF1 stands with those of recovering RF5 and RF10, and the undisturbed forest stands. Soil of the CF1 stands was sandy loam, while soils of both the recovering stands and the undisturbed stand were loamy sand. In grassland ecosystem, only the sand proportion varied significantly ($p<0.00$) among different recovery ages. It ranged between 69% and 92% depending on the age of recovery. Therefore, soil textural class varied among the recovering ages. It was a sandy loam in UG and MG, sandy in RG15, and loamy sand in RG50.
- In forest ecosystem, bulk density (BD) significantly ($p<0.01$) decreased with recovery ages. It was highest in CF1 stands (0. 51 g cm^{-3}) i.e. immediately after deforestation followed by the recovering RF5 (0.41 g cm^{-3}), RF10 (0.30 g cm^{-3}) and the old growth undisturbed forest stands (0.26 g cm^{-3}). In grassland ecosystem, it increased due to disturbance and showed an overall reduction as recovery proceeded. The mining affected MG sites had significantly ($p<0.00$) greater BD than the recovering RG15 and RG50 sites. However, porosity followed a reverse trend as expected in both forest and grassland ecosystems.
- Water holding capacity (WHC) also followed the same trend as that of BD. With the increase in bulk density WHC also increased. It decreased significantly ($p<0.00$) as recovery followed. WHC ranged from 12–38% in grassland and 18–60% in forest.
- In forest ecosystem, cation exchange capacity (CEC) was significantly ($p<0.05$) greater in CF1 stands than the recovering i.e. RF5 and RF10 and UF stands. Overall, the value reduced significantly ($p<0.05$) as recovery progressed. Similarly in grassland ecosystem, CEC decreased with increasing recovery age. It was highest in MG and was significantly greater ($p<0.00$) than the recovering RG15 and RG50 and the undisturbed grasslands.
- In forest ecosystem, soil temperature (ST) reduced significantly ($p<0.01$) as recovery progressed. It varied significantly ($p<0.00$) among the recovery ages. However in

grassland ecosystem, ST did not show any significant difference among MG, RG15, RG50 and the undisturbed grassland sites. It was significantly ($p<0.01$) higher in grassland ecosystem than forest ecosystem. ST varied significantly ($p<0.00$) among the seasons in forest ecosystem with highest during rainy season and lowest during winter season.

- Soil moisture content (SMC) was significantly ($p<0.01$) greater in CF1/MG stands than the recovering and undisturbed stands. The value reduced significantly ($p<0.01$) as recovery progressed. SMC was significantly ($p<0.00$) greater in forest ecosystem than grassland ecosystem. It ranged from 22.4 – 34.6% in forest ecosystem and 15.9 – 31.5% in grassland ecosystem.
- The soils were acidic in nature in both the ecosystems. In forest ecosystem, pH had a relatively narrow range of 4.8–4.9 than the grassland ecosystem (4.0–5.3). In grassland ecosystem the disturbed MG sites had significantly ($p<0.01$) lower pH than the recovering RG15 and RG50 sites, and the undisturbed sites. Overall, the value increased significantly ($p<0.01$) as recovery progressed. pH also varied significantly ($p<0.00$) among the seasons in grassland ecosystem with highest value during winter and lowest during rainy.
- In forest ecosystem, soil organic carbon (SOC) was significantly ($p<0.01$) greater in CF1 stands (4.5%) i.e. immediately after deforestation than the undisturbed stands (3.8%). It reduced till 5th year of deforestation (RF5: 4.3%), after which it started to increase (RF10: 4.6%) indicating that the system recovered after 5th year of disturbance. In grassland ecosystem, due to coal dumping SOC was significantly ($p<0.00$) greater in MG sites (4.9%) than the recovering RG15 (2.6%) and RG50 (2.4%) sites, and the undisturbed grassland sites (3.9%). As coal dumping was stopped and the recovery started, the SOC values decreased sharply. It varied significantly ($p<0.00$) among recovery ages in both forest and grassland ecosystem. SOC was significantly ($p<0.00$) higher in grassland ecosystem than forest ecosystem. It ranged between 3.8 – 4.6% in forest and 2.4 – 5.0% in grassland ecosystem. SOC varied significantly ($p<0.00$) among the seasons and was highest during rainy season and lowest during summer season in both the ecosystems.
- Total phosphorus (TP) varied between 217.6 and 363.8 $\mu g\ g^{-1}$ in forest ecosystem, and 153.1 and 297.2 $\mu g\ g^{-1}$ in grassland ecosystem. In forest ecosystem, TP was significantly ($p<0.01$) greater in CF1 stands than the recovering RF5 and RF10 and undisturbed forest stands. As recovery followed the TP content declined initially till 5th year of deforestation and increased thereafter. In grassland ecosystem, UG and

MG sites had significantly ($p<0.00$) higher TP than RG15 and RG50. It was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. TP varied significantly ($p<0.00$) among the seasons, and was highest during autumn and lowest during summer/rainy seasons.

- Total Kjeldahl nitrogen (TKN) in forest soil ranged between 0.25 and 0.48%, and 0.13 and 0.32% in grassland ecosystem. In forest ecosystem, TKN was significantly ($p<0.00$) greater in CF1 stands (0.48%) i.e. immediately after deforestation than RF5 (0.33%), RF10 (0.39%) and UF (0.25%) stands. In grassland ecosystem, TKN showed a sharp decline as recovery progressed. The mining affected MG (0.32%) and UG (0.25%) sites had significantly ($p<0.00$) greater values of TKN than the recovering RG15 (0.14%) and RG50 (0.13%) sites. It was significantly ($p<0.00$) higher in forest ecosystem than the grassland ecosystem. It varied among the seasons significantly ($p<0.00$) and was highest during winter and lowest during summer.
- In forest ecosystem, C/N ratio was significantly ($p<0.01$) lower in CF1 stands than RF5, RF10 and UF stands. As recovery proceeded, it showed an overall increasing trend. C/P also followed a more or less same trend with an overall increase along the recovery ages. In grassland ecosystem, C/N ratio was significantly ($p<0.01$) lower in MG than RG15, RG50 and UG sites. It showed an increasing trend as recovery progressed. In contrast, C/P declined as recovery progressed. It was significantly ($p<0.01$) greater in MG than UG.
- PCA revealed higher impact of soil WHC, C/P and TP on the stands than the other physico–chemical properties.

Vegetation characteristics during recovery

- In forest ecosystem, due to disturbance a reduced number of tree species were recorded from the recovering stands. In the 1st year of deforestation, i.e. in CF1 stands, 27 tree species were recorded. In the recovering stands RF5 and RF10, 27 tree species and 34 tree species, respectively were recorded. The number of species under herbs and shrubs increased after disturbance. The number of herb species in CF1, RF5 and RF10 was 31, 22 and 13, respectively. The corresponding figures for shrub species were 23, 32 and 15. Only 12 tree species (15%) were common to all the stands. Three species viz., *Lithocarpus fenestratus*, *Photinia* sp. and *Toddalia asiatica* were exclusively found in CF1, 5 species viz., *Albizia odoratissima*, *Beilschmiedia assamica*, *Brassaiopsis speciosa*, *Ficus neriifolia* and *Litsea salicifolia* in RF5 and only one species viz., *Viburnum simonsii* was found in RF10 stands. Thirty two species were exclusive to UF stand only.

- In grassland ecosystem, from MG sites, nine herbaceous species belonging to 9 genera and 5 families were recorded. The recovering sites RG15 and RG50 had 20 and 21 plant species, respectively. Only 6 plant species (19%) were common to all the sites. One plant species viz., *Themeda intermedia* was found only in RG50 while 7 species viz., *Aeginetia indica, Habenaria goodyeroides, Malaxis acuminata, Satyrium nepalense, Spiranthes sinensis, Themeda villosa, Tripogon trifidus* were found only in UG. No species was exclusive to MG and RG15.
- In forest ecosystem, Shannon's diversity index and evenness index for tree species reduced due to disturbance. However, as recovery followed the diversity and evenness gradually improved to attain a more diverse and equitable community. In case of herbs and shrubs, Shannon's diversity index increased immediately after disturbance and subsequently reduced. Pielou's evenness index was greater for herbs than shrubs and trees in all the stands. In grassland ecosystem, Shannon's diversity index was greater in the undisturbed site than the recovering sites. Disturbance reduced the species diversity, which improved as recovery followed. Pielou's evenness index exhibited quite high values in all the stands.
- The dominance–diversity curve followed a log normal distribution pattern i.e. low dominance or high equitability for trees in the undisturbed forest stands whereas in CF1, RF5 and RF10 the trees species showed low equitability or high dominance. The pattern of dominance–diversity curves for herbs and shrubs however, did not vary among the different recovery ages. In grassland ecosystem, the plant species exhibited a log normal distribution pattern in UG, RG15 and RG50 sites whereas MG sites had a steeper dominance–diversity curve depicting high dominance of a few species i.e. *Digitaria violascens* and *Arundinella khasiana.* The dominant species in all the vegetation components changed with recovery age in both the ecosystems.
- In forest ecosystem, tree density was lowest in CF1 (326 stems ha^{-1}) followed by RF5 (1052 stems ha^{-1}). The highest density value was in RF10 (1706 stems ha^{-1}) i.e. after 10 years of recovery. The tree density in the undisturbed forest stands was 1144 stems ha^{-1}. Tree basal area was lowest in CF1and increased as recovery followed. Densities of herb and shrub species increased due to disturbance. The density values reduced as recovery proceeded. In grassland ecosystem, disturbance reduced plant density. MG sites (27, 000 ha^{-1}) had significantly ($p<0.05$) lower density than the recovering RG15 (2,99,500 ha^{-1}) and RG50 (3,13,000 ha^{-1}) sites, and undisturbed

grassland site (3, 83,500 ha^{-1}). With increase in recovery age the density values increased.

- Tree species composition during different recovery ages of the forest ecosystem were assessed by PCA. *Quercus glauca* had higher impact on the CF1 stands and the recovering stands RF5 than the other species. *Symplocos glomerata* and *Neolitsea cassia* were found important for the recovering stands RF10. In grassland sites, *Digitaria violascens*, *Borreria articularis*, *Eulalia quadrinervis* and *Arundinella khasiana* were found important for mining affected MG sites. *Eragrostis nigra* and *Osbeckia capitata* had higher impact on recovering sites RG15 and RG50 respectively.

Microbial community structure and functions related to C–dynamics

- Disturbances impacted the total amount of soil organic matter in both the ecosystems, which had a direct effect on the biomass, population size and activities of microbial community. A decline in the biological properties associated with C cycle viz., enzyme activity, microbial biomass carbon (MBC), MBC/SOC ratio and basal respiration (BR) was observed following disturbance.
- MBC ranged between 324 $\mu g\ g^{-1}$ and 663 $\mu g\ g^{-1}$ in forest ecosystem and 206 $\mu g\ g^{-1}$ and 482 $\mu g\ g^{-1}$ in grassland ecosystem. It reduced significantly ($p<0.05$) in the disturbed sites in both the ecosystem and showed gradual increase with progress in recovery. MBC was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. It varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with highest during winter and lowest during rainy.
- MBC/SOC ratio ranged between 0.7% and 1.9% In forest ecosystem and 0.4% and 1.9% in grassland ecosystem. This ratio increased as recovery progressed and indicated accumulation of labile C in soil and favourable environment for microbial growth. As microbial populations are largely dependent on the soil labile C as their C source, low MBC/SOC ratio after disturbance was indicative of poor quality of organic matter.
- A decline in β–glucosidase activity following disturbance was observed. This indicated that the microbial populations allocated most of their energy towards maintenance of a minimum viable population size rather than enzyme production after disturbance. β–glucosidase ranged between 257.4 $\mu g\ g^{-1}$ soil h^{-1} and 309 $\mu g\ g^{-1}$

soil h^{-1} in forest ecosystem, and 116.7 $\mu g\ g^{-1}$ soil h^{-1} and 256.0 $\mu g\ g^{-1}$ soil h^{-1} in grassland ecosystem.

- Dehydrogenase (DHY) activity also followed the similar trend. The values of dehydrogenase ranged between 21.8 $\mu g\ g^{-1}$ soil h^{-1} and 36.6 $\mu g\ g^{-1}$ soil h^{-1} in forest ecosystem and 14.8 $\mu g\ g^{-1}$ soil h^{-1} and 29.9 $\mu g\ g^{-1}$ soil h^{-1} in grassland ecosystem. DHY activity was significantly higher ($p<0.00$) in forest ecosystem than grassland ecosystem. DHY activity varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with its peak during rainy season and trough during winter.
- Basal respiration ranged between 2.7 $\mu g\ g^{-1}$ soil h^{-1} and 3.8 $\mu g\ g^{-1}$ soil h^{-1} in forest ecosystem, and 1.8 $\mu g\ g^{-1}$ soil h^{-1} and 2.7 $\mu g\ g^{-1}$ soil h^{-1} in grassland ecosystem. It declined significantly ($p<0.05$) after disturbance due to the decrease in microbial population in both the ecosystem and showed gradual increase with recovery progress. Basal respiration was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. It varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystem with highest during rainy season and lowest during winter.
- The microbial metabolic quotient (qCO_2), a bioindicator of ecosystem development as well as of environmental stress on microbial communities, had greater values in the post disturbance sites. This was due to increased availability of readily degradable C indicating that a relatively large percentage of the substrate was decomposed to meet the energy demand of the microflora. As recovery progressed, the prevailing stress on soil microbes gradually subsided resulting in decreased qCO_2. It ranged between 7.0 and 13.9 in forest ecosystem, and 6.7 and 11.4 in grassland ecosystem.
- Among the soil biological parameters studied, dehydrogenase activity, glucosidase activity and basal respiration showed significant correlation with soil moisture and temperature. SOC showed a significant positive relationship with dehydrogenase activity. BR was significantly correlated with SOC and Bray's extractable P in forest ecosystem. MBC showed a significant positive correlation with total P and extractable P in forest ecosystem and with TKN in grassland ecosystem. Therefore, it may be concluded that the microbial populations were limited by P in forest ecosystem and by N in grassland ecosystem.

Microbial community structure and functions related to P–dynamics

- While total P increased immediately after the disturbance both in forest and grassland ecosystems, extractable P declined. The decline in extractable P after disturbance was partly attributed to weathering of soil due to removal of vegetation cover. Microbial biomass played a crucial role in P dynamics as it acted as a transient sink of labile P. The percentage contribution of microbial biomass P to total P (7–21%) was higher than the values reported by earlier workers (2.6–7.9%). This suggests that microbial communities in the present study sites have evolved a more complex system of substrate–use efficiency with the heterogeneous input of organic matter, enabling them to immobilise a greater portion of P in their biomass. This higher rate of immobilization of P in the microbial biomass observed in the present study might be a possible reason for the very low available P in these ecosystems.

- There was a decline in phosphatase activity during the initial years of recovery. After 5th year of recovery the activity increased showing net recovery. Acid phosphatase activity was highest during rainy season and lowest during dry season. Significant positive relationship of acid phosphatase activity with SOC and total N was obtained.
- The extractable P was higher during wet season than the dry seasons, which was attributed to the enhanced water filled pore space due to rainfall that restricted the diffusion of oxygen from the atmosphere to the soils creating anaerobic condition in soils. This anaerobic condition reduced the redox potential of soil promoting Fe reduction and the concomitant release of Fe–bound P. A negative correlation between the inorganic P fractions (water extractable P and Bray's extractable P) and acid phosphatase activity was due to the inhibition of acid phosphatase activity by available P (negative feedback regulation).
- MBP was positively correlated with total P ($p<0.00$) in forest ecosystem and with SOC in grassland ecosystem. The significant positive correlation between microbial biomass P and total P indicates that it could be used as an indicator of ecosystem recovery.

Phosphorus solubilising bacterial diversity during ecosystem recovery, and their relative efficiency

- A combination of approaches viz., morphological, biochemical and 16S rRNA gene sequences were used for the identification of PSB from undisturbed and recovering stands of forest and grassland ecosystems. A total of 47 PSB isolates were obtained after screening which were identified. The phosphate solubilization potential of PSB species was evaluated through *in vitro* experiments as well as crop application.
- The decrease in the population size and diversity of PSB after disturbance was observed. *Burkholderia* was the dominant genus involved in P solubilization in both

the ecosystems. In spite of growing in selective NBRIP medium, only a few isolates formed a halo around the colony. On the other hand, all the isolates growing on NBRIP medium reduced pH suggesting that pH reduction assay is a far superior tool to screen PSB.

- Crop application of selected PSB showed positive effects on the growth of maize plants. Inoculation with PSB made more soluble phosphates available to the growing plants and therefore, was the reason for improved growth of maize plants in pot experiment. The PSB species with inherent high solubilising efficiency in *in vitro* conditions failed to retain the similar efficiency when exposed to stress in terms of reduced nutrients presented by the pot experiment. In contrast, species with intermediate level of efficiency in *in vitro* conditions excelled in the stressed conditions. The stress originating from disturbance events such as mining related activities, resulted in occurrence of the most efficient strains of PSB e.g. *Burkholderia tropica* in the disturbed grassland ecosystem indicating the positive response of microbes to high intensity disturbances.
- Recovering stands varying in recovery age and the undisturbed stands had different PSB species composition. New groups of PSBs colonised each stand and no species was common to all the recovering/undisturbed stands. This pattern of species composition during different recovery ages indicates that PSB as a community exhibits a microbial succession model that is very close to the "Relay floristic model" as exhibited by higher plants during the succession process.

Factors affecting the distribution of PSB during ecosystem recovery

- The disturbance events resulted in reduction in PSB populations from 16 x 10^4 CFU g^{-1} soil to 29 x 10^4 CFU g^{-1} soil in forest ecosystem and 13 x 10^4 CFU g^{-1} soil to 27 x 10^4 CFU g^{-1} soil in grassland ecosystem.
- CCA revealed a significant association of PSBs with some plant species and soil parameters. *Castanopsis tribuloides*, *Quercus glauca*, *Elaeocarpus lanceifolius* and *Neolitsea cassia* were important tree species in forest ecosystem influencing PSB distribution. The PSB species *Burkholderia arboris* showed association with two tree species viz., *Castanopsis tribuloides* and *Quercus glauca*, PSB species *Burkholderia fungorum* was influenced by *Elaeocarpus lanceifolius* and *Castanopsis purpurella*, and the PSBs *Burkholderia phytofirmans* and *Burkholderia cepacia* showed association with *Symplocos glomerata* and *Neolitsea cassia* tree species. In grassland

ecosystem, *Arundinella khasiana*, *Cyanotis vaga*, *Eriocaulon cristatum* and *Themeda intermedia* were important plant species influencing PSB distribution. Only two species of PSB viz., *Burkholderia unamae* and *Burkholderia mimosarum* showed association with the grass species *Arundinella khasiana* and *Cyanotis vaga*.

- CCA also revealed that soil temperature, soil moisture, acid phosphatase activity, water extractable phosphorus and phosphorus mineralization were important for the distribution of PSB species among the different recovering stands. Two PSB species viz., *Burkholderia phytofirmans* and *Burkholderia phenoliruptrix* showed strong association with porosity, microbial biomass carbon (MBC) and phosphorus (MBP). A third species viz., *Burkholderia fungorum* was associated with total nitrogen (TKN).

Conclusion

- Notwithstanding the limitations of chronosequence studies, the study revealed that microbes did play a major role in both the ecosystems during post–disturbance recovery process. As stress resulting from ecosystem degradation reduced with recovery age, the functional diversity and activity of the soil microbial communities increased. The microbes influenced the recovery process by impacting various ecosystem processes and functions through their activities such as enzyme activities, mineralization and immobilization.
- Several structural and functional microbial parameters were identified as potential indicators for monitoring the recovery of C and P pools in grassland and forest ecosystems. Most of these parameters showed sufficient level of sensitivity to recovering vegetation and soil properties. Among these, the parameters which showed a consistent trend and greater percentage recovery were considered more sensitive indicators and were recommended for using them as indicators for monitoring the rate of ecosystem recovery. These indicators are: (i) plant density, Bray's extractable phosphorus, C/N, microbial biomass carbon, MBC/SOC, MBP/TP and metabolic quotient in both the ecosystems, (ii) soil moisture content and microbial biomass phosphorus only in forest ecosystem, and (iii) dehydrogenase activity in grassland ecosystem.

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DECLARATION

I, Debashree Nath, hereby declare that the subject matter of this thesis entitled "Role of soil microorganisms in C and P dynamics during recovery of degraded terrestrial ecosystems of Cherrapunjee plateau" is the record of work done by me. The contents of the thesis did not form basis of award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any University/Institute.

This is being submitted to the North-Eastern Hill University for the award of the degree of Doctor of Philosophy in Botany.

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CHAPTER 1
INTRODUCTION

Resource exploitation from earth's ecosystems by the humans has increased manifold during the last half century compared to the entire history of the planet (Steffen *et al.*, 2004; Ellis and Ramankutty, 2008; Rockstrom *et al.*, 2009; Ellis *et al.*, 2010). This has caused disturbance in many ecosystems and resulted in significant land use changes at landscape level. Land use changes pose threat to biological diversity, impact many ecological processes, and reduce resilience ability of an ecosystem (Fu *et al.*, 2000; Hacisalihoglu, 2007). Such changes are often associated with intensified land use that cause soil erosion and compaction, altering many soil physical and chemical properties (Islam and Weil, 2000; Chen *et al.*, 2001; Caravaca *et al.*, 2002; Celik, 2005; Wang *et al.*, 2006; Misir *et al.*, 2007). Soil bulk density increases following deforestation resulting in decrease in soil porosity and aggregate stability (Motavalli and McConnell, 1998; Riezebos and Loerts, 1998; Islam and Weil, 2000; Mojiri *et al.*, 2012). Organic C and total N content in soil decrease following conversion of forest land to other land uses (Mulugeta *et al.*, 2005).

Subsequent to a disturbance event, an ecosystem follows natural recovery process. The natural recovery process commences immediately if the intensity of disturbance gets reduced or the disturbance causing factors cease to operate. In fact, the rate of recovery during the post–disturbance period is a function of past disturbance history and the matrix of environmental factors that the ecosystem is exposed to. Ecosystem recovery encompasses restoration of soil characteristics as well as vegetation. Therefore, to monitor ecosystem recovery process, soil and vegetation characteristics are two important criteria. A number of soil physical, chemical and biological parameters act as good indicators of ecosystem recovery, since many of these are sensitive to reducing ecological stress during recovery stages. Some of the potential physico–chemical indicators of soil quality include, bulk density, water holding capacity, porosity, pH, cation exchange capacity and organic matter content (Larson and Pierce, 1991). However, significant changes in these properties occur only with a substantial time lag that ranges from a few years to decades. In contrast, soil biological properties such as soil microbial biomass, microbial respiration and enzyme activities respond very quickly to small changes in soil conditions and therefore, are increasingly being used as indicators to monitor recovery process following anthropogenic disturbances (Sicardi *et al.*, 2004; Izquierdo *et al.*, 2005; Lagomarsino *et al.*, 2009).

Anthropogenic disturbances leading to land use changes in an ecosystem induce substantial changes in microbial communities in terms of density and diversity, and also affect their dynamics and activities. Altered microbial functions in turn influence the biogeochemical cycles (Cleveland *et al.*, 2003), and impact the overall functioning of the ecosystem (Bascompte, 2009). Restoration of biogeochemical cycles is fundamental to recovery of degraded ecosystems, particularly for adequate availability of nitrogen (N), phosphorus (P) and other nutrients to the plants. Being substrate–specific, maintenance of the variety and variability among the microbes within the microbial community is essential as they mediate the release of a wide range of organically bound nutrients. In addition, maintenance of a minimum and critical size of various soil microbial populations is crucial to sustain the ecosystem functioning during recovery phase. The microorganisms derive the energy needed for their maintenance from soil organic matter, which is also the source of C for building the microbial biomass. Therefore, availability of C to the microbial community holds key to the restoration of biogeochemical cycles as well as to the overall recovery process.

Microorganisms play an important role in plant nutrient acquisition either directly through N fixation and nutrient mobilisation through mycorrhizal association (Sprent, 2001; Phiri *et al.*, 2003) or indirectly by converting the unavailable forms of various elements such as P into forms that can be assimilated by plants through specific biochemical reactions (Richardson and Simpson, 2011). They also influence soil respiration, carbon mineralization (Franchini *et al.*, 2007), decomposition of organic matter (DeAngelis *et al.*, 2013) and soil formation (Rillig and Mummey, 2006; Schulz *et al.*, 2013). Because all these processes are microbial–specific, maintenance of the diversity of soil microorganisms in the soil system is important. However, their capacity to maintain functional diversity of soil processes particularly following disturbances and during succession stages is more important than the taxonomic diversity as ecosystem productivity and stability are crucial for ecosystem recovery (Caldwell, 2004).

Soil organic C (SOC) being the most important source of C for soil heterotrophic microorganisms, cycling of C in soil compartments is important during ecosystem recovery. The growth and activity of soil microorganisms are mainly limited by carbon availability (De Nobili *et al.*, 2001; Ekblad and Nordgren, 2002; Ilstedt and Singh, 2005; Demoling *et al.*, 2007). In fact, the size, activity and diversity of the soil microbial community are closely linked to SOM content (Nsabimana *et al.*, 2004). Besides their role in active labile C pool in soil, microbes also impact C storage in soils by transforming C in decaying plants into humic substances, with varying degrees of recalcitrance (Jastrow *et al.*, 2007). Since soils contain about 75% of the C in the terrestrial ecosystem, knowing more about the microbial processes leading to long–term carbon storage will help sequestering atmospheric carbon.

Soil biological processes provide resiliency and buffering capacity to ameliorate stress in disturbed environments (Karlen *et al.*, 1992). It has been established that soil biological processes such as microbial biomass, soil enzyme activity and soil respiration respond more quickly to environmental changes than total soil organic matter (SOM)

(Doran *et al.*, 1996). This is due to the fact that about 95% of the total SOM is non–living and is relatively stable or resistant to change, therefore decades may be required to observe a measurable change in SOM (Masto *et al.*, 2006). On the other hand, microbial biomass responds rapidly to altered soil conditions, as it has a turnover time of less than 1 year (Paul, 1984). Therefore, selection of appropriate microbial parameters and microorganism–mediated processes is important that would act as useful indicators for monitoring ecosystem recovery. There is growing evidence that soil microbiological and biochemical parameters viz., microbial biomass, enzyme activities and labile organic matter may be used as early and sensitive indicators of soil ecological stress or restoration (Dick, 1997). The relative role of microorganisms can be evaluated by estimating the size and activity of specific soil microbial populations. However, due to the complex dynamics of soil ecosystems, no single property is satisfactory for studying microbial activity.

Microbial biomass carbon (MBC) is the living and active part of the soil organic matter, the source and sink of available nutrients, and builds up with increased accumulation of organic matter during soil development (Jenkinson and Ladd, 1981; Diaz–Ravina *et al.*, 1993). It is an important component for maintaining soil quality and plant productivity (Nogueira *et al.*, 2006). Increase in microbial biomass C is more important than the increase in the amount of soil organic C because microbial biomass is one of the most labile pools comprising organic matter, and an increase in microbial biomass carbon (MBC) is likely to better represent changes in the nutrient–supplying capacity of organic matter (Gunapala and Scow, 1998). Apart from MBC, MBC/SOC and qCO2 have been used to indicate the soil vulnerability to disturbance in terms of resilience and resistance (Seybold *et al.*, 1999). It is assumed that an ecosystem has low resistance if MBC is significantly decreased after disturbances, but high resilience if the MBC/SOC and the qCO2 are barely affected (Kaschuk *et al.*, 2010).

Soil microorganisms, and extracellular enzymes most of which have a microbial origin (Tabatabai, 1994), are responsible for the biological transformations sustaining soil functions and control the rate at which substrates are degraded and become available for uptake by microbes and plants (Marx *et al.*, 2001). Soil enzyme activities are one of the most suitable indicators of soil quality that are increasingly been studied (Pascual *et al.*, 2000; Zabaloy *et al.*, 2008; Tejada, 2009). Estimation of soil enzyme activities as recovery indicators has an edge over other microbial parameters because: (a) they are strictly linked to the microbial populations and soil nutrient dynamics, and therefore provide a direct measure of the soil microbial activity; (b) they respond to both natural and anthropogenic changes quite fast; (c) they can be easily monitored (Calderon *et al.*, 2000; Colombo *et al.*, 2002; Nannipieri *et al.*, 2002; Schimel and Weintraub, 2003). Soil enzymes most commonly analyzed are various hydrolases involved in the C, N, P and S cycles, as well as some oxidoreductases such as dehydrogenase. Analysis of soil hydrolases for example, β–glucosidase and phosphatase involved in C and P cycles respectively, provide some insight into the metabolic capacity of the soil (Shaw and Burns, 2006). The hydrolase enzymes also play a role in key processes in the decomposition of organic remains.

The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils because the enzyme is an integral part of intact cells, and does not accumulate extracellularly in the soil (Burns, 1978). Dehydrogenase enzyme is known to oxidise soil organic matter by transferring protons and electrons from substrates to acceptors. This process is a part of respiration pathway of soil microorganisms and is closely related to the type of soil and soil air–water conditions (Glinski and Stepniewski, 1985; Kandeler, 1996). Therefore, studies on the activities of dehydrogenase enzyme in the soil are very important.

Beta–glucosidase is a common and predominant enzyme in soils (Eivazi and Tabatabai, 1988; Tabatabai, 1994). It is named according to the type of bond that it hydrolyses. This enzyme plays an important role in soils because it is involved in catalysing the hydrolysis and biodegradation of various beta–glucosides present in plant debris decomposing in the ecosystem (Ajwa and Tabatabai, 1994; Martinez and Tabatabai, 1997). Its final product is glucose, an important C energy source of life to microbes in the soil (Esen, 1993). There is considerable evidence suggesting that a significant fraction of enzyme activity measured in soil originates from abiontic enzymes (enzymes of biological origin no longer associated with living cells) excreted into the soil solution or immobilised enzymes of microbial origin adsorbed to clays or humic colloids (Busto and Perez–Mateos, 2000). Beta–glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilise the soil organic matter, and can be used to detect management effect on soils (Bandick and Dick, 1999; Ndiaye *et al.*, 2000). Generally, beta–glucosidase activities can provide advanced evidence of changes in organic carbon long before it can be accurately measured by other routine methods (Dick, 1994; Dick *et al.*, 1996; Wick *et al.*, 1998). Beta–glucosidase enzyme is very sensitive to changes in pH, and soil management practices (Dick *et al.*, 1996; Kuperman and Carreiro, 1997; Bergstrom *et al.*, 1998; Bandick and Dick, 1999; Leiros *et al.*, 1999; Acosta–Martinez and Tabatabai, 2000; Madejon *et al.*, 2001). Therefore, estimation of beta–glucosidase activity can be a potential indicator of ecosystem recovery.

As enzymes are important indicators of the extent to which soil disturbance affects the immediate environment (Pascual *et al.*, 2000) and the subsequent recovery of the structural and functional properties of a soil following disturbance (Bending *et al.*, 2000; Breure, 2005), measurement of soil enzyme activities would provide invaluable information for soil quality and ecosystem recovery status. Although enzyme activities have been used for evaluating restoration success post disturbance (Izquierdo *et al.*, 2005; Jiang *et al.*, 2009), only a few studies have focused on them during spontaneous succession (Baldrian *et al.*, 2008).

Enzyme activities being directly related to the structure of the soil microbial community, as the enzymes are highly species–specific, estimation of soil enzyme activity would also indirectly provide the characteristic features of a microbial community. Many studies have highlighted the seasonal fluctuations of microbial community structure and function (Closa and Goicoechea, 2010; Kaiser *et al.*, 2010; Prevost–Bourea *et al.*, 2011; Koranda *et al.*, 2013; Thoms and Gleixner, 2013) but such studies during different stages of succession are extremely rare (Baldrian *et al.*, 2008; Moore–Kucera and Dick, 2008).

Unlike the Carbon cycle which is relatively open with large inputs and losses from the system, P cycling is rather recycled tightly within the ecosystems (Chapin *et al.*, 2002). Low availability of soil P is a major nutritional constraint for primary production in many ecosystems in the world (Raghothama, 1999). Batjes (1997) estimated that about 5.7 billion hectares of land worldwide is P–deficient. As such, the proportion of available P is extremely low (only 5–10%) in comparison to total soil P (Kucey *et al.*, 1989). The scarcity of available P (orthophosphates, $H_2PO_4^-$ and HPO_4^{2-}) in the soil has been attributed to: (i) immobilization of the soluble inorganic forms of P as a result of strong interactions with the components of weathered soils to form insoluble inorganic calcium, aluminum, and iron phosphates (Tan, 1993; Johnson and Loeppert, 2006), (ii) strong adsorption to the soil particles and precipitation as variscite and strongite in acid soils, or with Ca^{2+} in alkaline soils (Whitelaw, 2000), and (iii) loss of organic soluble P due to different reasons (Hedin *et al.*, 2003). In fact, organic P makes up a large fraction of soluble P, as much as 50% in soils with high organic matter content (Bayer *et al.*, 2001;

Gyaneshwar *et al.*, 2002). Phytate, a hexaphosphate salt of inositol, is the major form of P in organic matter contributing between 50 and 80% of the total organic P (Alexander, 1977; Wakelin *et al.*, 2004; Richardson *et al.*, 2005). Unfortunately, this labile organic P becomes insoluble as a result of forming complex molecules with Fe, Al and Ca in soil system (Turner *et al.*, 2007; Vincent *et al.*, 2012). All the factors mentioned above associated with frequent loss of available P due to extensive weathering, and leaching due to heavy precipitation before its uptake by the plants, make P a limiting factor to plant growth during the recovery process.

Therefore, important strategies for increasing phosphate availability in the soil should include: (i) controlling the leaching losses of organic soluble P, and (ii) mobilization of the orthophosphate from the insoluble and adsorbed complexes, mostly through microbial solubilization. P dynamics can be affected by microorganisms in several ways including the following: (1) an increase in biomass of phosphate solubilizing microorganisms (PSM) due to rhizo–deposition leading to increased P solubilization in the rhizosphere zone (Gyaneshwar *et al.*, 1998); (2) substantial release of P following the death of the microbial cells since microbes immobilize up to 10% of the total soil P (Richardson, 2001); and (3) mineralization of organic P to inorganic P through phosphatase activity (Kraemer and Green, 2000; Oehl *et al.*, 2001; Tarafdar and Claassen, 2003; Yadav and Tarafdar, 2003; Neble *et al.*, 2007; Zhang *et al.*, 2010).

Phosphate solubilising microorganisms are ubiquitous, though their numbers vary from soil to soil. Several soil bacteria, as well as fungi, e.g. *Burkholderia*, *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Enterobacter*, *Pantoea*, *Ralstonia*, *Flavobacterium*, *Corynebacterium*, *Streptomyces*, *Serratia*, *Aspergillus*, *Penicillium* and *Trichoderma* spp., are known to dissolve inorganic phosphorus (Kucey *et al.*, 1989; Seshadri *et al.*, 2002; Wakelin *et al.*, 2004; Chung *et al.*, 2005; Kim *et al.*, 2005; Souchie *et al.*, 2006; Vassilev *et al.*, 2006; Young *et al.*, 2006; Pérez *et al.*, 2007; Hamdali *et al.*, 2008; Kim *et al.*, 2008). Fungal isolates in general, exhibit greater P–solubilizing ability than bacteria in both liquid and solid media (Banik and Dey, 1982; Kucey, 1983). However in soil, PSB generally out–number P solubilizing fungi by 2–150 fold (Banik and Dey, 1982; Kucey, 1983; Kucey *et al.*, 1989). Most of the P solubilizing microrganisms display the ability to solubilize dicalcium phosphate (DCP), tricalcium phosphate (TCP), hydroxyapatite (HAP) and rock phosphate while a few have the ability to solubilize ferric and aluminium phosphates (Sperber, 1957; Bolan *et al.*, 1987; Kucey *et al.*, 1989; Halder *et al.*, 1991; Halder and Chakrabarty, 1993). Therefore, these microorganisms have been playing a significant role in supplying P to plants.

The main mechanism by which majority of PSM solubilizes insoluble phosphate complexes is by the secretion of mono–, di– and tri–carboxylic acids (Kucey *et al.*, 1989; Cunningham and Kuiack, 1992; Nahas, 1996; Jones, 1998; Reyes *et al.*, 2001; Richardson, 2001; Roelofs *et al.*, 2001; Khan *et al.*, 2007). Solubilization of phosphates may result from the drop in pH i.e P–mobilization by extrusion of protons (Roos and Luckner, 1984; Illmer and Schinner, 1995) or from cation chelation by organic acids (Puente *et al.*, 2004; Rodriguez *et al.*, 2006; Vassilev *et al.*, 2006).

Soil microorganisms, particularly in soils with low total P content, are capable of storing substantial amounts of P into their biomass above their own physiological P requirements which is referred to as microbial immobilization. However, through this process a substantial portion of soluble P fractions is prevented from being immobilized by soil components through complex formation with Al, Fe and Ca which are relatively more recalcitrant to solubilization in comparison to microbial immobilization fraction, which is regarded as an easily mineralisable P fraction of the soil (Redel *et al.*, 2008; Sugito *et al.*, 2010). Kondo *et al.* (1989) reported that almost 39% of the Olsen P originates from soil microbial biomass and Achat *et al.* (2010) found that 21.6 kg ha^{-1} of P in the soil microbial biomass. Although the P contained in soil microbial biomass cannot be directly used by the plants, the turnover of microbial biomass may release P slowly into the soil solution (Gichangi *et al.*, 2009). Thus, microbial biomass P particularly during the post–disturbance period, should function as a temporary P sink, retaining the nutrient that would otherwise be lost through leaching or converted into more recalcitrant form of unavailable P. However, empirical data on P availability or distribution of P in different compartments during ecosystem recovery are limited (Crews *et al.*, 1995; Olander and Vitousek, 2005).

In soil systems, phosphatases i.e. the group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid, mineralize organic P into inorganic P which subsequently is solubilized into available P fraction (Speir and Ross, 1978). Therefore, availability of phosphatase in soil which is an extracellular enzyme of specific microorganisms is crucial for ensuring P availability to the plants. The phosphatase enzymes are correlated with P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Dick and Tabatabai, 1992; Dick *et al.*, 2000; Xue *et al.*, 2006; He *et al.*, 2010) by linking biologically unavailable and bioavailable P pools in the soil (Speir and Ross, 1978) as mentioned above. Soil organic matter significantly influences the activities of acid and alkaline phosphatases (Guan, 1989; Jordan and Kremer, 1994; Aon and Colaneri, 2001). Another important factor that influences the rate of synthesis, release and stability of this enzyme is the soil pH (Juma and Tabatabai, 1977; Tabatabai, 1994; Acosta–Martínez and Tabatabai, 2000; Dick *et al.*, 2000; Sarapatka *et al.*, 2004). For example, phosphomonoesteraces inducibility and their exudation intensity by plant roots and microorganisms are determined by their orthophosphate need, which in turn is affected by soil pH (Skujins, 1976). It is, therefore, anticipated that practices that induce P stress in the rhizosphere should also affect the secretion of these enzymes in the ecosystem (Ndakidemi, 2006). Thus, measuring phosphatase activity during ecosystem recovery is important to understand the dynamics of P.

Much detailed studies of the factors regulating the nutrient dynamics, in particular the role of microbial biomass and their activities in nutrient acquisition of trees, have been conducted in tropical forests (Singh *et al.*, 1989; Campo *et al.*, 1998). However, studies on the microbial activities and nutrient cycles in the ecosystems undergoing recovery are not many (Wang *et al.*, 2011). Soil microbial diversity and ecosystem functioning are two facets which are regarded as intrinsically associated (Turbé *et al.*, 2010) even though this relationship has not been fully unravelled (Hooper *et al.*, 2005). Unravelling this relationship will provide key to a large number of questions such as the mechanisms and processes involved in the natural recovery process. This in turn, will help in designing appropriate strategies for restoration of highly degraded ecosystems where natural recovery process is very slow. To understand the relationship between microbial diversity and ecosystem functioning during recovery period, a thorough knowledge on nutrient cycling, and the structural and functional aspects of microbial communities is a pre–requisite.

The extent and the time required for a degraded ecosystem to recover will depend largely on the attributes of the past disturbance regime, degree of its impact i.e. high resistance low impact, and the resilience of the system. The limits of normal operating range of a system are dependent on the resistance of the system (i.e. the ability of the system to withstand disturbance) and its resilience (the ability of the system to recover after disturbance) (Seybold *et al.*, 1994; Wertz *et al.*, 2007; Allison and Martiny, 2008). Resistance is intrinsically related to functional redundancy, as more redundant communities may reveal unaltered process rates due to functionally redundant elements

(Pereira e Silva *et al.*, 2012). Wolters (2001) reported that many soil communities appear as functionally redundant for a range of functions. Highly resilient communities more readily return to their original function following a stress than poorly resilient communities (Pereira e Silva *et al.*, 2012).

In addition, post–disturbance prevailing micro–environmental conditions, particularly the edaphic factors influence the rate of recovery to a great extent. The response of microbial communities to restoration will therefore be a function of these factors. A number of investigations have detailed a significant impact of soil properties like soil type or structure (Wu *et al.*, 2008; Bach *et al.*, 2010a) and pH (Fierer and Jackson, 2006) on the structure of soil microbial communities. Aboveground vegetation has been reported to significantly influence the composition of microbial communities (Kowalchuk *et al.*, 2002; Carney and Matson, 2006; Bach *et al.*, 2010b; Snajdr *et al.*, 2013). Jangid *et al.* (2011) argued that landuse was a stronger determinant of the composition of microbial communities than vegetation and soil properties. However, a clear understanding of the relative influence of these factors on the development of soil microbial communities during recovery is still lacking.

Considering the importance of P and C cycles in recovery of degraded ecosystems and relatively less knowledge on the role of microbial communities in regulating their dynamics particularly during post–disturbance recovery period, it is essential to undertake studies to understand the patterns of change that take place in different ecosystems. Cherrapunjee plateau in Meghalaya, Northeast India is a fragile landscape and the top soil layer is thin. The soil is nutrient impoverished because of excessive nutrient leaching attributed to high rainfall. The two natural ecosystems of Cherrapunjee viz., forests and grasslands are under constant anthropogenic pressure. Over a period of time, most primary forests have been converted into secondary forests due to deforestation, and large tracts of grasslands have been converted into degraded ecosystems, primarily for mining activities.

Coal mining activities often results in removal of biologically rich top soil. The mine spoils are characterized by high bulk density, low pH, low nutrient availability, poor structure, low water holding capacity, and low biomass productivity (Harthill and Mckell, 1979; Jha and Singh, 1992). As a result, the subsoil becomes unfit for plant growth. On the other hand, deforestation causes accelerated soil erosion and soil compaction. Erosion problem leads to decline in essential nutrients and organic matter content and therefore, low microbial activity, which ultimately hinders the establishment of plant cover (Tejada *et al.*, 2009, 2010). Restoration of such systems is quite tedious. Even natural succession is very slow and recovery takes long time.

The interactions prevailing in early successional forests or young mine spoils are highly complex and dynamic in nature. Therefore, there has been a lot of emphasis laid on monitoring of biological properties along a time scale as recovery follows in order to achieve successful land reclamation. However, there are relatively few studies focusing such properties as indicators of ecosystem recovery (Trasar–Cepeda *et al.*, 2003; Frouz and Novakova, 2005; Fioretto *et al.*, 2009; Jiang *et al.*, 2009) and the information is even more scarce under subtropical climates, especially in humid subtropical regions of north–eastern India. Therefore, the landscape of Cherrapunjee plateau, dotted with a series of recovering ecosystems varying in recovery ages, offered an excellent opportunity to study the P and C dynamics and the role of microbial communities in recovery process of the degraded forest and grassland ecosystems. The objectives of the thesis are:

1. To study the changes in soil and vegetation characteristics during recovery process.
2. To elucidate the role of microorganisms in C and P dynamics during recovery period.
3. To identify a few P solubilising microbial strains based on their relative efficiency during different years of recovery.

CHAPTER 2
REVIEW OF LITERATURE

Land use change is a major ecological problem as it alters natural ecosystems, degrades vegetation and soil, and depletes soil nutrients. Degradation of high elevation vegetation is the beginning of land degradation process in higher reaches of mountains, as it has weak resilience. Therefore, degradation of vegetation, particularly in high altitude ecosystems, has been the main factor for converting the land into a non–reversible degraded state (Dhar, 2000). Blaikie and Sadeque (2000) through an experimental study in the Hindu Kush Himalayan (HKH) region have shown that the vegetation cover is important for reducing run–off losses and in minimizing nutrient loss and soil movements such as gullying, topsoil loss and mass wasting.

Land use changes also affect biodiversity through decline of natural habitats, elimination of species locally, and impacting ecosystem functioning (Lavorel *et al.*, 2007; Turner *et al.*, 2007a, 2007b). Many studies have evaluated the impact of decreased biodiversity on ecosystem functioning and ecosystem stability (McNaughton, 1977; Tilman, 1996; van der Heijden *et al.*, 1998; Wertz *et al.*, 2007). A number of researchers studied the impact of land use change on the soil system, and reported a reduction in soil nutrient availability and deterioration in microbiological properties (Chen *et al.*, 2000; Chen and Li, 2003; Wang and Wang, 2007; Yan *et al.*, 2008). Dupouey *et al.* (2002) assessed the impact of past land use on forest soil and biodiversity. They reported that land use imposed long–term changes in soil chemical and structural properties and suggested that such effects of past land use on forest biodiversity may be irreversible on an historical time scale. Araujo *et al.* (2010) investigated the effect of conversion of native savanna to *Eucalyptus grandis* forest on soil microbial biomass. They concluded that such changes in the soil altered the number and activity of soil microorganisms, which in turn, affected soil biochemical processes and influenced the plant growth. Similarly, Tejada *et al.*

(2010) reported that land use change led to decline in organic matter content and therefore, low microbial activity, which ultimately hindered the establishment of plant cover.

Post–disturbance establishment of plant cover is a prerequisite for ecosystem recovery as plant species influences ecosystem processes (Vinton and Burke, 1997) such as nutrient cycling (Hobbie, 1992), microbial biomass and composition (Bardgett *et al.*, 1999) and soil enzyme activity (Kourtev *et al.*, 2002). Rutigliano *et al.* (2004) evaluated the relationships between plant cover and soil characteristics, such as nutrient content, microbial biomass and activity in a forest ecosystem in the Mediterranean area of Southern Italy. They concluded that vegetation cover impacts significantly the soil properties, as it contributes to the input of organic matter to soil and protects the soil from erosion. Keller *et al.* (2013) found that plant species composition brings out important differences in soil properties and belowground biogeochemistry. Snajdr *et al.* (2013) observed significant impact of dominant tree species on microbial community composition and activity. Grayston *et al.* (1997) found re–vegetation stimulates the growth of soil microbes, as they obtained greater microbial biomass value in the re–vegetated site than the site without any vegetation. He attributed this to greater input of plant residues and plant roots in the re–vegetated site. Zhang *et al.* (2006) observed a decrease in soil microbial biomass from undisturbed vegetation site to severely degraded land, and explained it as a consequence of the loss of vegetation cover and SOC from the soil surface. Soil organic matter content (Wardle, 1992), soil nutrient status (Innes *et al.*, 2004) and soil texture (Groffman *et al.*, 1996) were found to influence plant microbe interactions and thus affect the ecosystem processes. Therefore, all these factors which alter the rates of ecosystem processes are important for the functioning of ecosystems and must attain pre–disturbance status for successful recovery of degraded ecosystems.

Several studies have demonstrated that microbial community composition and function are interrelated (Waldrop and Firestone, 2004; Balser and Firestone, 2005; Brant *et al.*, 2006; Strickland *et al.*, 2009; Paterson *et al.*, 2011) and shifts in microbial community following a disturbance event can effect microbial functions greatly (Brant *et al.*, 2006; Paterson *et al.*, 2011; Wickings *et al.*, 2011). Disturbances such as landuse change affect the microbial community and lower the diversity by altering the plant communities dramatically (Vitousek *et al.*, 1997). The direct effects of changes in plant community composition and/or diversity on ecosystem processes (Tilman and Downing 1994; McGrady–Steed *et al.*, 1997; Naeem and Li, 1997; Tilman, 1999; Lavorel and Garnier, 2002), as well as on microbial community (Nusslein and Tiedje, 1999; Wu *et al.*, 2011) who mediate the ecosystem processes have been evaluated in a number of studies. However, only a few studies exist that investigated how the microbial communities change during the recovery phase following the perturbation (Knelman *et al.*, 2012).

While evaluating the effect of disturbances on soil microbial populations in relation to ecosystem functioning, importance must be given to structural changes and not only on diversity, as diversity can attain its original value following the disturbance, whereas changes in community structure are permanent (Westergaard *et al.*, 2001). However, changes in soil microbial communities are complex and how soil microbial communities would respond to environmental changes is still largely unknown (Waldrop and Firestone, 2006; Balser and Wixon, 2009). Orwin and Wardle (2004) pointed out that a system's stability determines its ability to continue functioning under changing conditions. Stability of a system depends on the resistance i.e. the ability to withstand a stress, and resilience i.e. the ability to recover to pre–disturbance level (Griffiths *et al.*, 2000). Giller *et al.* (1997) suggested that more diverse a community, more stable it is against perturbations. A decrease in diversity of microbes in the soil, may cause a decline in resistance and resilience of the system. Degens *et al.* (2001) hypothesized that soil functional stability is primarily generated by the inherent functional redundancy present in the microbial diversity. These authors found that an arable soil with low catabolic diversity and organic C was less resistant to stress (low pH, high salinity, heavy metal contamination) or disturbance. Similarly, Griffiths *et al.* (2001) reported greater functional stability to copper and heat stresses in a grassland soil and an organically managed agricultural soil than in an intensively managed soil.

Urbanska (1997) in the context of ecological restoration emphasized the importance of interactions of plants with soil structure and nutrient status for successful plant colonisation. Dimitriu *et al.* (2010) in their study on response of enzyme activities and microbial community composition to restoration of surface–mined soil in the Athabasca oil sands region of northern Alberta reported that the response of microbial populations to restoration is governed by soil abiotic properties. While Gros *et al.* (2004) suggested that it is also important to consider the co–evolution between plants and their biotic environment, particularly microbial activities as plant cover degradation negatively affects soil microbial activity (Garcia *et al.*, 2002). However, Walker and Reddell (2007) suggested that no single approach is likely to improve the health of a landscape and in most cases a combination of approaches needs to be employed. While taking such an approach, belowground system i.e. soil system has to be in focus as it is the most likely approach to succeed. In fact, Doran and Safley (1997) and Van Bruggen and Semenov (2000), proposed a list of soil properties, currently recognized as indicators for monitoring the effect of land use changes. The suggested soil properties were: *physical*: bulk density, texture, porosity; *chemical*: pH, electric conductivity, cation exchange capacity, organic C and N; and *biological*: microbial biomass, enzymes activities, soil respiration, microbial density, microfaunae diversity, presence of detectable microbial pathogens.

Change of land use can cause significant variations in biogeochemical cycles and soil properties (Conant, *et al.*, 2003; Fraterrigo *et al.*, 2005; Hacisalihoglu, 2007; Saraswathy *et al.*, 2007). Most changes in land use affect the amount of carbon held in vegetation and soil either releasing carbon dioxide to the atmosphere or removing it from the atmosphere. Upadhyay *et al.* (2005) reported that in fragile ecosystems, landuse changes and soil degradation add to increased greenhouse gas emissions. The forests of Asia are net emitters of CO_2 currently, mostly due to deforestation (Dixon *et al.*, 1994). Post and Kwon (2000) opined that landuse changes and vegetation types significantly influence C fixation and flux in the soil. In general, forest ecosystems store 20–100 times more C per unit area than other systems and hence play a critical role in reducing ambient CO2 levels, by sequestering atmospheric C in the growth of woody biomass through the process of photosynthesis and thereby increasing the SOC content (Brown and Pearce, 1994). Soils of grassland and agricultural lands also have great potential to sequester C (Lal *et al.*, 1998). Therefore, Lal (1999) suggested that restoration of degraded ecosystems and soils has a potential for sequestering C in terrestrial ecosystems globally.

Soil organic matter (SOM) is the largest terrestrial store of biologically active carbon (Amundson, 2001). Land use change is known to be a major factor influencing the soil organic carbon (SOC) content (Houghton *et al.*, 2000). Nsabimana *et al.* (2004) concluded that land use had substantial effects on the size, activity and diversity of the soil microbial community and these changes could be broadly related to changes in SOM content. Karlen *et al.* (1992) demonstrated that soil biological processes provide resiliency and buffering capacity to ameliorate stress in disturbed environments. Besides, many of these processes could be effective indicators of stress environment as well as recovery process. Doran *et al.* (1996) established that soil biological processes such as microbial biomass, soil enzyme activity and soil respiration respond more quickly to environmental changes than total soil organic matter. This is due to the fact that about

95% of the total SOM is non–living and is relatively stable or resistant to change, therefore decades may be required to observe a measurable change in SOM (Masto *et al.*, 2006). On the other hand, as microbes are living organisms, they respond more quickly to the changes in soil conditions than SOM (Brookes *et al.*, 2008).

As microbes play critical role in ecosystem processes, alterations in soil microbial communities may have implications for ecosystem functioning. In fact, a few recent studies have related microbial community composition to rates of microbial mediated processes (Burns *et al.*, 1999; Cavigelli and Robertson 2000; Waldrop *et al*, 2000; Carney *et al.*, 2004; Carney and Matson, 2005). The findings of Carney and Matson (2006) provide evidence for human alteration of soil microbial communities via modification in the plant community composition and diversity, and those changes are mediated in part by changes in soil carbon quality. Ralte *et al.* (2005) reported that anthropogenic activities coupled with high rainfall in the buffer zone of the Nokrek biosphere reserve cause depletion of microbial biomass carbon (MBC) and reduction in dehydrogenase activity in the soil. SOC being the most important source of C for soil heterotrophic microorganisms, cycling of C in soil compartment is important during ecosystem recovery period. Since soils contain about 75% of the C in the terrestrial ecosystem, knowing more about the microbial processes taking place there will lead to a better understanding of long–term C storage in soils. Among the SOM constituents, polysaccharides are rapidly decomposed while lignin and its derivatives are incorporated into stable soil C pools (Martin *et al.*, 1980). Lignin concentration is an important predictor of C mineralization potential (Berg and Laskowski, 2006). While highly modified, unrecognizable lignin decomposition products, such as phenol, may accumulate in clay fractions, distinct, unmodified lignin macromolecules do not accumulate. Stable soil C pools are dominated instead by lipids and carbohydrates, mostly produced during microbial turnover (Grandy and Neff, 2008).

Microorganisms play a crucial role in most biogeochemical cycles in soil as well as in the formation of soil structure (Roldan *et al.*, 1994). Microbes being central to ecosystem function, soil microbial indices have been extensively used in various studies such as comparison of functional properties of microbial communities from different ecosystems (Balser and Firestone, 2005; Brant *et al.*, 2006; Strickland *et al.*, 2009; Paterson *et al.*, 2011), ecosystem responses to disturbances (Sicardi *et al.*, 2004; Bossio *et al.*, 2005; Tan *et al.*, 2008) and restoration success (Mummey *et al.*, 2002; Jiang *et al.*, 2009). Since soil microbial communities play a critical role in the recovery of structural and functional properties of a soil following a disturbance (Bending *et al.*, 2000; Breure, 2005), measurements of the characteristics of the microbial community provide invaluable information on soil quality and ecosystem recovery. The soil microbial biomass is the labile fraction of organic matter (Jenkinson and Ladd, 1981) and act as both source and sink of plant nutrients (Singh *et al.*, 1989). Soil microbial biomass being the living part of SOM acts as an important ecological indicator and is responsible for the decomposition and mineralization of plant and animal residues present in the soil (Marinari *et al.*, 2006). Thus, changes in soil microbial biomass may lead to the changes in important functions, such as organic matter decomposition and nutrient cycling.

Garcia *et al.* (2002) suggested that MBC/TOC ratio is a useful measure for monitoring the SOM and provides a more sensitive index than organic C measured alone. It serves as an indicator of the quality and availability of organic substances, and of C turnover in soils. Anderson and Domsch (1989) hypothesised that larger ratios imply an increased availability of fresh substrates, while smaller ratios implied reduced availability. Wardle (1992) pointed out that this ratio can provide an effective early warning of the deterioration of soil quality. Sparling (1997) found it useful for identifying trends over time and to compare different soils. This ratio increases or decreases as the input of organic matter in soil increases or decreases (Anderson and Domsch, 1989).

Bohme and Bohme (2006) reported that this ratio increases with the decomposability of soil organic matter. Landgraf (2001) observed that MBC/SOC ratio increases with time during succession.

Another sensitive indicator is microbial metabolic quotient (qCO_2) or the ratio of basal respiration to microbial biomass carbon. Yan *et al.* (2003) suggested that metabolic quotient is a valid indicator of the efficiency of energy use by microbes i.e. how efficiently the microbial biomass is utilizing available C for biosynthesis. It also provides a method to relate both the size and activity of soil microbial populations (Anderson and Domsch, 1993). Moscatelli *et al.* (2007) demonstrated that in disturbed ecosystems, the presence of competition induces microorganisms to use more C energy for cell integrity and maintenance than for growth and therefore qCO_2 can be a much more sensitive indicator of stress. Maeder *et al.* (2002) concluded that a decrease in qCO_2 indicated greater microbial community diversity or greater energy use efficiency and better soil quality. Landgraf (2001) showed that the soil qCO_2 decreased with time of succession. Though Wardle and Ghani (1995) have questioned the use of qCO_2 as a bioindicator, as it failed to distinguish between effects of disturbance and stress, qCO_2 has been widely applied as an indicator in the assessment of the cultivation regime (Anderson and Domsch, 1990), pollution gradients (Ohtonen, 1994), effect of temperature (Anderson and Domsch, 1986; Anderson and Gray, 1991), acidification (Wolters, 1991) and ecosystem restoration (Jiang *et al.*, 2009).

Soil enzyme activities have been suggested as an index of microbial functional diversity (Nannipieri *et al.*, 2002). Soil enzyme activities are one of the most suitable indicators of soil quality and are increasingly being studied recently (Zabaloy *et al.*, 2008; Tejada, 2009; Sardans and Peñuelas, 2010). Pascual *et al.* (2000) reported that enzymes are potential indicators of the extent to which soil disturbance by a given activity may affect the immediate environment. Caravaca *et al.* (2003) suggested that enzyme activity is an early and sensitive indicator for reflecting the degree of recovery of a soil system. Klose and Tabatabai (2000) reported decreasing enzymatic activity in the absence of vegetation cover as it decreased the amount of organic matter and suppressed microbial biomass. Garcia *et al.* (2002) also found that the values of enzyme activities were less in the soils without vegetation cover. Therefore, soil enzyme activities may be considered early and sensitive indicators to measure the degree of soil degradation. They also provide invaluable information on soil quality and the recovery status of a degraded ecosystem. Izquierdo *et al.* (2005) used soil enzymes to assess the success of reclamation of mining areas and suggested that they are very sensitive indicators of the improvement in soil quality resulting from revegetation.

Estimation of soil enzyme activities as a recovery indicator has an edge over other microbial parameters because: (a) they are a measure of the soil microbial activity and therefore they are strictly related to the nutrient cycles and transformations; (b) they rapidly respond to the changes caused by both natural and anthropogenic factors; (c) they are easy to measure (Gianfreda and Bollag, 1996; Nannipieri *et al.*, 2002). Soil enzyme activities may be sensitive to both natural and human–induced disturbances, and measurements of the activities of a range of enzymes may provide a valid estimation of the metabolic response of soils to management practices and environmental stress (Dick *et al.*, 1988; Nannipieri, 1994). However, the activity of a single enzyme does not reflect the soil fertility status (Skujins, 1978). Of all the enzymes involved in soil, dehydrogenase, β–glucosidase (E.C.3.2.1.21) and N–acetyl– β–D–glucosaminidase (E.C.3.2.1.30) are considered important in C cycle, and acid phosphatase (E.C.3.1.3.2) is an important enzyme in P cycle. The activity of these enzymes is often highly correlated with other enzymes involved in these cycles (Deng and Tabatabai, 1997).

Dehydrogenase activity reflects the total oxidative activity of the microbial biomass (Nannipieri *et al.*, 1990; Gianfreda *et al.*, 2005). A study by Brzezinska *et al.*

(1998) suggested that soil water content and temperature influence dehydrogenase activity indirectly by affecting the soil redox status. The redox transformations are closely connected with respiration activity of soil microorganisms. They may serve as indicators of the microbiological redox systems in soils and can be considered a possible measure of microbial oxidative activity (Tabatabai, 1982; Trevors, 1984; Glinski and Stepniewski, 1985; Gunnison *et al.*, 1985). Quilchano and Marañón (2002) found that site factors such as soil pH, available nutrients, and soil texture, and sampling season greatly influenced dehydrogenase activity. Therefore, this enzyme activity could be a sensitive indicator of recovery progress of a degraded ecosystem as the above mentioned factors are influenced by disturbance and are modified during recovery stages. In addition, this enzyme could be an indicator of the effect of land degradation and subsequent recovery on soil microbial activity as it is usually related to the presence of viable microorganisms.

β–glucosidase is an important enzyme in terrestrial C cycle catalyzing the hydrolysis of the non–reducing terminal ends of ß–glucosides with the release of glucose, that constitutes an important energy source for microbial biomass (Tabatabai, 1994). Thus determination of β–glucosidase activity has been suggested as a good indicator of soil quality among other hydrolytic enzyme activities (Dick *et al.*, 1996). Acosta–Martinez and Tabatabai (2000) reported β–glucosidase as sensitive to pH changes. This property makes this enzyme a good biochemical indicator for measuring ecological changes resulting from soil acidification. Various studies in the context of soil disturbances and ecosystem restoration have estimated β–glucosidase activity as it is sensitive to both natural and anthropogenic disturbances (Tan *et al.*, 2008; Baldrian *et al.*, 2008; Jiang *et al.*, 2009). Waldrop *et al.* (2003) and Hassett and Zak (2005) reported that disturbances resulting in decline of vegetation cover reduced extracellular enzyme activities involved in litter decomposition e.g. β–glucosidase.

Zak and Kling (2006) found correlation between enzymes and the molecular structure or turnover dynamics of SOM. Sollins *et al.* (1996) pointed out that extracellular soil enzymes are directly responsible for the initial processing of detrital carbon and organic–bound nutrients while Baldrian (2008) highlighted their role in acquisition of nutrients from organic resources. Although hydrolytic enzymes control the rate at which substrates are degraded and become available for uptake by microbes and plants (Marx *et al.*, 2001), only a few studies have focused on their production during spontaneous recovery following disturbance (Baldrian *et al.*, 2008; Wang *et al.*, 2011).

The microbial indices and their relationships with various factors have been studied in temperate (Priha, 1999; Leirós *et al.*, 2000; Priha *et al.*, 2001,) and tropical (Srivastava and Lal, 1994; Salamanca *et al.*, 2002; Dinesh *et al.*, 2003; Ilstedt and Singh, 2005; Gnankambary *et al.*, 2008) forests. Such studies in subtropical forests are however very few (Arunachalam and Arunachalam, 2000; Devi and Yadava, 2006).

Phosphorus (P) is an essential macronutrient needed for the growth and development of plants. Batjes (1997) estimated that about 5.7 billion hectares of land worldwide is P–deficient. Low availability of soil P is a major nutritional constraint for primary production in many ecosystems in the world (Raghothama, 1999). Most of the soil P is not immediately available for plant uptake due to its physiochemical properties and soil chemistry (Halford, 1997). P is poorly soluble in soil and possesses a low diffusion potential in comparison to other nutrients such as NO_3^- and K^+ (Barber, 1995). This mineral is most affected by the degradation processes of the soils (Rosas *et al.*, 2006). In contrast to C, N and S which accumulate with time (Allison *et al.*, 2007), the availability of mineral P declines during successional process due to weathering and drainage loss (Crews *et al.*, 1995).

Soils generally contain 100–3000 mg P kg^{-1} soil, of which about 15–80% is present in organic forms (Condron *et al.*, 2005) and cannot be utilized by plants. Further, the affinity of iron oxides and hydroxides for P in acidic and highly weathered soils lead to transformation of soluble forms of P into forms of poor solubility, particularly Fe–P and Al–P complexes, thereby decreasing its availability to plants (Johnson and Loeppert, 2006; Noemi *et al.*, 2006). The presence of these complexes reduces the nutrient capacity of these soils for sustaining plant and microbial growth (Richardson, 2001). The P deficiency caused by all these processes can eventually limit biomass production (Wardle *et al.*, 2004). However, many ecosystems are still able to support biomass production and biodiversity on highly weathered soils despite their P deficiency (Kitayama, 2005) due to the various adaptive mechanisms. Several workers have focused their studies on these adaptive mechanisms to P limitation: the tight recycling of P through ectomycorrhizal and fine root systems (Lambers *et al.*, 2008), the higher P utilization efficiencies of plants (Kitayama *et al.*, 2004), and the use of non–labile P through root exudation of organic acids and hydrolytic enzymes (i.e., phosphatase) (Liu *et al.*, 2006). Phosphatase catalyses the release of inorganic P from organic–bound P returned to soil as litter and other organic debris, and this activity plays an important role in the P cycling. Soil phosphatase activity has often been used to describe soil microbial activity (Garcia *et al.*, 1994), while other methods include soil MBC and N, basal respiration and ATP content (Nannipieri *et al.*, 1990).

Soil P cycling and availability is controlled by a combination of biological processes (mineralisation–immobilization) and chemical processes (adsorption–desorption and dissolution–precipitation) (Frossard *et al.*, 2000). Microorganisms can affect P dynamics in several ways including the following: (1) an increase in biomass of phosphate solubilizing microorganisms (PSM) (Gyaneshwar *et al.*, 1998); (2) Substantial release of P following the death of the microbial cells since microbes immobilize up to 10% of the total soil P (Richardson, 2001); and (3) mineralization of organic P to inorganic P through phosphatase activity (Oehl *et al.*, 2001).

Rhizodeposition increases P solubilization in the rhizosphere zone through promoting the growth of PSMs. Phosphatases, originating from bacteria, fungi and root exudates, catalyze the hydrolysis of ester bonds between phosphate and carbon compounds in organic substrates to enhance P availability to ecosystems (Turner and Haygarth, 2005). The production of phosphatase would be increased when the requirement for P by ecosystems is increased (Olander and Vitousek, 2000). These enzymes therefore play an important role in maintaining and controlling the rate of P cycling in ecosystems. Of the phosphatases, acid phosphatase is predominant in acidic soils due to their pH optima (Juma and Tabatabai, 1988). Its activity can provide useful information on organic P mineralization potential and biological activity of soils (Speir and Ross, 1978; Dick and Tabatabai, 1993; Kramer and Green, 2000). Acid phosphatase activity has been used as an indicator in several studies to evaluate P limitation in ecosystems (Schneider *et al.*, 2001; Gress *et al.*, 2007).

Bacteria colonizing P–deficient environments should exhibit high P–mobilizing abilities in order to sustain their own growth. So, plants capable of growing under such harsh conditions must be harboring one or more unique populations of PSM in their rhizosphere including phosphate solubilizing bacteria (PSB) that may contribute to the P nutrition of plants. Son *et al.* (2006) studied the mobilization of insoluble phosphates by PSB in harsh environments. PSB not only aid in the P nutrition of plants, but also facilitate the growth of plants by accelerating the accessibility of other trace elements and by synthesizing important growth promoting substances (Mittal *et al.*, 2008), including siderophores (Wani *et al.*, 2007) and antibiotics (Lipping *et al.*, 2008), and providing protection to plants against soil borne pathogens (Hamdali *et al.*, 2008).

Knowledge of this mutual relationship between plants and PSB encouraged many workers to screen for efficient microbial strains from various habitats (Chen *et al.*, 2006; Perez *et al.*, 2007; Kumar *et al.*, 2010; Crespo *et al.*, 2011) and explore their biofertilizer potential to improve crop yield (Fankem *et al.*, 2008). A large number of heterotrophic and autotrophic microorganisms, such as bacteria (Gothwal *et al.*, 2006), fungi (Achal *et al.*, 2007), actinomycetes and cyanobacteria (Yandigeri and Pabbi, 2005) are reported to solubilize insoluble phosphate. Among the soil bacterial communities strains from the bacterial genera *Pseudomonas*, *Burkholderia*, *Bacillus*, *Rhizobium* and *Enterobacter* and *Aspergillus* and *Penicillium* from fungal genera (Wakelin *et al.*, 2004; Perez *et al.*, 2007; Xiao *et al.*, 2011) have been reported as powerful phosphate solubilisers. Apart from these, the other bacterial genera reported as efficient phosphate solubilizers include *Agrobacterium*, *Achromobacter*, *Micrococcus*, *Aerobacter*, *Flavovobacterium*, *Erwinia* (Rodriguez and Fraga, 1999), *Azotobacter*, *Klebsiella* (Chung *et al.*, 2005), *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Delftia* sp. (Chen *et al.*, 2006), *Ralstonia* and *Pantoea* (Perez *et al.*, 2007).

The phenotype exhibited by phosphate solubilising bacteria (PSB) has been traditionally associated with the production of low–molecular–weight organic acids in varying concentration and types (Deubel *et al.*, 2000). Commonly reported organic acids produced by microorganisms for mineral phosphate solubilization are gluconic acid and keto–gluconic acids (Song *et al.*, 2008). Other organic acids, such as acetic, citric, lactic, propionic, glycolic, oxalic, malonic, succinic, fumaric, tartaric acid etc. have also been identified among phosphate solubilizers (Ahmed and Shahab, 2011). These acids diffuse freely outside the cells as they are produced in the periplasm of many gram negative bacteria and may release high amounts of soluble P from mineral phosphates, by supplying both protons and metal complexing organic acid anions (Gadd, 1999). Apart from this, the other proposed mechanisms include the production of chelating substances, the release of protons originated by NH_4^+ assimilation and the production of inorganic acids (Rodriguez and Fraga, 1999). The key processes involved in the solubilization of phosphate compounds by organic acids are attributed to acidification, chelation, and exchange reactions (Chung *et al.*, 2005; Gulati *et al.*, 2010).

Organic acids dissociate in a pH dependent equilibrium into their respective anion(s) and proton(s) and thereby lower the solution pH. In addition to pH reduction, organic acid anions can solubilize P through chelation reactions. Chelation involves the formation of two or more coordinate bonds between an anionic or polar molecule and a cation, resulting in a ring structure complex (Whitelaw, 2000). The organic acids have the ability to form stable complexes with cations such as Al^{3+}, Fe^{2+}, Fe^{3+}, Ca^{2+} through the oxygen containing carboxyl and hydroxyl groups, and release the bound phosphates (Jones, 1998). Lastly, through competition with phosphate anions that are adsorbed to crystalline $Fe(OH)_3$ and $Al(OH)_3$ surfaces, organic acid anions can contribute to P mobilization through ligand exchange reactions (Jones, 1998; Trolove *et al.*, 2003).

Several studies have revealed that PSB could increase growth and yield in several plants including walnut (Yu *et al.*, 2011), apple (Aslantas *et al.*, 2007), maize (Hameeda *et al.*, 2008), soybean (Fernandez *et al.*, 2007), and peanut (Taurian *et al.*, 2010). Although there have been many successful results from soil inoculations of PSB, the results have been highly variable in terms of plant growth and crop yields (Gyaneshwar *et al.*, 2002). In a study by Dwivedi *et al.* (2004), it was found that the inoculation of *Aspergillus awamori* to wheat and rice seedlings, increased the crop response to Musoorie rock phosphate over the non–inoculated treatments. However, many organisms that solubilise insoluble phosphates effectively in the laboratory are unable to do so in the natural field conditions (Richardson, 2001). Gyaneshwar *et al.* (2002) suggested that these variations in effectiveness may be due to the reduced ability of inoculated PSB to survive and colonize the rhizosphere, coupled with competition for resources with native soil microorganisms. Therefore, knowledge related to the practical and efficient use of microorganisms to increase the solubility of insoluble and bound phosphates would improve the P nutritional needs of plants and aid recovery progress of degraded ecosystems which are P–limited.

Several workers have shown that revegetation and natural regeneration through succession can restore structural and functional properties of degraded soil (Singh and Tripathi, 1992; Campbell *et al.*, 1994; Izquierdo *et al.*, 2005; Baldrian *et al.*, 2008; Wang *et al.*, 2011). However, regeneration through succession, particularly dynamics of microbial community and nutrients along the recovery ages has not received much attention. An understanding of the dynamics of C and P in the soil system during different stages of recovery and the role of different microorganisms at each stage would be helpful in designing restoration strategies for degraded ecosystems. However, no work has so far been undertaken on P dynamics in ferric–iron–rich, acidic and P–deficient soils as are found in North East India. Similarly, the role of different microbes in SOM mineralization has not been evaluated, particularly during recovery process.

CHAPTER 3
STUDY SITE

The study was conducted in Cherrapunjee plateau (Latitude 25^0 16.49' N and Longitude 091^0 43.59' E; altitudinal range 1480–1690 m a.s.l) in East Khasi Hills District of Meghalaya, which is about 50 km south of Shillong, the capital city of Meghalaya. The terrain of Cherrapunjee is mountainous and the land surface is characterized by thin topsoil and exposed rocks. The two dominant natural ecosystems viz., forests and grasslands are under constant anthropogenic pressure. Conversion of forest lands to other land uses through deforestation, and human dependency on forest and forest–products are the causes of degradation of forest ecosystem in Cherrapunjee. In contrast, mining of coal, limestone and sand are the causes of degradation of grassland ecosystem. Despite all the disturbances, the ecosystems of Cherrapunjee exhibit a high degree of resilience. This resiliency of Cherrapunjee landscape offers an interesting case study to understand the underlying processes and mechanism that result in the ecosystem stability.

The recovery pattern of ecosystems was studied following a chronosequence approach. In other words, the site selection was based on an age–series of recovering stands following disturbance. Two replicate stands for each recovery age and undisturbed forest/grassland category in each ecosystem were selected (Tables 3.1 and 3.2). The four categories of stands in forest ecosystem were: i) old–growth undisturbed forest stands (UF), and a chronosequence of three clear–cut and regrowing stands viz., ii) freshly clear–cut forest representing the post–disturbance stands (CF1), iii) 5–year old recovering forest stands (RF5) and iv) 10–year old recovering forest stands (RF10). The four categories of stands in grassland ecosystem were: i) undisturbed grassland stands (UG), and a chronosequence of three mining affected and recovering stands viz., ii) mining affected grassland stands (MG), iii) 15–year old recovering grassland stands (RG15) and iv) 50–year old recovering grassland stands (RG50).

The undisturbed forest (UF) and undisturbed grassland (UG) stands in this study represented the pre–disturbance vegetation and soil scenarios because all the chronosequence stands selected were once part of the same continuous undisturbed forest/grassland, which got fragmented at different points of time due to disturbance events. In fact, all the sites selected under the forest and grassland ecosystems were within 2.7 km radius at Sohrarim and Laitryngew villages of Cherrapunjee plateau and had the similar vegetation type. This implies that all the stands had a common undisturbed stage before clear–cutting/mining related activities. This was further confirmed by the local people in a meeting arranged to confirm the history of the selected experimental sites. The study was initiated immediately after the clear–cutting of the undisturbed forest for fuel wood purpose by the local people at two sites which were designated as CF1. Similarly, two freshly mining affected sites (mostly dumping of coal) which were previously undisturbed grasslands were selected as MG stands. Since clear–cutting of an undisturbed forest for experimentation is not feasible and also recreating a mine affected area on an undisturbed grassland is not possible, such an approach was the only alternative. Based on the physical observation and with the help of the local people, ages of the two regenerating/recovering stand categories were determined in both forest and grassland ecosystems, and were designated as RF5, RF10, and RG15, RG50, respectively.

GEOLOGY

Meghalaya represents the remnant of the ancient plateau of Pre–Cambrian Indian peninsula and forms a prominent geomorphic unit stretching across the Garo, Khasi and Jaintia hills in east–west direction. The central and northern part of Meghalaya plateau is made up of "Archaean Gnessic Complex" which is essentially highly metamorphosed crystalline rocks of Pre–Cambrian origin. This complex is made up of gneisses (biotic gneiss, biotic granulite, quartz–sillimanite gneiss, cordierite, garnet and chondrodite) and Schistose (mica schist, quartz–sillimanite schist and metabasite) members of varying composition (Anon, 1974; Murthy *et al.*, 1976).

Table 3.1: Characteritics of study sites selected under forest ecosystem in Cherrapunjee.

Stages of recovery	Replicate stands	Past land use	Recovery period (years)	Latitude	Longitude	Elevation (m)	Range of slope angle (°)	Slope aspect
Undisturbed forest (**UF**)	UF1	Forest	>200	N 25°19.895'	E 091°43.889'	1635	40–45	North–east
	UF2	Forest	>200	N 25°19.854'	E 091°44.117'	1682	35–40	South–east
1st year of deforestation (**CF1**)	CF1–1	Forest	1–3	N 25°21.795'	E 091°44.655'	1695	35–40	North–east
	CF1–2	Forest	1–3	N 25°21.354'	E 091°44.415'	1687	40–45	North–east
Five years after deforestation (**RF5**)	RF5–1	Forest	4–7	N 25°21.229'	E 091°44.483'	1686	40–45	North–east
	RF5–2	Forest	4–7	N 25°21.168'	E 091°44.290'	1674	30–35	South–east
Ten years after deforestation (**RF10**)	RF10–1	Forest	9–11	N 25°21.836'	E 091°44.622'	1679	55–60	North–east
	RF10–2	Forest	9–11	N 25°21.820'	E 091°44.535'	1672	55–60	North–east

Table 3.2: Characteritics of study sites selected under grassland ecosystem in Cherrapunjee.

Stages of recovery	Replicate stands	Past land use	Recovery period (years)	Latitude	Longitude	Elevation (m)	Range of slope angle (°)	Slope aspect
Undisturbed grassland (**UG**)	UG1	Grassland	>70	N 25°20.089'	E 091°44.198'	1640	25–30	North–east
	UG2	Grassland	>70	N 25°20.211'	E 091°43.836'	1639	25–30	North–east
Mining affected grassland (**MG**)	MG1	Grassland	8–10	N 25°20.089'	E 091°44.198'	1648	15–20	North–east
	MG2	Grassland	8–10	N 25°20.229'	E 091°44.274'	1662	20–25	South–east
Recovering grassland 15 yrs. old (**RG 15**)	RG15–1	G and CM	15–20	N 25°19.293'	E 091°44.115'	1637	30–35	South–east
	RG15–2	G and CM	15–20	N 25°19.353'	E 091°44.171'	1658	25–30	North–east
Recovered Grassland 50 yrs. old (**RG 50**)	RG50–1	G and CM	50–55	N 25°19.134'	E 091°44.009'	1634	15–20	North–east
	RG50–2	G and CM	50–55	N 25°19.198'	E 091°43.875'	1636	15–20	North–east

Note: G– Grassland; CM– Coal mining

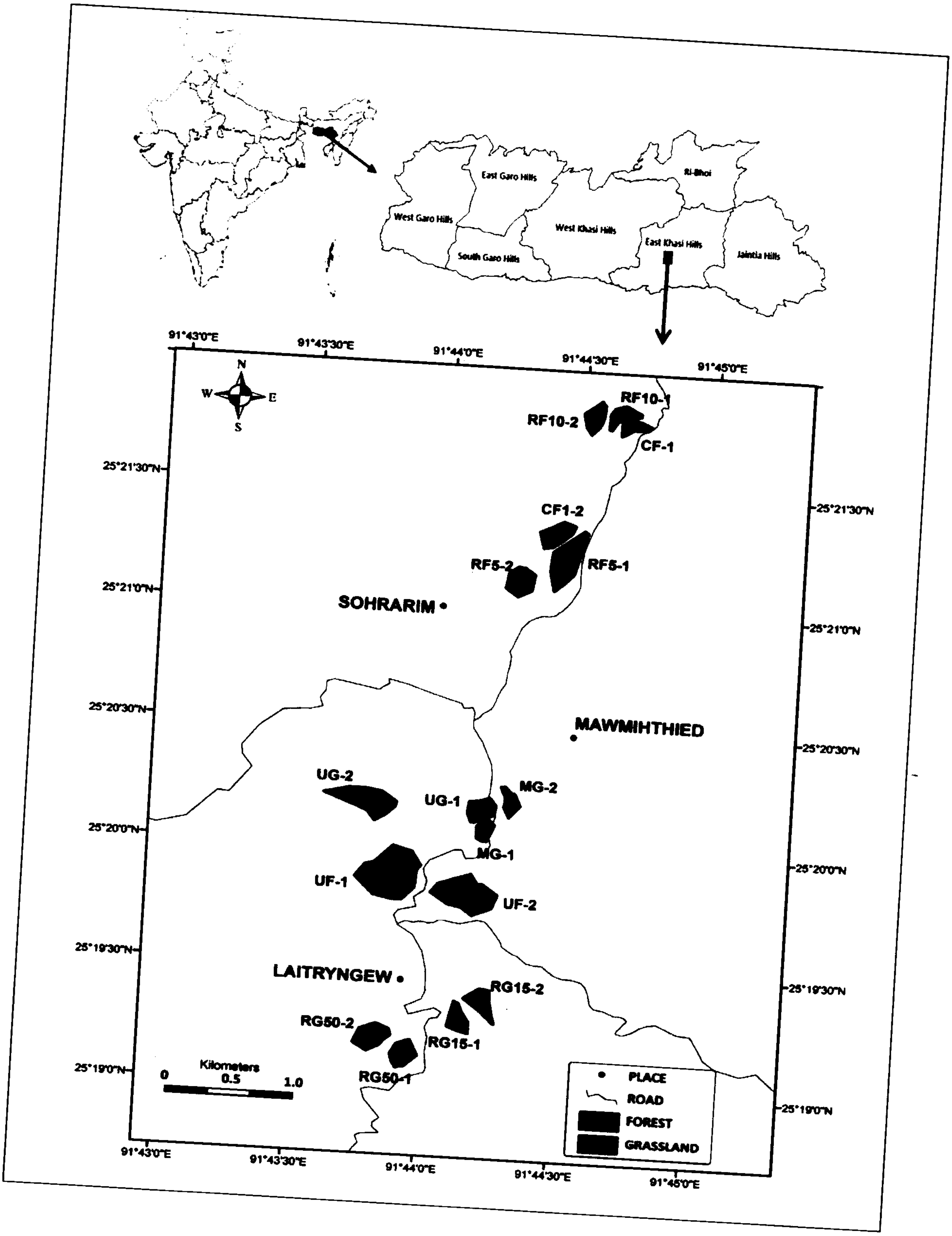

Fig. 3.1: Location of study sites in Cherrapunjee.

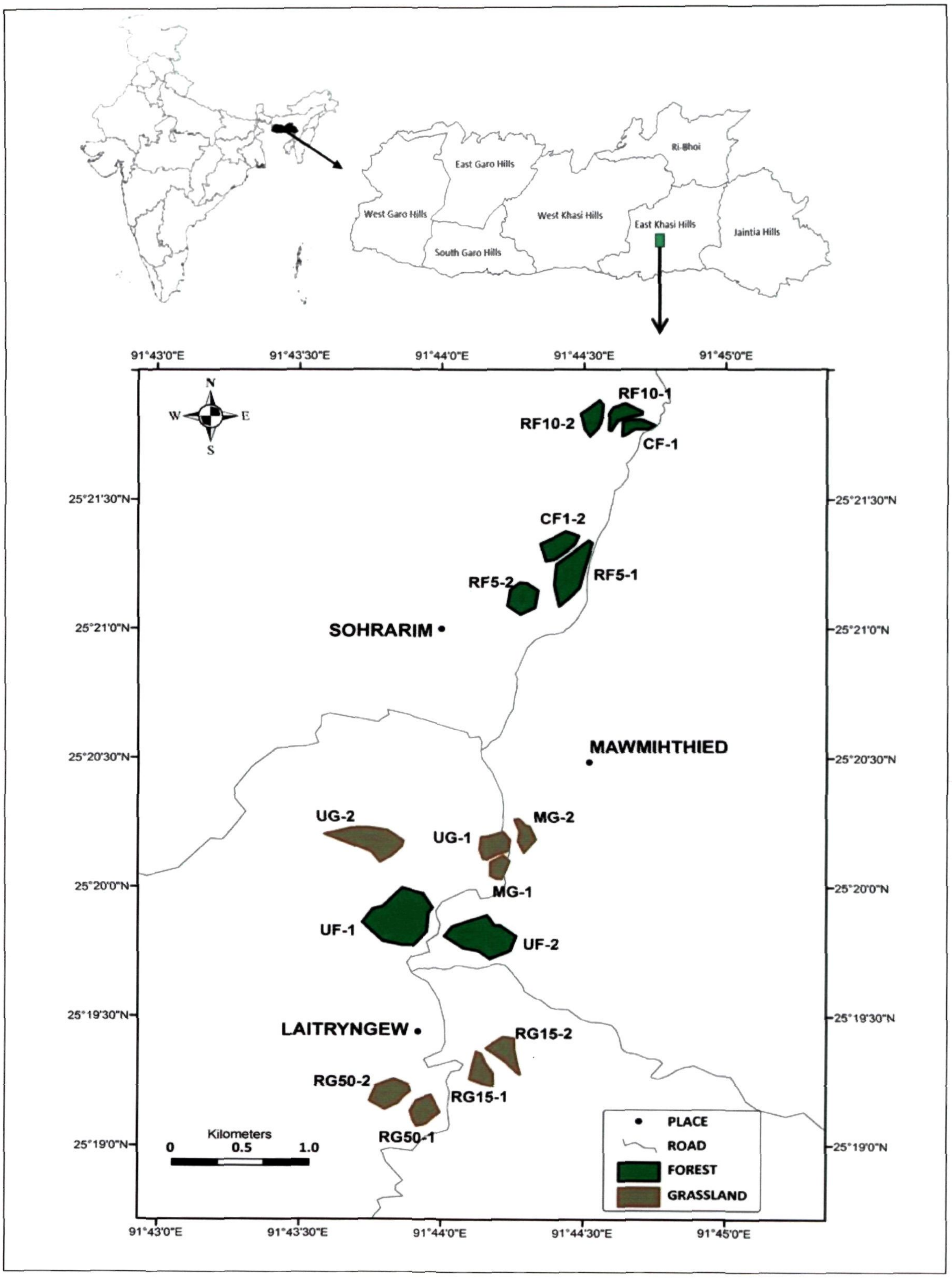

Fig. 3.1: Location of study sites in Cherrapunjee.

Fig. 3.2: Site representing 1st year of deforestation (CF1)

Fig. 3.3: RF5 site showing recovery after five years of deforestation

Fig. 3.4: RF10 Site showing recovery after ten years of deforestation

Fig. 3.5: Old growth undisturbed forest (UF)

Fig. 3.6: Grassland affected by mining related activities (MG)

Fig. 3.7: 15 years old recovering grassland after cessation of mining related activities (RG15)

Fig. 3.8: 50 years old recovering grassland after cessation of mining related activities (RG50)

Fig 3.9: Undisturbed grassland (UG)

CLIMATE

The climatic variables like temperature, rainfall and humidity vary widely from place to place in the state due to wide variation in topography. Cherrapunjee has a mild subtropical highland climate, with monsoonal influences. It receives both the southwest and northeast monsoonal winds, giving it a single monsoon season. It lies on the windward side of the Khasi Hills, so the resulting orographic lift enhances precipitation. In the winter months it receives the northeast monsoon showers that travel down the Brahmaputra valley. Based on the climatic conditions, the year may be divided into spring, summer, autumn and winter seasons. The summer is characterized by relatively high temperature, occasional thunderstorms with high rainfall and high wind velocity. The summer or the rainy season commences with the onset of southwest monsoon in May and continues up to September. Three fourth of the total rainfall is received during this season. Rainy season is followed by a brief autumn during October and November. Rainfall and temperature sharply decline during this period. The winter season extends from December to February. Morning fog and frost, and dry weather are the characteristic features of this season. March and April represent the spring season.

The meteorological data were collected from Cherrapunjee meteorological station. During the study period, the annual rainfall recorded was 11,415 mm during 2008, 9,038 mm during 2009 and 13,474 mm during 2010. 80% of the total rainfall was received during the rainy season. During wet season monthly rainfall ranged from a maximum of 3615.8 mm in July to a minimum of 400.7 mm in May. Though winter season received some rainfall but it was almost negligible. The mean monthly temperature varied from a maximum of 24.8°C in the month of September to a minimum of 7.7°C in the month of December (Fig. 3.2). Relative humidity varied markedly with a peak (90%) during May–September and minimum (65%) in December–February.

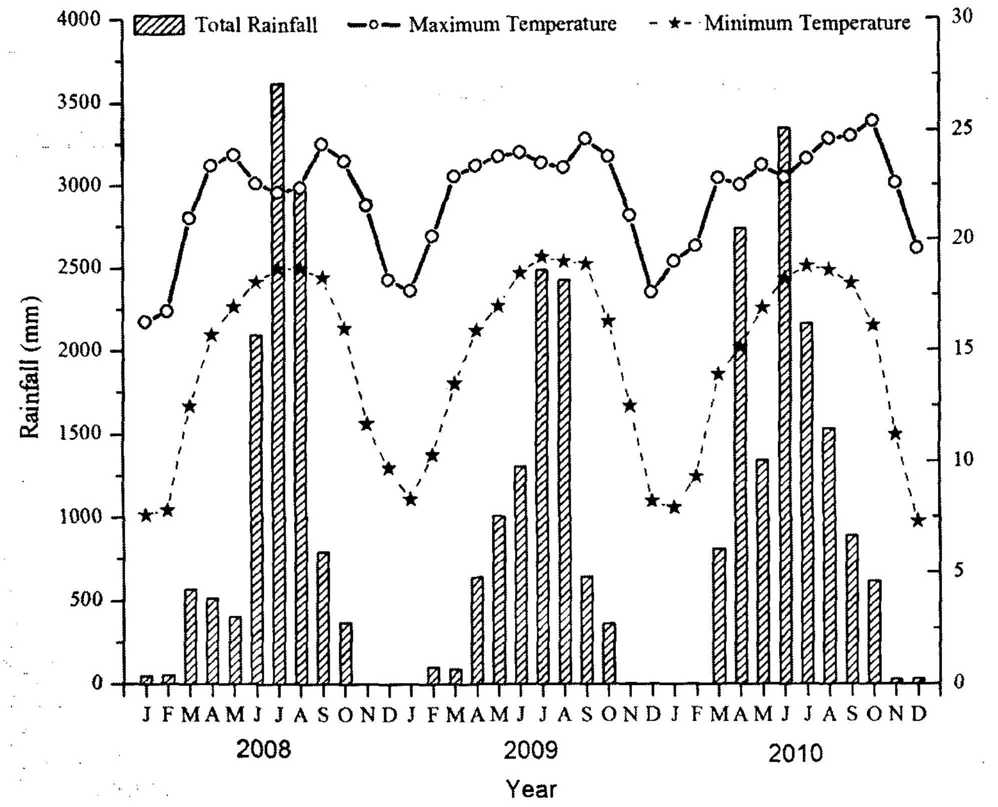

Fig. 3.10: Monthly rainfall and mean monthly minimum and maximum temperature at Cherrapunjee during the study period i.e. 2008–2010.

SOIL

The soils of Meghalaya are derived from the underlying gneisses, schists and granites. These have been grouped under latosol (Oxisol) type (Pascoe, 1950). Soils of Cherrapunjee plateau are poor owing to deforestation and subsequent washout caused by heavy rains. The soils are acidic (pH 3 to 6.5) in nature. Acidity is attributed to the leaching of cations like Ca, Mg and K from the soil due to high rainfall and undulating topography. Total N content is low (ca. 0.4%), deficient in available P (28 $\mu g\ g^{-1}$ soil), average in available K (13 $\mu g\ g^{-1}$ soil) and rich in organic carbon (4%). Soils are not suitable for intensive cultivation due to their poor base (35%) saturation (Singh, 1996). Soil layer is very thin and in some places its thickness is even less than 20 cm.

VEGETATION

The vegetation in Cherrapunjee plateau is very sparse. This area is dominated by undulating grasslands and patches of forest here and there. According to Champion and Seth (1968), the forests found in this high rainfall area are humid subtropical evergreen broad–leaved type and are characterized by a patchy distribution. The vegetation of the area is largely dominated by *Castanopsis purpurella* (Miq.) N.P.Balakr., *Quercus glauca* Thunb., *Quercus kamroopii* D.Don, *Lithocarpus dealbatus* (Hk. f. & Th. Ex Miq) Rehder, and *Neolitsea cassia* (L.) Kosterm. among trees, *Rubus*, *Osbeckia*, *Psychotria symplocifolia* Kurz. and *Eurya japonica* Thunb. among shrubs and the herbaceous community is dominated by species like *Begonia laciniata* Roxb., *Arisaema tortuosum* (Wall.) Scott, *Bidens pilosa* L. *Carex baccans* Nees and grasses such as *Digitaria violascens* Link, *Arundinella khasiana* Nees ex Steud., *Cyanotis vaga* (Lour.) Schult. & Schult.f., *Osbeckia capitata* Benth. ex Naudin and *Drosera peltata* Thunb.

CHAPTER 4

CHANGES IN SOIL AND VEGETATION CHARACTERISTICS DURING ECOSYSTEM RECOVERY

INTRODUCTION

Disturbances play an important role in shaping the structures of populations, communities and ecosystems, and significantly impact ecosystem functioning. The structure of plant communities in many natural ecosystems is largely influenced by anthropogenic as well as natural disturbances (Kwit and Platt, 2003; Bennett and Adams, 2004; Elderd and Doak, 2006). Disturbance can have negative impacts on plant diversity by destroying climax assemblages and introducing instability in the system (Clements, 1936). One of the positive effects of disturbance is enhanced species diversity, particularly in the communities exposed to intermediate level of disturbance (Lubchenco, 1978; Huston, 1979).

Besides changing the plant community structure, disturbances can cause significant alteration in soil properties and biogeochemical cycles (Conant *et al.*, 2003; Tate *et al.*, 2004; Fraterrigo *et al.*, 2005; Hacisalihoglu, 2007). Disturbances may also influence the spatial distribution of soil nutrients (Diekmann *et al.*, 2007) including the carbon stocks in vegetation and soil. The greatest flux of carbon has been observed when forests are converted into open lands and vice–versa (Houghton and Goodale, 2004). In an oak forest of Iran, Hajabbasi *et al.* (1997) found that even after 20 years of recovery following deforestation and subsequent tillage practices, in top soil layer, there was a 20% increase in bulk density and 50% decrease in SOM in comparison to the forest soil before disturbance. In a semi–arid grassland of southern China, Su *et al.* (2004) observed that overgrazing resulted in loss of soil organic C, total N, P, and depletion in soil enzyme activities resulting in permanent degradation of land and destruction of the ecosystem.

Recovery of a degraded ecosystem is a complex process in which the functioning and services of an ecosystem are expected to regain most of the desirable attributes to pre–disturbance condition through natural processes (Meffe *et al.*, 1997; Tilman *et al.*, 2006; Walker and del Moral, 2009). Following anthropogenic disturbances, different ecosystems vary in their capacities to recover. The factors that determine the rate of recovery are: initial ecosystem structure and function, and prevailing environmental conditions (Brearley *et al.*, 2004), disturbance intensity (van der Maarel, 1993; Turner *et al.*, 1998; Collins *et al.*, 2001; Brearley *et al.*, 2004), length of time since abandonment (Brearley *et al.*, 2004) and post–disturbance factors that impact the ecosystem (Swanson *et al.*, 2011). Soil properties and vegetation characteristics are two important indicators of recovery process. Soil physico–chemical characteristics and biological processes in particular, influence the post–disturbance biogeochemical cycles and also regulate vegetation recovery process to a great extent. The vegetation in turn, also influences the soil characteristics and processes. Therefore, during recovery process, soil and vegetation components of ecosystems are interlinked through a variety of direct and indirect interactions (Kardol and Wardle, 2010).

Plant species can have significant impacts on soil physico–chemical properties and on the quality of substrate for soil microbes (Binkley and Giardina, 1998; Lavorel and Garnier, 2002; Kamei *et al.*, 2009). Growing evidences suggest that plant species also have significant impacts on the composition of soil microbial community (Porazinska *et al.*, 2003; Bartelt–Ryser *et al.*, 2005; Ushio *et al.*, 2008). The quantity and quality of soil organic matter is largely regulated by the autotrophic plant communities, because the major fraction of it originates from plant residues viz., above– and below– ground litter, root exudates and leachates, and the chemical composition of these residues varies according to the plant species (Quideau *et al.*, 2001; Marschner *et al.*, 2004; Aneja *et al.*, 2006; Kazakou *et al.*, 2006).

Although several edaphic changes are associated with disturbance, soil compaction and alteration in soil profile are two major impacts of most disturbance events. Many studies have demonstrated a long–term negative impact of soil compaction on the soil properties and plant growth rates (Rab *et al.*, 1992; Croke *et al.*, 2001; Gomez *et al.*, 2002; Tan *et al.*, 2005). Several researchers found that soil compaction and disturbance to forest floor affect most soil physical and chemical properties, particularly soil porosity, aeration, water content, temperature, and substrate availability (Tew *et al.*, 1986; Zabowski *et al.*, 1994; Gomez *et al.*, 2002; Tan *et al.*, 2005). Soil profile disturbance redistributes organic matter (Walker, 1999; Rab, 2004) and thus, influences the nutrient availability to plants during recovery phase. However, there are not many studies depicting temporal changes in soil nutrients in an ecosystem during the recovery process following a disturbance event such as deforestation and mining related activities (Li *et al.*, 2005; Nyberg *et al.*, 2012; Maharana and Patel, 2013).

Since the soil nutrient status is largely impacted by the above ground vegetation and vice–versa, a study on soil and vegetation characteristics during the recovery phase would not only help understanding the changes that take place in an ecosystem during post disturbance period but also would provide valuable insight into the vegetation–soil interactions during recovery process. Therefore, the study had the following objectives:

- To assess the change in soil physical and chemical properties during different years of recovery following the disturbances in forest and grassland ecosystems, and
- To enumerate the species composition and analyze different vegetation characteristics during recovery of ecosystems.

METHODS

Soil and vegetation sampling

Soil samples were collected on a seasonal basis for two years (2008–2010). Sampling for autumn season (October – November) was done in the month of October, for winter (December – February) in January, for spring (March – April) in April and for rainy (May–September) in the month of July. The structure of plant community was analysed by studying tree, shrub and herb components in forest ecosystem and only herb component in grassland ecosystem. While tree and shrub components were sampled once only during rainy season (August–September) of 2009, herbs were studied during all the four seasons.

In forest ecosystem, each site was divided into 10 m x10 m grids. For soil sampling, four grids were selected randomly from each site. Therefore, four composite soil samples were collected from each site. The number of soil samples was kept at four per site keeping in view the logistic constraints of analysis of a large number of soil samples involving several soil physical, chemical and biological parameters. For tree sampling, twenty five grids were randomly selected for each site. Shrubs were sampled in ten random 5 m x 5 m quadrats within the selected 10 m x10 m grids. Similarly, herbs were sampled in 1 m x 1 m quadrats within each shrub quadrat.

In grassland ecosystem, each site was divided into considering the homogeneity of vegetation ten grids of 1 m x 1 m size were randomly selected after dividing each site into 1 m x 1 m grids. Soil samples were collected from four of the above grids selected based on random numbers.

Soil processing

The soil samples were transferred to the laboratory in sealed plastic bags on ice. The samples were homogenized and sieved through a 2 mm mesh. The soil was then separated into two portions: A portion of the soil was stored at 4°C for analysis of soil moisture content, soil pH and soil biological parameters, and the remaining was air dried and stored for chemical analysis. The air dried samples were further sieved through appropriate mesh size for specific physical and chemical analyses.

Soil analyses

The soil physical parameters studied were soil texture, bulk density (BD), water holding capacity (WHC), porosity, soil temperature and soil moisture content (SMC). Soil chemical properties analyzed were: soil pH, soil organic carbon (SOC), soil organic matter (SOM), available phosphorus comprising of Bray's phosphorus and water extractable phosphorus, total phosphorus (TP), and total Kjeldahl nitrogen (TKN).

Soil texture was determined by Bouyoucos hydrometer method (Allen *et al.*, 1974). Twenty five ml of 5% sodium hexa–metaphosphate (calgon) was added to fifty grams of air dried sieved (2 mm mesh) soil sample. The soil suspension was shaken for two hours at 100 rpm on a shaker after making the volume to 250 ml with tap water. The suspension was transferred into 1,000 ml graduated cylinder, and the volume was made upto the mark. The first reading was recorded after one minute of stirring. The soil suspension was allowed to stand undisturbed for five hours before measuring the second reading. Textural class was determined by using the International Society of Soil Science Triangle. Soil texture was calculated using the following formulae:

$$\text{Clay (\%)} = \frac{\text{A x 100}}{50 - \text{(Moisture weight)}} - 1$$

$$\text{[Silt + Clay] (\%)} = \frac{\text{B x 100}}{50 - \text{(Moisture weight)}} - 1$$

$$\text{Silt (\%)} = \text{[Silt + Clay] (\%)} - \text{Clay (\%)}$$

$$\text{Total Sand (\%)} = 100 - \text{[Silt + Clay] (\%)}$$

where, B = Hydrometer reading (g l^{-1}) after 4 minutes 48 seconds; A = Hydrometer reading (g l^{-1}) after 5 hours; 1 = Calgon correction factor

Soil moisture content (SMC) was determined by gravimetric method (Allen *et al.*, 1974). Ten grams of fresh sieved (2mm mesh) soil was taken in a petri plate and oven dried at 105^{o}C till constant weight. Plates were removed from oven, cooled and weights were recorded. SMC was determined by the following formula:

$$\text{SMC (\%)} = \frac{\text{Initial weight (fresh)} - \text{final weight (oven dried)}}{\text{Final weight of soil}} \times 100$$

Bulk density was estimated by gravimetric method (Allen *et al.*, 1974). Soil corer was pressed into the soil to fill the inner metal cylinder. The corer was taken out ensuring an intact sample and the soil was pushed out from the cylinder. Weight of the soil was recorded before and after oven drying at 105^{o}C till constant weight. Bulk density of soil was determined using the formula as follows:

$$\text{BD (g cm}^{-3}\text{)} = \frac{\text{Initial weight (fresh)} - \text{final weight (dry)}}{\text{Volume of metal corer}}$$

Porosity was calculated using bulk density data and particle density value as 2.65 g cm^{-3}.

$$\text{Porosity (\%)} = 1 - \frac{\text{Bulk density}}{\text{Particle density}} \times 100$$

Water holding capacity (WHC) was determined by Keen's box method, by using copper cups of 5.6 cm internal diameter and 1.6 cm height (Piper, 1942). WHC was calculated as follows:

$$\text{WHC (\%)} = \frac{W2 - W3 - W4}{W3 - W1} \times 100$$

where, W1 = Weight of Keen's cup + dry filter paper; W2 = Weight of Keen's cup + saturated filter paper + saturated soil; W3 = Weight of Keen's cup + saturated soil after oven drying; W4 = Weight of Keen's cup + saturated filter paper

Soil temperature was measured by a digital soil thermometer (Sisedco multi thermometer).

A digital pH meter (Professional Meter, PP–20, Sartorius) was used to determine the pH in 1:2.5 w/v suspension of soil in deionized water, which was stirred in a magnetic stirrer for about 15–20 minutes and then left undisturbed overnight for recording pH (Anderson and Ingram, 1993). Two buffer solutions of pH 4.0 and 7.0 were used as standards.

Cation exchange capacity (CEC) was estimated by extracting the exchangeable bases from the soil with 1 M ammonium acetate (pH 7.0). Excess ammonium was removed with ethanol, followed by replacement and leaching of exchangeable ammonium with protons from HCl (Horneck *et al.*, 1989). The concentration of ammonium nitrogen (NH_4–N) in the leachate was determined by indophenol–blue method (Allen *et al.*, 1974). An aliquot of 5 ml was taken and to it 8 ml of sodium potassium tartrate solution, 1 ml of sodium nitroprusside solution, 2 ml of freshly prepared sodium phenate reagent and 1 ml of 0.16% w/v sodium hypochlorite solution were added. The solutions were mixed thoroughly and made upto volume with distilled water. After mixing, the beakers were kept in a water bath (40 °C) for 20 minutes for full colour development. Reagent blanks and standard solutions were prepared accordingly without adding soil samples. Transmittance was recorded at 625 nm using Spectrophotometer (Lambda–35, UV/VIS, Perkin Elmer, USA). The CEC was calculated using the following formula:

$$\text{CEC (meq 100 g}^{-1}\text{ dry soil)} = \frac{\text{C x 0.25 x 100}}{\text{14 x sample weight}}$$

where, C = Concentration of NH_4–N (mg) obtained from standard graph

Soil organic carbon was determined by colorimetric method (Anderson and Ingram, 1993). One gram of air dried, sieved (0.2 mm mesh) soil was mixed with 10 ml of 5% potassium dichromate solution. Twenty ml of concentrated H_2SO_4 was added and kept for 30 minutes for cooling. After cooling, 50 ml of 0.4% $BaCl_2$ was added, mixed thoroughly and allowed to stay overnight so as to leave a clean supernatant. One reagent blank and standard solutions were prepared similarly without adding soil samples. Transmittance was recorded at 600 nm using Spectrophotometer (Lambda–35, UV/VIS, Perkin Elmer, USA). SOC was calculated as follows:

$$\text{SOC (\%)} = \frac{\text{K x 0.1}}{\text{W x 0.74}}$$

where, K = Concentration of carbon obtained from standard graph; W = Sample dry weight (g)

Total nitrogen and total phosphorus were determined by Kjeldahl digestion method followed by colorimetric analysis. Ammonium gas–diffusion technique was applied (application notes, AN–5222) for estimation of total nitrogen and vanado–molybdate method was applied (application notes, AN–5249) for total phosphorus, using the automated Spectrophotometric Flow Injection Analyser (FIAstar, Model 5000–Analyser, 5027–Sampler, AB, FOSS, Hoganas, Sweden). One gram of air dried sieved (0.2 mm mesh) soil samples were added with 10 ml concentrated sulphuric acid using one FOSS Kjeldahl tablet CU 3.5 (1.5 g K_2SO_4/0.15 g $CuSO_4$ x $5H_20$) as catalyst, and digested at 360°C for three hours on a Tecator Digestor (Model–20, FOSS, Hoganas, Sweden). Reagent blanks and standard solutions for analysis were prepared as per the manual. The samples were then analysed following the application notes for each element as outlined in the manufacturer's manual. Total nitrogen and total phosphorus in soil were calculated according to the following formula:

$$\text{Total N or Total P (\%)} = \frac{\text{(C x 0.001 x 0.1)}}{\text{W}} \text{ x 100}$$

where, C = Concentration of N (mg l^{-1}) or P (mg l^{-1}) obtained from standard graph; W = Soil dry weight (g)

Vegetation Analyses

To study the species composition at each site, specimens were collected and herbaria were prepared. The specimens were identified with the help of available floras and existing herbarium records of Botanical survey of India (BSI), Eastern Circle, Shillong.

Methods outlined in Mishra (1968) and Mueler–Dombois and Ellenberg (1974) were employed to determine the density, frequency, abundance, basal cover and importance value index (IVI) of all the enumerated species. Vegetation indices such as Shannon's diversity index (Shannon, 1948), and Pielou's evenness index (Pielou, 1966) were calculated.

Statistical Analyses

Statistica version 7.0 (Statistical Software Inc. 2001) and origin version 7.0 (Origin Lab Corporation, USA) softwares were used to analyze the data. Appropriate ANOVA models were used to test the variation in soil physico–chemical properties among land uses, recovery ages, and seasons. The data were subjected to tests of normality, homogeneity of variance, and additivity using appropriate tests before undertaking ANOVA. Wherever necessary, the data were log or square root transformed. The correlation between soil nutrients and the related parameters was calculated and tested for statistical significance. The minimum significance level for all statistical tests was at $p<0.05$ level.

RESULTS

Soil Physico–Chemical Properties

Soil texture: In the forest ecosystem, the soil of CF1 stands, i.e. immediately after deforestation had the highest clay content (16.4%). As recovery progressed, a significant (Tukey's test, $p<0.00$) reduction in the clay content was observed (Table 4.1). One–way ANOVA and subsequent Tukey's test revealed a significant variation ($p<0.01$) in the proportion of sand and clay fractions in soils of CF1 stands with those of recovering RF5 and RF10, and the undisturbed forest stands. Soil of the CF1 stands was sandy loam, while soils of both the recovering stands and the undisturbed stand were loamy sand. In grassland ecosystem, only the sand proportion varied significantly ($p<0.00$) among different recovery ages (Table 4.2). It ranged between 69% and 92% depending on the age of recovery. Therefore, soil textural class varied widely among the recovering ages. It was a sandy loam in UG and MG, sandy in RG15, and loamy sand in RG50.

Bulk density, porosity and Water holding capacity: In forest ecosystem, bulk density (BD) significantly (Tukey's test, $p<0.01$) decreased with recovery ages. It was highest in CF1 stands (0. 51 g cm^{-3}) i.e. immediately after deforestation followed by the recovering RF5 (0.41 g cm^{-3}), RF10 (0.30 g cm^{-3}) and the old growth undisturbed forest stands (0.26 g cm^{-3}) (Table 4.1). In grassland ecosystem, it increased due to disturbance and showed an overall reduction as recovery proceeded (Table 4.2). The mining affected MG sites had significantly (Tukey's test, $p<0.00$) greater BD than the recovering RG15 and RG50 sites. In both forest and grassland ecosystem, it varied significantly ($p<0.00$) among the ages of recovery. Water holding capacity (WHC) also followed the same trend as that of BD. However, porosity followed a reverse trend as expected. With the increase in bulk density WHC also increased. It decreased significantly ($p<0.00$) as recovery followed. WHC ranged from 12–38% in grassland and 18–60% in forest.

Cation exchange capacity: In forest ecosystem, cation exchange capacity (CEC) was significantly (Tukey's test, $p<0.05$) greater in CF1 stands than the recovering i.e. RF5 and RF10 and UF stands. Overall, the value reduced significantly (Tukey's test, $p<0.05$) as recovery progressed (Table 4.1). Similarly in grassland ecosystem, CEC decreased with increasing recovery age. It was highest in MG and was significantly greater (Tukey's test, $p<0.00$) than the recovering RG15 and RG50 and the undisturbed grasslands (Table 4.2).

Table 4.1: Mean values (± standard error) of soil physico–chemical properties in the four categories of chronosequence stands selected under forest ecosystem in Cherrapunjee.

Sites	Sand (%)	Clay (%)	Silt (%)	BD (g cm^{-3})	WHC (%)	Porosity (%)	CEC (Meq /100g soil)
CF1	67.6 ± 1.20^{b}	16.4 ± 0.69^{b}	16.0 ± 0.69^{b}	0.51 ± 0.02^{b}	60.1 ± 2.44^{b}	80.9 ± 0.81^{b}	0.46 ± 0.03^{b}
RF5	80.0 ± 1.24^{c}	8.6 ± 0.69^{a}	11.3 ± 1.58	0.41 ± 0.02^{c}	40.6 ± 0.98^{c}	84.4 ± 0.80^{c}	0.35 ± 0.01^{ac}
RF10	80.9 ± 0.69^{c}	8.0 ± 0.69^{a}	11.1 ± 1.39	0.30 ± 0.01^{a}	22.3 ± 1.55^{a}	88.6 ± 0.49^{a}	0.39 ± 0.00^{c}
UF	85.4 ± 0.60^{a}	8.0 ± 0.69^{a}	6.6 ± 0.35^{a}	0.26 ± 0.02^{a}	18.0 ± 2.62^{a}	90.0 ± 0.62^{a}	0.28 ± 0.00^{a}

BD– Bulk density; WHC – Water holding capacity; CEC – Cation exchange capacity

Table 4.2: Mean values (± standard error) of soil physico–chemical properties in the four categories of chronosequence stands selected under grassland ecosystem in Cherrapunjee.

Sites	Sand (%)	Clay (%)	Silt (%)	BD (g cm^{-3})	WHC (%)	Porosity (%)	CEC (Meq /100g soil)
MG	74.1 ± 1.20^{b}	10.7 ± 0.69^{a}	15.2 ± 0.69^{a}	0.48 ± 0.03^{a}	38.0 ± 1.44^{a}	81.8 ± 0.98^{a}	0.53 ± 0.00^{b}
RG15	91.8 ± 0.59^{c}	5.8 ± 0.34^{b}	2.4 ± 0.34^{b}	0.27 ± 0.01^{b}	12.5 ± 1.34^{b}	89.9 ± 0.40^{b}	0.11 ± 0.00^{c}
RG50	79.8 ± 0.68^{d}	5.8 ± 0.68^{b}	14.4 ± 1.19^{a}	0.30 ± 0.01^{b}	16.8 ± 1.63^{b}	86.6 ± 0.31^{b}	0.09 ± 0.00^{d}
UG	68.9 ± 0.90^{a}	12.6 ± 0.68^{a}	18.4 ± 1.56^{a}	0.43 ± 0.02^{a}	35.3 ± 1.35^{a}	87.3 ± 0.73^{a}	0.28 ± 0.00^{a}

BD– Bulk density; WHC – Water holding capacity; CEC – Cation exchange capacity

Soil temperature: In forest ecosystem, soil temperature (ST) reduced significantly (Tukey's test, $p<0.01$) as recovery progressed. It varied significantly ($p<0.00$) among the recovery ages (Table 4.3). However in grassland ecosystem, ST did not show any significant difference among MG, RG15, RG50 and the undisturbed grassland sites. It was significantly ($p<0.01$) higher in grassland ecosystem than forest ecosystem. ST varied significantly ($p<0.00$) among the seasons in forest ecosystem with highest during rainy season and lowest during winter season (Fig. 4.1).

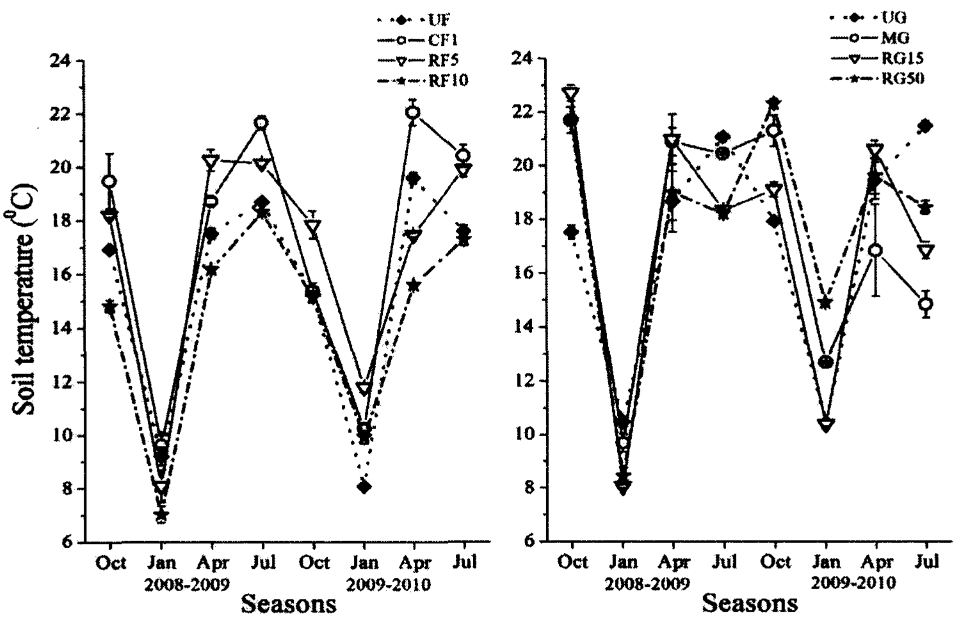

Fig. 4.1: Seasonal variation in soil temperature in the undisturbed and recovering stands of forest and grassland ecosystems.

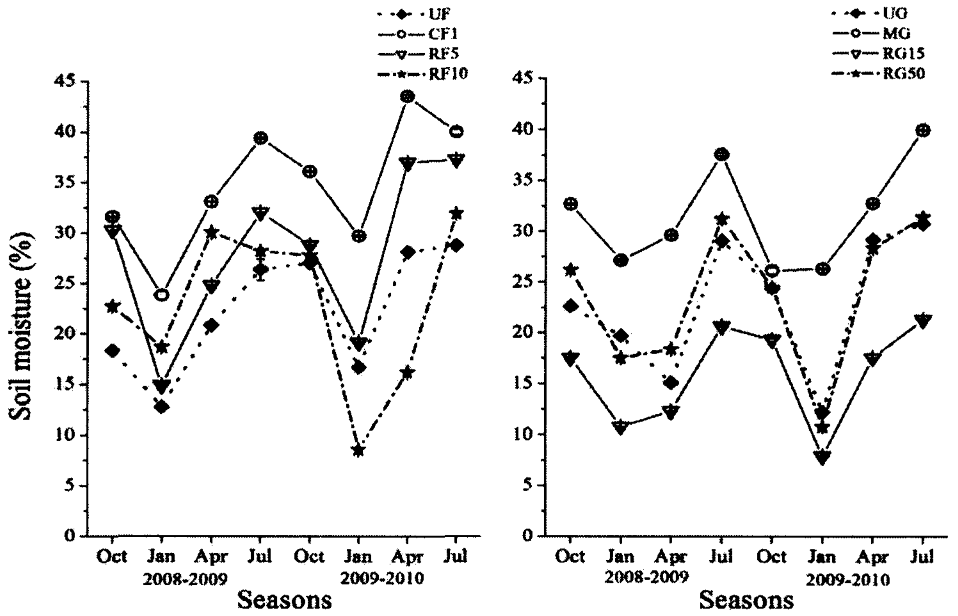

Fig. 4.2: Seasonal variation in soil moisture content in the undisturbed and recovering stands of forest and grassland ecosystems.

Soil moisture content: Soil moisture content (SMC) in forest ecosystem was significantly (Tukey's test, $p<0.01$) greater in CF1 stands than the recovering RF5 and RF10 and UF stands. The value reduced significantly (Tukey's test, $p<0.01$) as recovery progressed. Similarly in grassland ecosystem, soil moisture content was significantly

(Tukey's test, $p<0.00$) greater in MG than the other grassland sites. Overall, the value reduced significantly (Tukey's test, $p<0.00$) as recovery progressed. It varied significantly ($p<0.00$) among recovery ages in both the land uses (Tables 4.3 and 4.4). SMC was significantly ($p<0.00$) greater in forest ecosystem than grassland ecosystem. It ranged from 22.4 – 34.6% in forest ecosystem and 15.9 – 31.5% in grassland ecosystem. SMC varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystem. During rainy season, the values were highest and lowest values were recorded during winter season (Fig. 4.2).

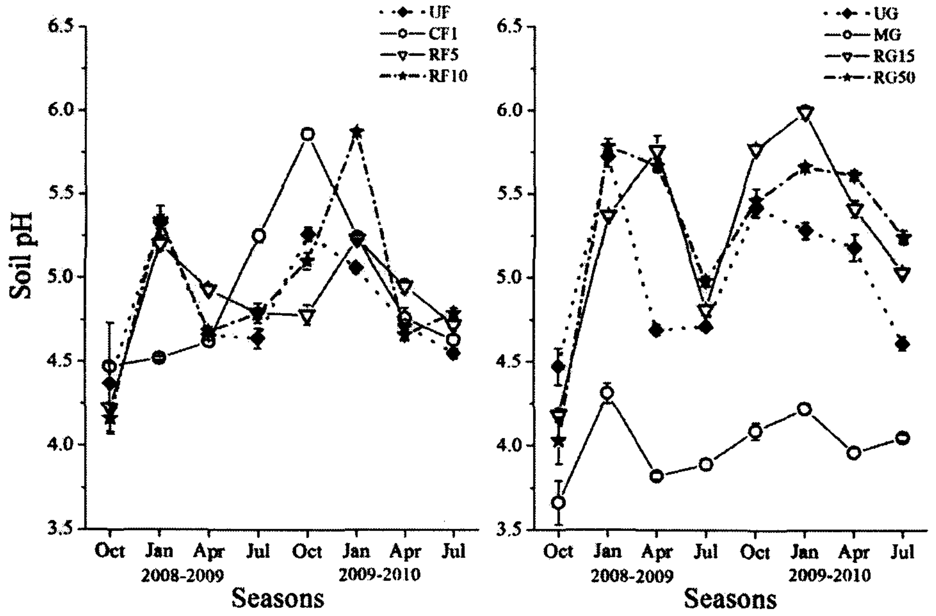

Fig. 4. 3: Seasonal variation in soil pH in the undisturbed and recovering stands of forest and grassland ecosystems.

Soil pH: The soils were acidic in nature in both the ecosystems. In forest ecosystem, pH had a relatively narrow range of 4.8–4.9 than the grassland ecosystem (4.0–5.3). While there was no significant difference in soil pH among the forest stands, in grassland ecosystem the disturbed MG sites had significantly (ANOVA, $p<0.01$) lower pH than the recovering RG15 and RG50 sites, and the undisturbed sites (Tables 4.4). Overall, the value increased significantly (Tukey's test, $p<0.01$) as recovery progressed.

pH also varied significantly (p<0.00) among the seasons in grassland ecosystem with highest value during winter and lowest during rainy (Fig. 4.3).

Table 4.3: Results of two–way ANOVA to test the effect of recovery age and season on soil physico–chemical parameters in forest ecosystem.

Parameters	df	Recovery Age		Season	
		F	p	F	p
ST	3	40.7***	0.00	485.5***	0.00
SMC	3	86.9***	0.00	109.4***	0.00
pH	3	1.0	0.42	17.6***	0.00
SOC	3	44.2***	0.00	26.1***	0.00
TKN	3	124.3***	0.00	12.7***	0.00
TP	3	39.9***	0.00	17.5***	0.00
C/N	3	61.9***	0.00	13.1***	0.00
C/P	3	16.9***	0.00	34.5***	0.00

ST – Soil Temperature; SMC – Soil Moisture Content; pH – Soil pH; SOC – Soil Organic Carbon; TKN – Total Kjeldahl Nitrogen; TP – Total Phosphorus; C/N – Carbon to Nitrogen ratio; C/P – Carbon to Total phosphorus ratio. ***significant at p<0.001

Table 4.4: Results of two–way ANOVA to test the effect of recovery age and season on soil physico–chemical parameters in grassland ecosystem.

Parameters	df	Recovery Age		Season	
		F	p	F	p
ST	3	1.2	0.33	237.2***	0.00
SMC	3	184.0***	0.00	142.2***	0.00
pH	3	53.0***	0.00	10.2***	0.00
SOC	3	184.1***	0.00	2.7*	0.05
TKN	3	150.8***	0.00	4.3**	0.01
TP	3	57.1***	0.00	17.3***	0.00
C/N	3	21.2***	0.00	8.3***	0.00
C/P	3	10.9***	0.00	50.6***	0.00

ST – Soil Temperature; SMC – Soil Moisture Content; pH – Soil pH; SOC – Soil Organic Carbon; TKN – Total Kjeldahl Nitrogen; TP – Total Phosphorus; C/N – Carbon to Nitrogen ratio; C/P – Carbon to Total phosphorus ratio; *,** and *** significant at p<0.05, 0.01 and 0.001 respectively.

Soil organic carbon: In forest ecosystem, soil organic carbon (SOC) was significantly (Tukey's test, p<0.01) greater in CF1 stands (4.5%) i.e. immediately after deforestation than the undisturbed stands (3.8%). It reduced till 5th year of deforestation (RF5: 4.3%), after which it started to increase (RF10: 4.6%) indicating that the system recovered after 5th year of disturbance (Fig. 4.4). In grassland ecosystem, due to coal dumping SOC was significantly (Tukey's test, p<0.00) greater in MG sites (4.9%) than the recovering RG15 (2.6%) and RG50 (2.4%) sites, and the undisturbed grassland sites (3.9%). As coal dumping was stopped and the recovery started, the SOC values decreased sharply (Fig. 4.4). It varied significantly ($p<0.00$) among recovery ages in both forest and grassland ecosystem (Tables 4.3 and 4.4). SOC was significantly ($p<0.00$) higher in grassland ecosystem than forest ecosystem. It ranged between 3.8 – 4.6% in forest and 2.4 – 5.0% in grassland ecosystem. SOC varied significantly ($p<0.00$) among the seasons and was highest during rainy season and lowest during summer season in both the ecosystems (Fig. 4.4).

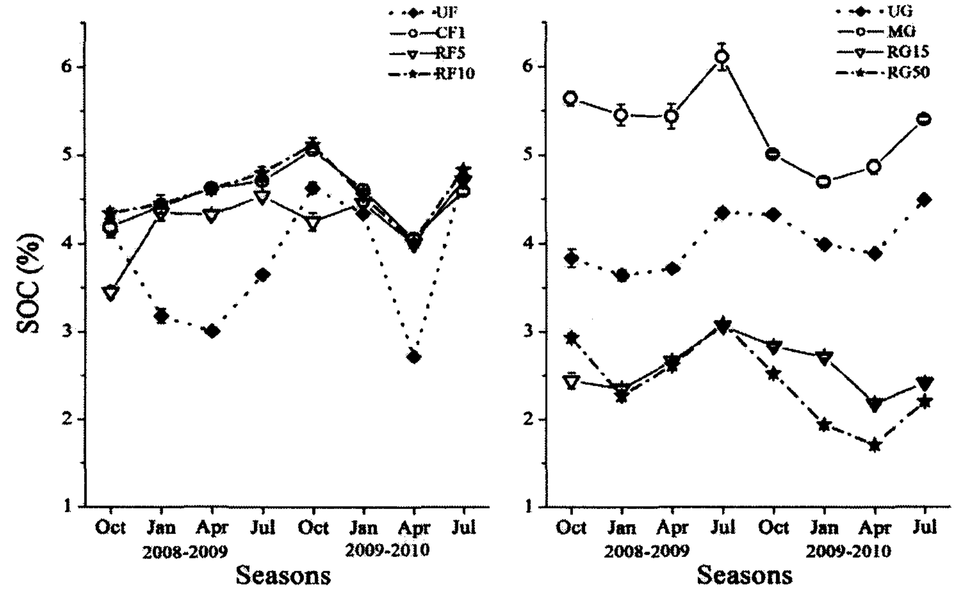

Fig. 4.4: Seasonal variation in soil organic carbon in the undisturbed and recovering stands of forest and grassland ecosystems.

Total phosphorus: Total phosphorus (TP) varied between 217.6 and 363.8 μg g^{-1} in forest ecosystem, and 153.1 and 297.2 μg g^{-1} in grassland ecosystem. In forest ecosystem, TP was significantly (Tukey's test, $p<0.01$) greater in CF1 stands than the recovering RF5 and RF10 and undisturbed forest stands (Fig. 4.5 and Table 4.3). As recovery followed the TP content declined initially till 5th year of deforestation and increased thereafter. In grassland ecosystem, UG and MG sites had significantly (Tukey's test, $p<0.00$) higher TP than RG15 and RG50 (Fig. 4.5 and Table 4.4). It was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. TP varied significantly ($p<0.00$) among the seasons, and was highest during autumn and lowest during summer/rainy seasons (Fig. 4.5).

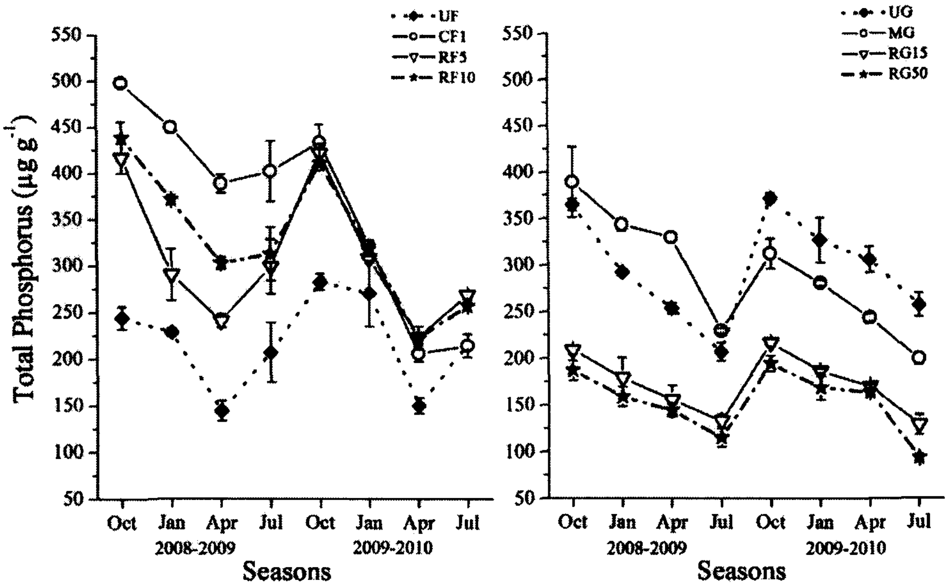

Fig. 4.5: Seasonal variation in total phosphorus in the undisturbed and recovering stands of forest and grassland ecosystems.

Total Kjeldahl nitrogen: Total Kjeldahl nitrogen (TKN) in forest soil ranged between 0.25 and 0.48%, and 0.13 and 0.32% in grassland ecosystem. In forest ecosystem, TKN was significantly (Tukey's test, $p<0.00$) greater in CF1 stands (0.48%) i.e. immediately after deforestation than RF5 (0.33%), RF10 (0.39%) and UF (0.25%) stands (Table 4.3 and Fig. 4.6). In grassland ecosystem, TKN showed a sharp decline as recovery progressed (Fig. 4.6). The mining affected MG (0.32%) and UG (0.25%) sites had significantly (Tukey's test, $p<0.00$) greater values of TKN than the recovering RG15 (0.14%) and RG50 (0.13%) sites (Table 4.4). It was significantly ($p<0.00$) higher in forest ecosystem than the grassland ecosystem. It varied among the seasons significantly ($p<0.00$) and was highest during winter and lowest during summer (Fig. 4.6).

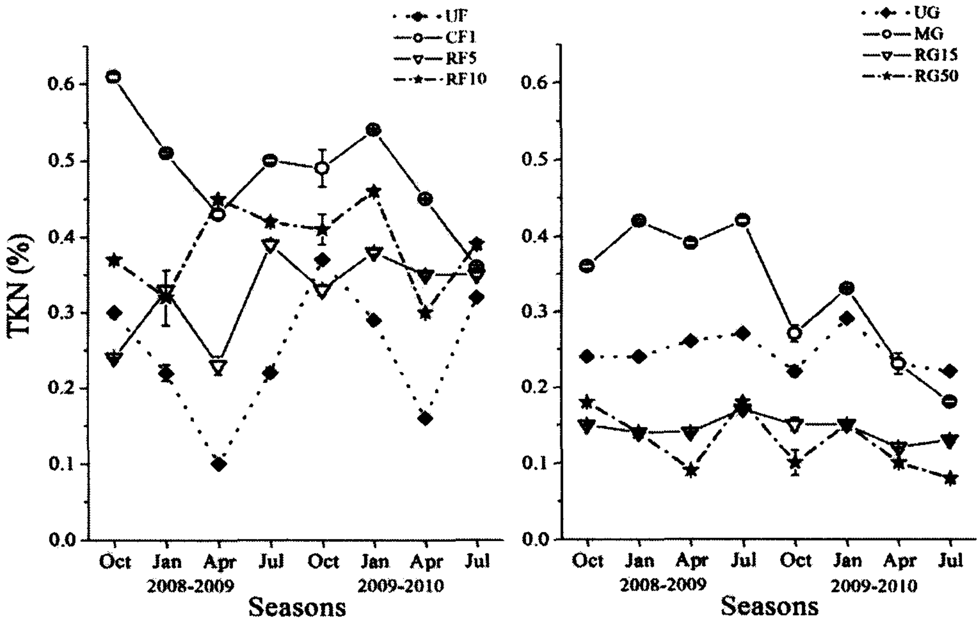

Fig. 4.6: Seasonal variation in total Kjeldahl nitrogen in the undisturbed and recovering stands of forest and grassland ecosystems.

In forest ecosystem, C/N ratio was significantly (Tukey's test, $p<0.01$) lower in CF1 stands than RF5, RF10 and UF stands. As recovery proceeded, it showed an overall increasing trend. C/P also followed a more or less same trend with an overall increase along the recovery ages. In grassland ecosystem, C/N ratio was significantly (Tukey's test, $p<0.01$) lower in MG than RG15, RG50 and UG sites. It showed an increasing trend as recovery progressed. In contrast, C/P declined as recovery progressed. It was significantly (Tukey's test, $p<0.01$) greater in MG than UG.

Relative contribution of different soil physico–chemical properties to recovery of the forest stands were assessed through PCA (Fig. 4.7). The results for the first two axes were reported because the first and second axis of PCA (PC1 and PC2) explained a high proportion of the variance (PC1 and PC2 explained 93.9% and 3.5% of variance, respectively). The factor scores in the biplot indicated higher impact of WHC, C/P and TP on the forest stands than the other physicochemical properties.

The principal component analysis for different soil physico–chemical properties in grassland ecosystem showed that the first two components accounted for 76.9% of the total variance (Fig. 4.8). The same factors as that of forest ecosystem were found important in grassland ecosystem as well.

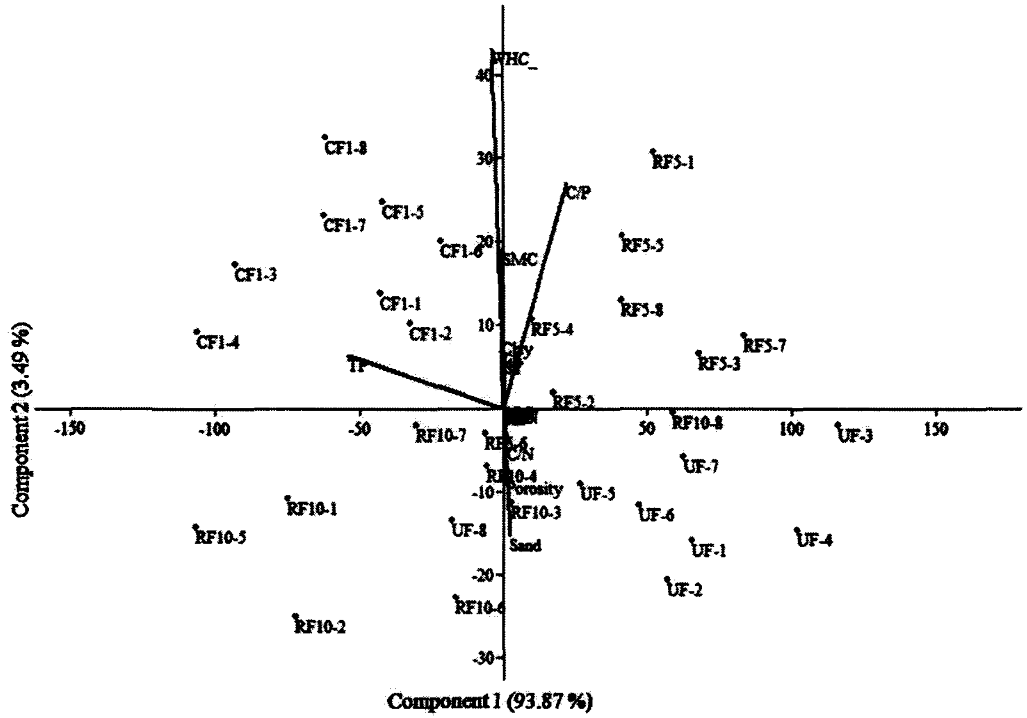

Fig. 4.7: PCA ordination of soil physico–chemical properties, and the undisturbed and recovering stands of forest ecosystem.

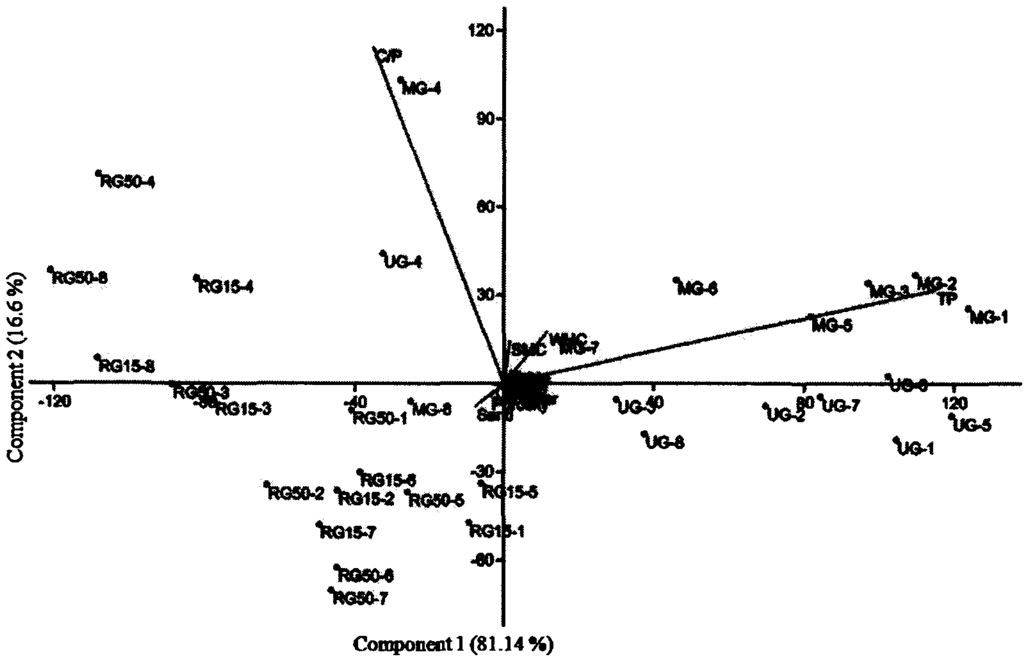

Fig. 4.8: PCA ordination of soil physico–chemical properties, and the undisturbed and recovering stands of grassland ecosystems.

Vegetation Characteristics

Floristic composition: In forest ecosystem, the undisturbed stands had 66 tree species belonging to 46 genera and 29 families, 14 shrub species belonging to 14 genera and 11 families, and 16 herb species under 16 genera and 16 families. Due to disturbance the species composition was altered and a reduced number of tree species were recorded from the recovering stands. In the 1st year of deforestation, i.e. in CF1 stands, 27 tree species were recorded. In the recovering stands RF5 and RF10, 27 tree species and 34 tree species respectively were recorded. The number of species under herbs and shrubs increased after disturbance compared to the undisturbed stand. The number of herb species in CF1, RF5 and RF10 was 31, 22 and 13, respectively. The corresponding figures for shrub species were 23, 32 and 15 (Table 4.5). Across the stands, the number of herbaceous species was highest during rainy season and lowest during winter. Fagaceae had the highest number of tree species in both undisturbed and recovering forests. However, dominant families for herbs and shrubs varied among the stands. Rosaceae had the highest number of shrub species in CF1. Rubiaceae dominated the RF5 and UF stands and Melastomataceae dominated RF10. Among herbs, Cyperaceae dominated CF1, Asteraceae RF5, Begoniaceae, Cyperaceae and Violaceae RF10 and the members of Selaginellaceae dominated UF.

Only 12 tree species (15%) were common to all the stands. Three species viz., *Lithocarpus fenestratus*, *Photinia* sp. and *Toddalia asiatica* were exclusively found in CF1, 5 species viz., *Albizia odoratissima*, *Beilschmiedia assamica*, *Brassaiopsis speciosa*, *Ficus neriifolia* and *Litsea salicifolia* in RF5 and only one species viz., *Viburnum simonsii* was found in RF10 stands. Thirty two species were exclusive to UF stand only (Table 4.6). Among shrubs, only two species (4%) were common to all the stands. Nine species were found only in CF1, 17 species in RF5 and 6 species in UF only. No shrub species was exclusive to RF10 (Table 4.7). Among herbs only 5 species (9%) were common to all the stands. The numbers of species exclusive to CF1, RF5, RF10 and UF were 14, 6, 1 and 8, respectively (Table 4.8).

Table 4.5: Floristic composition of plant communities in the undisturbed and recovering stands of forest ecosystem in Cherrapunjee.

	GENERA				SPECIES				FAMILY			
	CF1	**RF5**	**RF10**	**UF**	**CF1**	**RF5**	**RF10**	**UF**	**CF1**	**RF5**	**RF10**	**UF**
Trees	26	25	30	46	31	27	34	66	20	17	24	29
Shrubs	18	28	14	14	23	32	15	14	15	21	12	11
Herbs												
Winter	22	17	9	14	22	18	9	14	18	14	9	14
Spring	28	20	10	14	28	21	10	14	22	16	9	14
Rainy	31	21	13	16	31	22	13	16	25	16	12	16
Autumn	31	21	13	16	31	22	13	16	25	16	12	16

Table 4.6: Tree species present exclusively in different recovery age categories and species common to all the stands in the forest ecosystem.

Lithocarpus fenestratus (Roxb.) Rehder	*Albizia odoratissima* (L.f.) Benth.	*Viburnum simonsii* Hook.f.& Thomson	*Acer oblongum* Wall. ex DC.	*Acer laevigatum* Wall.
Photinia sp.	*Beilschmiedia assamica* Meisn.		*Castanopsis armata (Roxb.)*Spach.	*Camellia caduca* C.B. Clarke ex Brandis
Toddalia asiatica (L.) Lam.	*Brassaiopsis speciosa* Decne & Planch.		*Castanopsis kurzii (Hance)*S. N. Biswas	*Castanopsis purpurella* (Miq.) N.P.Balakr.
	Ficus neriifolia Sm.		*Cinnamomum glanduliferum* (Wall.) Meisn.	*Castanopsis tribuloides* (Sm.) A.DC.
	*Litsea salicifolia (*Roxburgh ex Nees) Hook.f.		*Coffea jenkinsii* Hook. f.	*Garcinia cowa* Roxb. ex Choisy
			Diospyros kaki Thunb.	*Glochidion acuminatum* Mull.Arg.
			Elaeocarpus acuminatus Wall. ex Mast.	*Lithocarpus dealbatus* (Hk. f. & Th. Ex Miq) Rehder
			Elaeocarpus braceanus Watt ex C.B.Clarke	*Myrica esculenta* Buch.–Ham.ex D.Don
			Ficus hispida L.f.	*Neolitsea cassia* (L.) Kosterm.
			Ficus nemoralis Wall. ex Miq.	*Quercus glauca* Thunb.
			Glycosmis arborea (Roxb.) DC.	*Schima khasiana* Dyer
			Maesa indica (Roxb.) A. DC.	*Syzygium tetragonum* (Wight) Wall. ex Walp.
			Magnolia pterocarpa Roxb.	
			Magnolia sp.	
			Manglietia caveana Hook. f. & Thomson	
			Manglietia insignis (Wall.) Blume	
			Michelia sp.	
			Neolitsea umbrosa (Nees) Gamble	
			*Persea kingii (Hook.f.)*Kosterm.	
			Picrasma javanica Blume	
			Prunus cerasoides Buch.–Ham. ex D. Don	
			Pseudobrassaiopsis mitis	

			(C.B.Clarke)R.N. Ban. *Psychotria symplocifolia* Kurz *Quercus griffithii* Hook.f. & Thomson ex Miq. *Quercus kamroopii* D.Don *Quercus semecarpifolia* Sm. *Randia fasciculata (Roxb.)* DC. *Rhododendron arboreum* Sm. *Rhus hookeri* Sahni & Bahadur *Skimmia laureola* (DC.) Siebold & Zucc. ex Walp. *Symplocos ramosissima Wall. ex G. Don* *Wendlandia wallichii* Wight & Arn.	
3	5	1	32	12

Table 4.7: Shrub species present exclusively in different recovery age categories and species common to all the stands in the forest ecosystem.

Shrub [illegible]	Shrub [illegible]	Shrub [illegible]	Common Shrubs [illegible]
Callicarpa rubella Lindl.	*Berberis wallichiana* DC.	*Breynia rhamnoides* Mull. Arg.	*Lasianthus lancifolius* Hook.f.
Daphne involucrata Wall.	*Coffea jenkinsii* Hook.f	*Luculia pinceana* Hook.	*Viburnum foetidum* Wall.
Mahonia pycnophylla (Fedde) Takeda	*Euonymus theifolius* Wall.ex M.A.Lawsen	*Macropanax dispermus* (Blume) Kuntze	
Osbeckia sp.	*Gleichenia* sp.	*Raphidophora decursiva (Roxb.) A.*Schott	
Rubus ellipticus Sm.	*Goniothalamus sesquipedalis* (Wall.)Hk.f. & Th.	*Styrax hookeri* C.B.Clarke.	
Solanum myriacanthum	*Jasminum calycinum* Wall. Ex Voigt	*Tupidanthus calyptratus* Hk. f. & Th.	
Solanum sisymbriifolium Lam.	*Lasianthus lucidus* Blume		
Viburnum simonsii Hk. f. & Th.	*Lyonia ovalifolia* (Wall.) Drude		
Xanthoxylum sp.	*Macrosolen psilanthus* (Hk.f.) Danser		
	Mucuna bracteata DC.		
	Mussaenda roxburghii Hk.f		
	Paederia foetida L.		

	Piper khasianum C. DC. *Polygonum chinense* L. *Sarcandra glabra* (Thunb.) Nakai. *Solanum torvum* Buch.Ham. ex Wall. *Strychnos wallichiana* Steud. Ex A.DC.		
9	17	6	2

Table 4.8: Herb species present exclusively in different recovery age categories and species common to all the stands in the forest ecosystem.

[illegible]	[illegible]	[illegible]	[illegible]	Common [illegible]
Anaphalis adnata Wall. ex DC.	*Alyxia fascicularis* (Wall. ex G.Don) Hook.f.	*Monotropa uniflora*	*Anoetochilus roxburghii* (Wall) Lindl.	*Arisaema tortuosum* (Wall.) Scott
Brunella vulgaris L.	*Bidens biternata* (Lour.) Merr. & Sherff.		*Balanophora dioica* R. Br. ex Royle	*Begonia laciniata* Roxb.
Centella asiatica (L.) Urb.	*Melodinus monogynus* Roxb.		*Dicranopteris linearis* (Burm.f.) Underw.	*Carex baccans* Nees
Commelina benghalensis L.	*Paederia* sp.		*Hemiphragma heterophyllum* Wall.	*Hedychium spicatum* Sm.
Crassocephalum crepidioides (Benth.) S.Moore	*Pteris* sp		*Selaginella sp.*	*Viola tricolor* L.
Cuscuta reflexa Roxb.	*Rhaphidophora lancifolia* Schott.		*Setaria palmifolia* (J.Koenig) Stapf.	
Emilia sonchifolia (L.) DC. ex DC.			*Smilax myrtillus* A.DC.	
Fimbristylis quinquangularis Kunth			*Sonerilia sp.*	
Gentiana sp.				
Plantago major L.				
Polygonum bistorta L.				
Pouzolzia hirta (Blume) Blume ex Hassk.				
Pratia begonifolia (Wall.) Lindl.				
Urena lobata L.				
14	6	1	8	5

In grassland ecosystem, the undisturbed site had 30 plant species belonging to 29 genera and 16 families. The species composition changed and the number of species declined following disturbance. From MG sites, nine herbaceous species belonging to 9 genera and 5 families were recorded. The recovering sites RG15 and RG50 had 20 and 21 plant species, respectively (Table 4.9). The family Poaceae dominated all the grassland sites. Only 6 plant species (19%) were common to all the sites. One plant species viz., *Themeda intermedia* was found only in RG50 while 7 species viz., *Aeginetia indica, Habenaria goodyeroides, Malaxis acuminata, Satyrium nepalense, Spiranthes sinensis, Themeda villosa, Tripogon trifidus* were found only in UG (Table 4.10). No species was exclusive to MG and RG15.

Table 4.9: Floristic composition of plant communities in the undisturbed and recovering stands of grassland ecosystem in Cherrapunjee.

	GENERA				SPECIES				FAMILY			
	MG	RG15	RG50	UG	MG	RG15	RG50	UG	MG	RG15	RG50	UG
Winter	5	17	16	20	5	17	17	21	1	12	12	12
Spring	7	18	17	24	7	18	18	25	4	13	13	14
Rainy	9	20	20	29	9	20	21	30	5	15	16	16
Autumn	9	20	20	28	9	20	21	29	5	15	15	15

Table 4.10: Plant species present exclusively in different recovery age categories and species common to all the stands in the grassland ecosystem.

Grassland species (RG50)	Grassland species (UG)	Common Grassland species
Themeda intermedia (Hack.) Bor	*Aeginetia indica* L.	*Arundinella khasiana* Nees ex Steud.
	Habenaria goodyeroides D. Don	*Borreria articularis* (L.f.) F.N.Williams
	Malaxis acuminata D. Don	*Cyanotis vaga* (Lour.) Schult. & Schult.f.
	Satyrium nepalense D. Don	*Eragrostis nigra* Nees ex Steud.
	Spiranthes sinensis (Pers.) Ames	*Fimbristylis hookeriana* Boeckeler
	Themeda villosa (Poir.) A.Camus	*Polygonum bistorta* L.
	Tripogon trifidus Munro ex Hook.f.	
1	7	6

Table 4.11: Shannon's diversity index and Pielou's evenness index in the undisturbed and recovering stands of forest ecosystem in Cherrapunjee.

	SHANNON				EVENNESS			
	CF1	RF5	RF10	UF	CF1	RF5	RF10	UF
Trees	2.91	2.83	3.16	3.64	0.68	0.60	0.69	0.58
Shrubs	2.83	3.34	2.55	2.52	0.90	0.96	0.94	0.96
Herbs								
Winter	2.87	2.76	2.15	2.53	0.93	0.96	0.98	0.96
Spring	3.03	2.90	2.22	2.53	0.91	0.95	0.97	0.96
Rainy	3.07	2.95	2.47	2.67	0.89	0.95	0.96	0.96
Autumn	3.23	2.96	2.46	2.67	0.94	0.96	0.96	0.96

Table 4.12: Shannon's diversity index and Pielou's evenness index in the undisturbed and recovering stands of grassland ecosystem in Cherrapunjee.

	SHANNON				EVENNESS			
	MG	RG15	RG50	UG	MG	RG15	RG50	UG
Winter	1.53	2.79	2.36	2.85	0.948	0.986	0.834	0.937
Spring	1.76	2.85	2.53	3.00	0.904	0.987	0.874	0.932
Rainy	2.05	2.95	3.01	3.18	0.932	0.984	0.989	0.935
Autumn	2.04	2.95	2.94	3.11	0.927	0.984	0.966	0.923

Species diversity: In forest ecosystem, Shannon's diversity index and evenness index for tree species reduced due to disturbance (Table 4.11). In other words, the undisturbed site had greater number of species than the recovering stands. The species were also more evenly distributed in the undisturbed stand compared to the recovering and disturbed stands. However, as recovery followed the diversity and evenness gradually improved to attain a more diverse and equitable community. In case of herbs and shrubs, Shannon's diversity index increased immediately after disturbance and subsequently reduced as evident from the values in RF5, RF10 and UF stands. Pielou's evenness index was greater for herbs than shrubs and trees in all the stands. In grassland ecosystem, Shannon's diversity index was greater in the undisturbed site than the recovering sites (Table 4.12). Disturbance reduced the species diversity, which improved as recovery followed. Pielou's evenness index exhibited quite high values in all the stands.

Dominance–diversity pattern: The dominance–diversity curve using log IVI values followed a log normal distribution pattern i.e. low dominance or high equitability for trees in the undisturbed forest stands whereas in CF1, RF5 and RF10 the trees species showed low equitability or high dominance (Fig. 4.9). While most species in UF shared the IVI values equitably, a high proportion of total IVI values were concentrated in *Castanopsis purpurella* species in CF1 (21.5%), *Lithocarpus dealbatus*, *Castanopsis tribuloides*, *Castanopsis purpurella* and *Quercus glauca* in RF5 (55.6%), and *Castanopsis purpurella* and *Lithocarpus dealbatus* in RF10 (30.9%). Thus, dominance pattern among the species varied along the different recovery ages. The pattern of dominance–diversity curves for herbs and shrubs however, did not vary among the different recovery ages (Figs. 4.10 and 4.11). The dominant shrub species in CF1, RF5, RF10 and UF was *Rubus niveus* (IVI– 26.6), *Psychotria symplocifolia* (IVI– 13.5), *Eurya japonica* (IVI– 28.0) and *Osbeckia stellata* (IVI– 26.2), respectively. Among the herbs, *Carex baccans* (IVI– 25.2), *Bidens pilosa* (IVI– 19.9), *Begonia laciniata* (IVI– 23.4) and *Selaginella* sp. (IVI– 22.8), dominated CF1, RF5, RF10 and UF, respectively (Tables 4.13, 4.14, 4,15 and 4.16). In grassland ecosystem, the plant species exhibited a log normal distribution pattern in UG, RG15 and RG50 sites whereas MG sites had a steeper dominance–diversity curve depicting high dominance of a few species i.e. *Digitaria violascens* and *Arundinella khasiana* (26.3%) (Fig. 4.12). *Cyanotis vaga* (IVI– 13.4) was dominant in RG15 site, *Osbeckia capitata* (IVI– 14.4) in RG50 and *Drosera peltata* (IVI– 18.9) in UG sites (Table 4.17). The dominant species in all the vegetation components changed with recovery age in both the ecosystems.

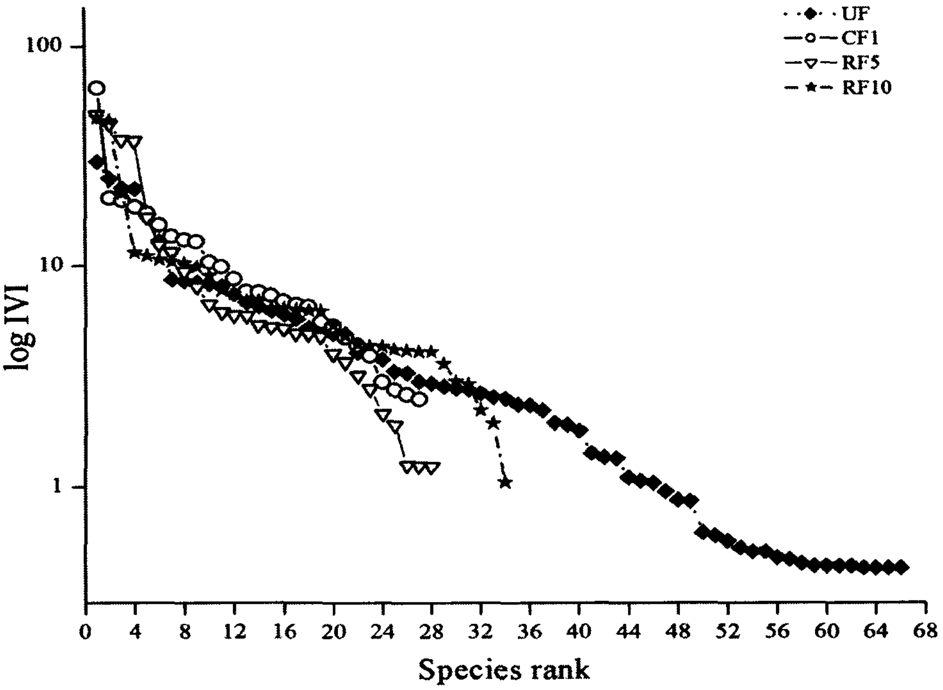

Fig. 4.9: Diversity–dominance curves for tree species in the undisturbed and recovering stands of forest ecosystem in Cherrapunjee. The species are ranked from most abundant on the left to least abundant on the right.

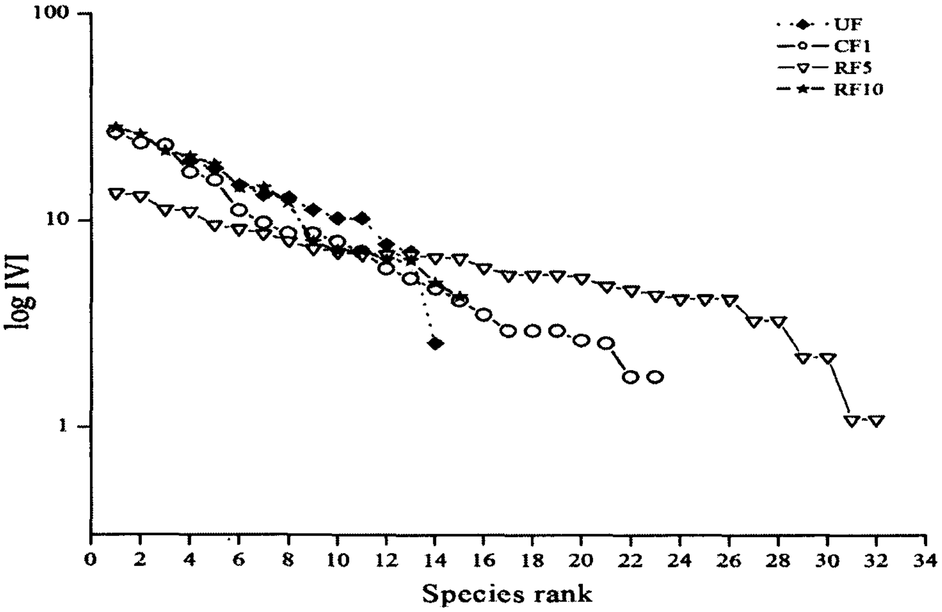

Fig. 4.10: Diversity–dominance curves for shrub species in the undisturbed and recovering stands of forest ecosystem in Cherrapunjee. The species are ranked from most abundant on the left to least abundant on the right.

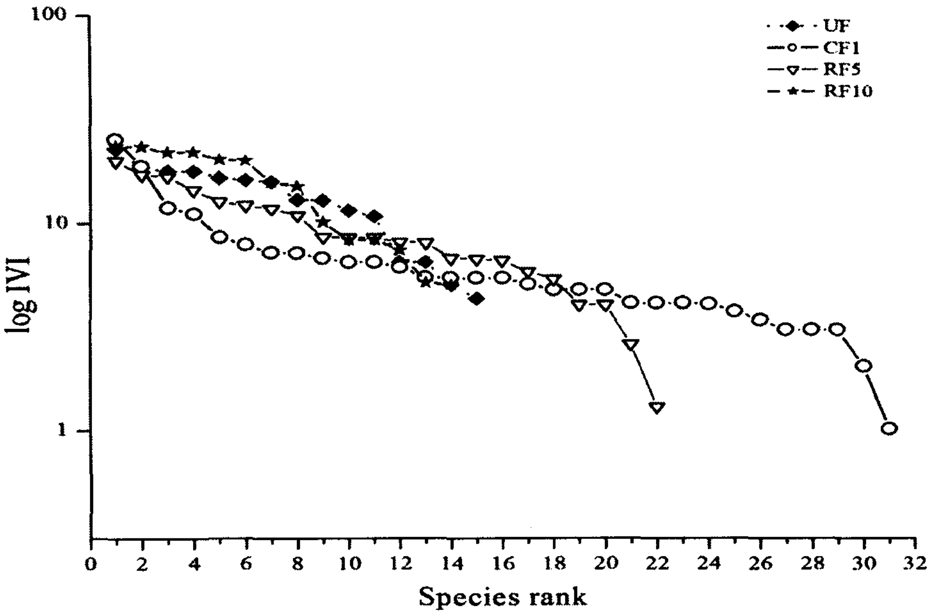

Fig. 4.11: Diversity–dominance curves for herb species in the undisturbed and recovering stands of forest ecosystem in Cherrapunjee. The species are ranked from most abundant on the left to least abundant on the right.

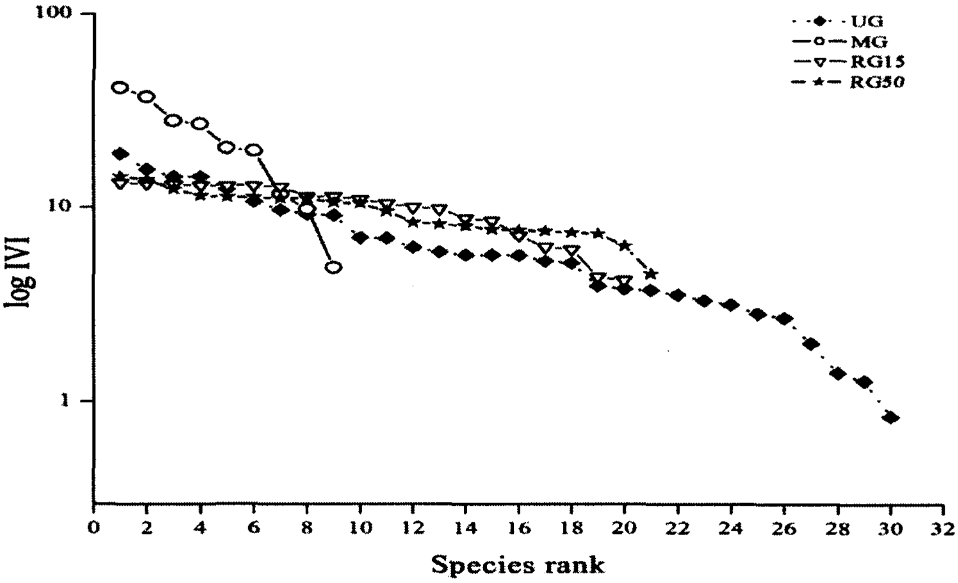

Fig. 4.12: Diversity–dominance curves for plant species in the undisturbed and recovering stands of grassland ecosystem in Cherrapunjee. The species are ranked from most abundant on the left to least abundant on the right.

Table 4.13: Density (ha^{-1}) and importance value index (IVI) of ten dominant species in CF1forest stands.

[illegible]	Family	[illegible]	[illegible]
Castanopsis purpurella (Miq.) N.P.Balakr.	Fagaceae	70	64.5
Lithocarpus dealbatus (Hk. f. & Th. Ex Miq) Rehder	Fagaceae	18	20.3
Schima khasiana Dyer	Theaceae	28	19.8
Quercus glauca Thunb.	Fagaceae	12	18.5
Neolitsea cassia (L.) Kosterm.	Lauraceae	18	17.3
Rhus javanica L.	Anacardiaceae	20	15.3
Lyonia ovalifolia (Wall.) Drude	Ericaceae	14	13.5
Cinnamomum zeylanicum Blume	Lauraceae	16	13.1
Symplocos spicata Roxb.	Symplocaceae	16	12.8
Elaeocarpus lanceifolius Roxb.	Elaeocarpaceae	10	10.3
[illegible]	[illegible]	[illegible]	[illegible]
Rubus niveus Thunb.	Rosaceae	1160	26.6
Osbeckia sp.	Melastomataceae	880	23.6
Rubus rugosus Sm.	Rosaceae	840	23.0
Rubus ellipticus Sm.	Rosaceae	480	17.1
Daphne involucrata Wall.	Thymelaeaceae	500	15.6
Eurya japonica Thunb.	Pentaphylacaceae	360	11.2
Viburnum simonsii Hk. f. & Th.	Adoxaceae	260	9.7
Melastoma sp.	Melastomataceae	320	8.6
Solanum myriacanthum	Solanaceae	320	8.6
Xanthoxylum	Rutaceae	180	7.9
[illegible]	[illegible]	[illegible]	[illegible]
Carex baccans Nees	Cyperaceae	19000	25.2
Ophiopogon intermedius D. Don.	Asparagaceae	14500	18.8
Viola tricolor L.	Violaceae	8500	11.9
Pratia begonifolia (Wall.) Lindl.	Campanulaceae	12000	11.1
Paspalum orbiculare G.Forst.	Poaceae	6500	8.5
Gentiana sp.	Gentianaceae	6500	7.9
Commelina benghalensis L.	Commelinaceae	6500	7.2
Emilia sonchifolia (L.) DC. ex DC.	Compositae	5500	7.2
Arisaema tortuosum (Wall.) Scott	Araceae	4000	6.8
Bidens pilosa L.	Compositae	4500	6.5

Table 4.14: Density (ha^{-1}) and importance value index (IVI) of ten dominant species in RF5 forest stands.

[illegible]	[illegible]	[illegible]	[illegible]
Lithocarpus dealbatus (Hk. f. & Th. Ex Miq) Rehder	Fagaceae	152	48.5
Castanopsis tribuloides (Sm.) A.DC.	Fagaceae	140	44.1
Castanopsis purpurella (Miq.) N.P.Balakr.	Fagaceae	104	37.2
Quercus glauca Thunb.	Fagaceae	138	36.8
Myrica esculenta Buch.–Ham.ex D.Don	Myricaceae	78	16.6
Ficus nerifolia Sm.	Moraceae	46	12.4
Helicia nilagirica Bedd.	Proteaceae	52	11.5
Persea duthiei (King) Kosterm	Lauraceae	38	9.4
Schima khasiana Dyer	Theaceae	26	8.0

Litsea salicifolia Roxb.	Lauraceae	26	6.6
[illegible]			
Psychotria symplocifolia Kurz.	Rubiaceae	340	13.5
Viburnum foetidum Wall.	Adoxaceae	320	13.1
Rubus rugosus Sm.	Rosaceae	240	11.3
Smilax sp	Smilacaceae	260	11.1
Jasminum calycinum Wall. Ex Voigt	Oleaceae	220	9.5
Rubus niveus Thunb.	Rosaceae	200	9.1
Piper khasianum C. DC.	Piperaceae	180	8.6
Senecio cappa Buch.–Ham.ex D.Don	Compositae	180	8.0
Lasianthus lancifolius Hook.f.	Rubiaceae	180	7.3
Goniothalamus sesquipedalis (Wall.)Hk.f. & Th.	Annonaceae	140	7.1
[illegible]			
Bidens pilosa L.	Compositae	13000	19.9
Viola tricolor L.	Violaceae	10000	17.1
Carex baccans Nees	Cyperaceae	10500	16.7
Ophiopogon sp	Asparagaceae	8000	14.4
Dicranopteris sp	Gleicheniaceae	8000	12.7
Botrychium lanuginosum Wall. ex Hook & Grev.	Ophioglossaceae	6500	12.1
Begonia laciniata Roxb.	Begoniaceae	7000	11.7
Globba multiflora Wall. ex Baker	Zingiberaceae	7000	10.9
Pteris sp	Pteridaceae	4500	8.5
Paederia sp.	Rubiaceae	3500	8.5

Table 4.15: Density (ha^{-1}) and importance value index (IVI) of ten dominant species in RF10 forest stands.

[illegible]	Family	[illegible]	[illegible]
Castanopsis purpurella (Miq.) N.P.Balakr.	Fagaceae	260	47.0
Lithocarpus dealbatus (Hk. f. & Th. Ex Miq) Rehder	Fagaceae	226	45.6
Neolitsea cassia (L.) Kosterm.	Lauraceae	128	21.3
Symplocos glomerata King ex C. B. Clarke	Symplocaceae	74	11.4
Persea odoratissima (Nees) Kosterm.	Lauraceae	68	11.1
Ligustrum robustum (Roxb.) Blume	Oleaceae	62	10.6
Schima khasiana Dyer	Theaceae	50	10.4
Quercus glauca Thunb.	Fagaceae	42	10.2
Syzygium tetragonum (Wight) Wall. ex Walp.	Myrtaceae	52	9.8
Eurya acuminata DC.	Theaceae	60	8.9
[illegible]			
Eurya japonica Thunb.	Pentaphylacaceae	420	28.0
Osbeckia stellata Buch.–Ham. ex Ker Gawl.	Melastomataceae	440	25.9
Daphne papyracea Wall. ex W.W.Sm. & Cave	Thymelaeaceae	320	21.6
Rubus rugosus Sm.	Rosaceae	300	20.4
Viburnum foetidum Wall.	Adoxaceae	280	18.7
Lasianthus lancifolius Hook.f.	Rubiaceae	200	14.4
Psychotria symplocifolia Kurz.	Rubiaceae	160	14.4
Smilax myrtillus A.DC.	Smilacaceae	140	12.2
Senecio cappa Buch.–Ham.ex D.Don	Compositae	100	7.9
Ficus gasparriniana Miq.	Moraceae	80	7.2

[illegible]	[illegible]	[illegible]	[illegible]
Begonia laciniata Roxb.	Begoniaceae	7000	23.4
Arisaema tortuosum (Wall.) Scott	Araceae	6000	23.2
Carex baccans Nees	Cyperaceae	7000	21.9
Viola tricolor L.	Violaceae	7000	21.9
Impatiens chinensis L.	Balsaminaceae	6000	20.1
Tetrastigma serrulatum (Roxb.) Planch	Vitaceae	5000	19.9
Jasminum sp	Oleaceae	3500	15.6
Hedychium spicatum Sm.	Zingiberaceae	4000	15.0
Ophiopogon sp	Asparagaceae	3000	10.0
Dicranopteris sp	Gleicheniaceae	2000	8.3

Table 4.16: Density (ha^{-1}) and importance value index (IVI) of ten dominant species in old growth undisturbed forest (UF) stands.

[illegible]	[illegible]	[illegible]	[illegible]
Quercus kamroopii D.Don	Fagaceae	38	29.7
Neolitsea cassia (L.) Kosterm.	Lauraceae	120	25.0
Lithocarpus dealbatus (Hk. f. & Th. Ex Miq) Rehder	Fagaceae	96	22.6
Castanopsis purpurella (Miq.) N.P.Balakr.	Fagaceae	42	22.4
Myrica esculenta Buch.–Ham.ex D.Don	Myricaceae	68	17.4
Elaeocarpus braceanus Watt ex C.B.Clarke	Elaeocarpaceae	50	12.9
Garcinia cowa Roxb. ex Choisy	Clusiaceae	40	8.6
*Exbucklandia populnea (R.*Br. ex Griff.) R.W.Br.	Hamamelidaceae	22	8.4
Persea odoratissima (Nees) Kosterm.	Lauraceae	46	8.4
Schima khasiana Dyer	Theaceae	28	8.2
[illegible]			
Osbeckia stellata Buch.–Ham. ex Ker Gawl.	Melastomataceae	340	26.2
Coffea khasiana (Korth.) Hook.f.	Rubiaceae	260	23.9
Lasianthus lancifolius Hook.f.	Rubiaceae	240	22.9
Viburnum foetidum Wall.	Adoxaceae	200	19.3
Smilax sp	Smilacaceae	200	17.7
*Raphidophora decursiva (Roxb.) A.*Schott	Araceae	140	14.8
Daphne papyracea Wall. ex W.W.Sm. & Cave	Thymelaeaceae	140	13.2
Tupidanthus calyptratus Hk. f. & Th.	Araliaceae	100	12.8
Breynia rhamnoides Mull. Arg.	Euphorbiaceae	100	11.3
Ficus gasparriniana Miq.	Moraceae	80	10.3
[illegible]			
Selaginella sp.	Selaginellaceae	9000	22.8
Begonia laciniata Roxb.	Begoniaceae	7000	18.5
Arisaema tortuosum (Wall.) Scott	Araceae	6500	17.8
Carex baccans Nees	Cyperaceae	7500	17.8
Hedychium spicatum Sm.	Zingiberaceae	5500	16.4
Sonerilia sp.	Melastomataceae	8500	16.0
Impatiens chinensis L.	Balsaminaceae	5000	15.7
Ophiopogon intermedius D. Don.	Asparagaceae	4000	12.9
Viola tricolor L.	Violaceae	5000	12.8
Anoetochilus roxburghii (Wall) Lindl.	Orchidaceae	3000	11.5

Table 4.17: Density (ha^{-1}) and importance value index (IVI) of ten dominant species in recovering (MG, RG15 and RG50) and the undisturbed (UG) stands of grassland ecosystem.

[illegible]	[illegible]	[illegible]	[illegible]
Digitaria violascens Link	Poaceae	5500	41.6
Arundinella khasiana Nees ex Steud.	Poaceae	6000	37.4
Borreria articularis (L.f.) F.N.Williams	Rubiaceae	3500	28.1
Ischaemum hirtum Hack.	Poaceae	4000	26.9
Eulalia quadrinervis (Hack.) Kuntze	Poaceae	3000	20.2
Polygonum bistorta L.	Polygonaceae	2000	19.5
Eragrostis nigra Nees ex Steud.	Poaceae	1500	11.6
Cyanotis vaga (Lour.) Schult. & Schult.f.	Commelinaceae	1000	9.8
Fimbristylis hookeriana Boeckeler	Cyperaceae	500	4.9
[illegible]			
Cyanotis vaga (Lour.) Schult. & Schult.f.	Commelinaceae	21000	13.4
Ischaemum hirtum Hack.	Poaceae	18000	13.2
Fimbristylis hookeriana Boeckeler	Cyperaceae	16500	13.1
Mazus pumilus (Burm. f.) Steenis	Phrymaceae	21000	12.9
Gnaphalium luteoalbum L.	Compositae	17000	12.9
Smithia ciliata Royle	Leguminosae	19500	12.9
Emilia sonchifolia (L.) DC. ex DC.	Asteraceae	16500	12.7
Osbeckia capitata Benth. ex Naudin	Melastomaceae	20000	11.3
Eragrostis nigra Nees ex Steud.	Poaceae	13500	11.3
Gentiana quadrifaria Blume	Gentianaceae	17500	10.9
[illegible]			
Osbeckia capitata Benth. ex Naudin	Melastomaceae	26000	14.4
Eriocaulon cristatum Mart.	Eriocaulaceae	23000	13.9
Cyanotis vaga (Lour.) Schult. & Schult.f.	Commelinaceae	21000	12.4
Themeda intermedia (Hack.) Bor	Poaceae	18000	11.5
Gnaphalium luteoalbum L.	Compositae	13500	11.3
Mazus pumilus (Burm. f.) Steenis	Phrymaceae	16000	11.2
Eragrostis nigra Nees ex Steud.	Poaceae	15500	11.1
Polygonum bistorta L.	Polygonaceae	15500	11.1
Drosera peltata Thunb.	Droseraceae	21500	10.5
Polygonum nepalense Meisn.	Polygonaceae	16000	10.4
[illegible]			
Drosera peltata Thunb.	Droseraceae	43500	18.9
Arundinella khasiana Nees ex Steud.	Poaceae	36500	15.7
Osbeckia capitata Benth. ex Naudin	Melastomaceae	28000	14.4
Hypoxis aurea Lour.	Hypoxidaceae	26000	14.3
Smithia ciliata Royle	Leguminosae	27000	12.4
Polygonum bistorta L.	Polygonaceae	17000	10.7
Cyanotis vaga (Lour.) Schult. & Schult.f.	Commelinaceae	16500	9.6
Emilia sonchifolia (L.) DC. ex DC.	Asteraceae	13000	9.2
Eriocaulon cristatum Mart.	Eriocaulaceae	16000	9.1
Gentiana quadrifaria Blume	Gentianaceae	16500	7.0

Density and Abundance: In forest ecosystem, tree density was lowest in CF1 (326 stems ha^{-1}) followed by RF5 (1052 stems ha^{-1}). The highest density value was in RF10 (1706 stems ha^{-1}) i.e. after 10 years of recovery. The tree density in the undisturbed forest stands was 1144 stems ha^{-1}. Tree basal area was also lowest in CF1 and increased as recovery followed. Densities of herb and shrub species increased due to disturbance. The density values reduced as recovery proceeded. In grassland ecosystem, disturbance reduced plant density. MG sites (27, 000 ha^{-1}) had significantly ($p<0.05$) lower density than the recovering RG15 (2,99,500 ha^{-1}) and RG50 (3,13,000 ha^{-1}) sites, and undisturbed grassland site (3, 83,500 ha^{-1}). With increase in recovery age the density values increased.

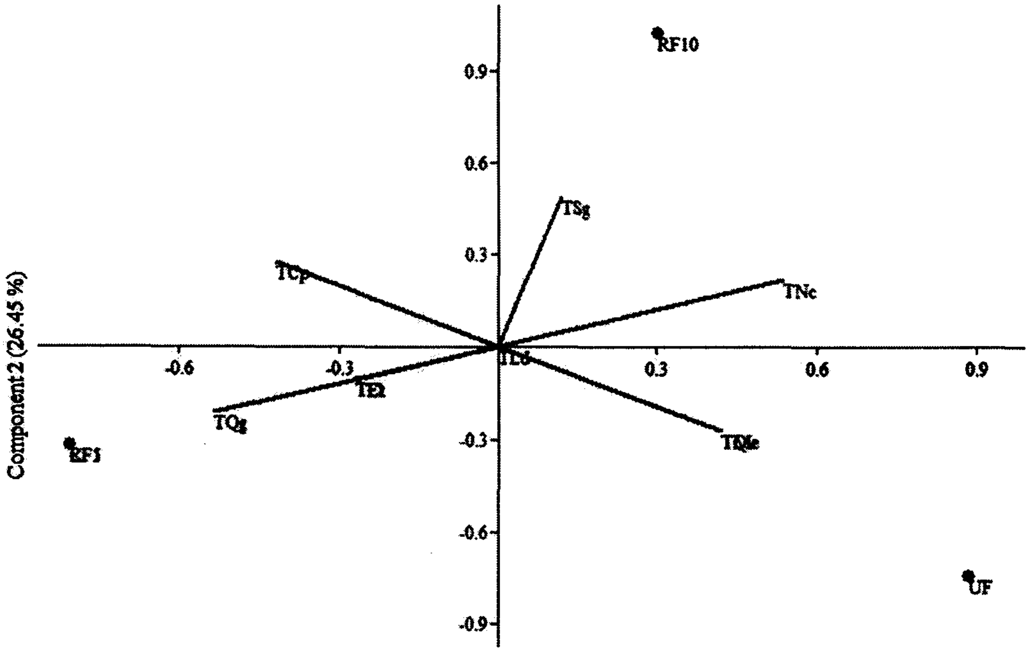

Fig. 4.13: PCA ordination of tree species in the undisturbed and recovering stands of forest ecosystem.

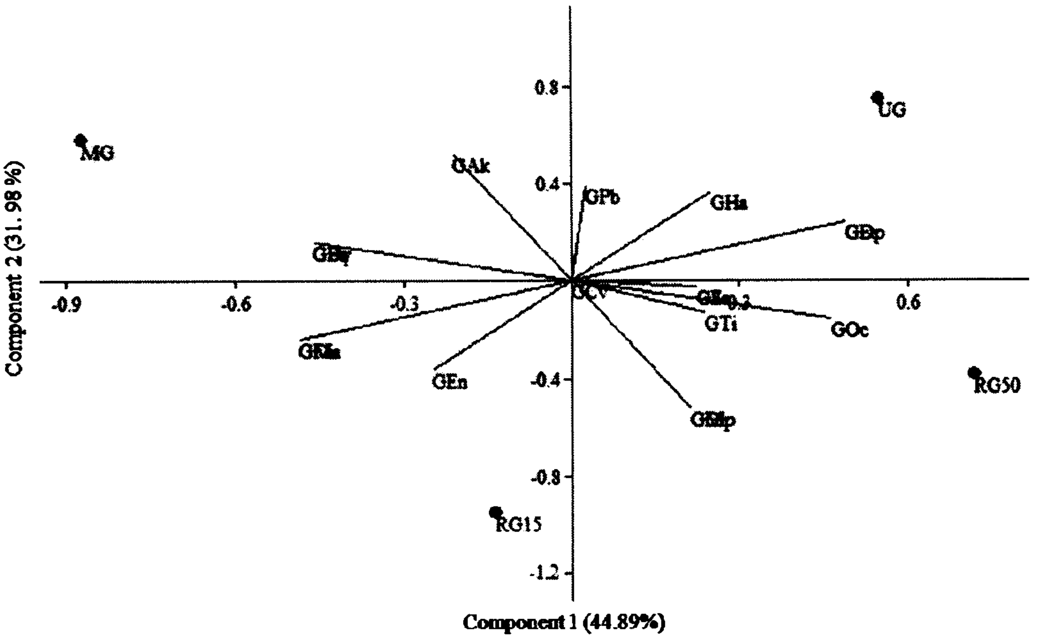

Fig. 4.14: PCA ordination of plant species in the undisturbed and recovering stands of grassland ecosystem.

Tree species associated with recovery age of the forest stands were assessed by PCA (Fig. 4.13). The results for the first two axes were reported because the first and second axis of PCA (PC1 and PC2) explained a high proportion of the variance (PC1 and PC2 explained 58.2% and 26.5% of variance, respectively). The factor scores in the biplot indicated a clear separation between the forest stands. The relatively greater factor score (i.e., vector length) of *Quercus glauca* indicated higher impact of this species on the CF1 stands and the recovering stands RF5 than the other species, while *Symplocos glomerata* and *Neolitsea cassia* were found important for the recovering stands RF10.

The principal component analysis applied to plant species in grassland ecosystem showed that the first two components accounted for the 76.9% of the total variance distributed between PC1 (44.9%) and PC2 (32.0%) (Fig. 4.14). The factor scores in the biplot clearly seggregated the grassland sites. *Digitaria violascens*, *Borreria articularis*, *Eulalia quadrinervis* and *Arundinella khasiana* were found important for mining affected MG sites. *Eragrostis nigra* and *Osbeckia capitata* had higher impact on recovering sites RG15 and RG50 respectively.

DISCUSSION

Changes in vegetation composition and structure during recovery ameliorate soil conditions and assist further vegetation development (Isermann, 2005). Vegetation development enhances the accumulation of carbon and nutrients in the soil (Walker and del Moral, 2009), and these processes improve the soil conditions for subsequent species colonization and ecosystem development (Frouz *et al.*, 2008). Plant communities that develop during the post–disturbance recovery phase differ from the primary forests due to the modification of soil properties (Compton and Boone, 2000) and the relative colonization rates of species (Brunet and Oheimb, 1998). Therefore, knowledge of the likely changes in vegetation development and soil parameters during recovery provides valuable insights of ecosystem development after disturbance and help to gain a more predictive understanding of recovery dynamics.

Effect of recovery process on soil physical properties

Improvement in the physical properties of the soil, particularly bulk density, affects soil biological and biochemical activities (Giusquiani *et al.*, 1995). In the present study, there was an increase in bulk density following deforestation. Similar observations were also made by Hajabbasi *et al.* (1997). The higher bulk density in mining affected MG site may be attributed to soil compaction which decreases the pore space in *soil* and thereby increasing *bulk density*. However, the higher values of bulk density gradually reduced as recovery followed. This could be due to the increasing root biomass and incorporation of organic material from the growing vegetation that made the soil more porous and hence reduced the soil bulk density along the recovery ages. The increased water holding capacity and decreased porosity after disturbance could be due to higher bulk density and compact texture of the recently disturbed sites. This could also be due to increase in clay content that have inherently greater water holding capacity (Voroney, 2007) and therefore, reduced porosity.

Forest floor and mineral soil temperatures are influenced by the incoming solar radiation and soil surface characteristics (Oliver *et al.*, 1987). Soil temperature was significantly higher in grassland ecosystem. It increased significantly due to deforestation and coal dumping because removal of the vegetation cover increased the amount of radiation reaching the soil surface. As recovery followed, vegetation density and canopy cover improved and therefore, soil temperature gradually decreased (Oliver *et al.*, 1987; Balisky and Burton, 1995; Griffiths and Swanson, 2001).

Soil moisture content (SMC) was significantly higher in forest than grassland ecosystem, which was due to greater vegetation cover and higher accumulation of litter in forest ecosystem. An increase in moisture content due to disturbance as recorded in the present study may be attributed to decreased evapo–transpiration (Elliot *et al.*, 1998). This finding was consistent with Cadenasso *et al.* (1997) and Redding *et al.* (2003). The higher value of SMC in the mining affected grassland may be due to the coal dump which is capable of retaining moisture in it. The increase in bulk density also aided to retention of moisture by the soil (Redding *et al.*, 2003).

Effect of recovery process on soil chemical properties

Cation exchange capacity (CEC) is a measure of the capacity of the soil to exchange ions. Cations have the ability to be exchanged for another positively charged ion from the surfaces of clay minerals and organic matter. Sandy soils with low organic matter generally have low CEC values indicating that the soil is inefficient in holding nutrients, thereby limiting the availability of mineral nutrients to the plants. The CEC values reported in the present study were much lower than the earlier reported values for acidic soils (Izquierdo *et al.*, 2005; Closa and Goicoechea, 2010), which may be due to high sand content. The CEC varied among the various sites depending on their clay content which is generally known to be important in adsorbing ions because of its high surface area to volume ratio. The disturbed sites had higher CEC values than the recovering sites owing to their high clay content.

A significant drop of pH was observed in the mining affected site which may be due to the coal dumps as the chemical nature of overburden materials can strongly influence soil pH (Tucker *et al.*, 1987). Coal contains pyrite (FeS_2), a reduced form of iron which oxidises to sulphuric acid and ferric hydroxide when exposed to air (Bohn *et al.*, 1985). Leaching of this sulphuric acid also called acid mine drainage from the coal dumps into the surrounding soil leads to acidification of the soil. As recovery follows the soil pH increases as the accumulated acid is washed out or diluted by the rain. The lower pH during rainy season in comparison to winter could be mainly due to leaching of basic cations by rain water. This was in agreement with Wild (1996) who concluded that excessive leaching of bases like Al^{3+} by rain water could lead to a drop in pH during rainy season.

In forest ecosystem, soil organic carbon (SOC) and the major soil nutrients viz. total N and total P increased significantly after deforestation. These results were consistent with other studies (Hyvönen *et al.*, 2000; de Dieu Nzila *et al.*, 2002; O'Connell *et al.*, 2004; Palviainen *et al.*, 2004; Chen and Xu, 2005). This could be due to the fact that after clear–cutting a considerable amount of nutrients (C, N and P) are contributed by the slash residues to the soil. Slash residues generated after clear cutting consisting of fresh leaves, twigs, branches and log chunks generally contain higher concentration of nutrients than the natural litter. Deposition of large quantities of these materials on the soil surface influences the nutrient content and the soil biological processes. The leaf component of slash residues decay more rapidly than the same fractions in natural litter (O'Connell, 1987). In fact, retention of slash residues could actually increase the rate of mineralization of the existing soil organic matter stocks (Moroni *et al.*, 2007; Vanguelova *et al.*, 2010). Moreover, the higher soil temperatures due to greater exposure of the soil surface and the reduced water use due to lack of vegetation creates favourable environmental conditions i.e. elevated soil temperature and moisture for enhanced decomposition and mineralization. Also reduced uptake by plant roots leads to accumulation of nutrients in the soil surface. The accumulated nutrients are subject to leaching losses due to the absence of vegetation cover. However, Palviainen (2005) suggested that nutrients released from slash residues are initially retained on site primarily through soil processes and microbial immobilization. Also the increased bulk density and the compactness of the soil post disturbance reduce leaching losses.

As recovery followed a decline in the concentration of all the nutrients was evident till 5th year of recovery. This decline during the initial years of recovery might be due to uptake of nutrients by the largely growing herbaceous vegetation and coppice growth from stumps. In fact, the nutrient demand is very high in young stands before canopy closure (Jacobson *et al.*, 2000; Prescott, 2002; Smolander *et al.*, 2008; Helmisaari *et al.*, 2011; Palviainen and Finér, 2012). Moreover, the majority of soil nutrients available for plant uptake are derived from turnover of soil organic matter and the subsequent mineralization of organically bound nutrients. The rate at which nutrients become available is dependent partly on the quality of soil organic matter. Due to the absence of vegetation cover, the recovering RF5 stands lacks a litter layer. Also, the fine residues from slash which are the most important source of nutrients got decomposed during the first year of clear– cutting. Only the coarse residues are retained on the forest floor as they are more resistant to decay (Hyvönen *et al.*, 2000). As recovery progressed, litter inputs from the growing vegetation led to the formation of a litter layer which helped in meeting the nutrient demands of the regenerating forest stands.

Mining related activities compresses the soil and damages the field layer and plant root systems. Decomposition of the resulting foliage and dead roots might be the reason for the increased concentrations of SOC and TKN in the mining affected MG sites.

Differences in texture may also explain the higher SOC and TKN concentrations. Though erosion effects are high on the top soil due to removal of plant cover, the increase in bulk density due to disturbance made the soil more compact reducing the pore spaces and thereby, limiting the loss of nutrients from the deeper soil layers. Moreover, clay content has been found to have positive impact on nutrient retention in soils (Knops and Tilman, 2000). Presence of fine particles in soils with higher clay content supports organic matter protection owing to the building of organo–mineral complexes that are resistant to microbial degradation (Franzluebbers *et al.*, 1996; Muller and Hoper, 2004). Additionally, the young recently disposed mine spoils that lack pedogenic organic matter, contain substantial amount of geogenic organic C originating from lignite particles present in the dumped overburden (Rumpel *et al.*, 1998).

The high levels of total Kjeldahl nitrogen during winter season might be attributed to reduced mineralization rate as well as plant uptake. This trend is in agreement with Wolfhard and Reinhard (1998), and Rahaman *et al.* (2013), who concluded that nitrogen was usually built up during dry seasons. The higher concentration of total phosphorus during autumn as compared to that of summer and rainy seasons may be due to greater litter input during autumn. Chen *et al.* (2008) concluded that P recycling was mainly driven by plant P demand and sustained by root litter inputs in the grassland and leaf litter inputs in the forest.

Because of the close connection between C and N, and C and P, indices such as the C:N and C:P ratios in the terrestrial systems are often used to indicate the fertility of the soil and changes in the index can indicate differences in the rates of litter decomposition (Van Minnen, 2008). The C/N and C/P ratio of the organic matter largely determines whether N and P are mineralized or immobilized within the system (Johansson, 1994; Berg and McClaugherty, 2008; Brady and Weil, 2008). The low C/N and C/P ratios of CF1 stands stimulated decomposition and could be a possible reason for the greater concentration of nutrients in this stands than the recovering RF5 and RF10 stands and the old growth undisturbed forest stands. Similarly, in case of grassland ecosystem the lower C/N ratio in the mining affected MG sites stimulated decomposition and possibly responsible for the greater nitrogen concentrations in comparison to the recovering and undisturbed grassland sites.

Effect of recovery process on vegetation characteristics and species composition

Species richness and diversity varied along the recovery ages. With disturbance, species richness and diversity of tree species declined in forest ecosystem as well in grassland ecosystem. However, shrub and herb species richness and diversity increased following disturbance. The CF1 stands i.e. immediately after disturbance, had minimum tree species richness and diversity. This finding was in conformity with Rao *et al.* (1990), Ramirez–Marcial *et al.* (2001), Bhuyan *et al.* (2003) and Mishra *et al.* (2004) who found that species diversity and richness markedly declined from undisturbed to the highly disturbed stands. Disturbance regimes often increase the invasibility of herbaceous vegetation (Hobbs and Huenneke, 1992). The higher richness and diversity of the herbaceous species in the CF1 stands may be attributed to this reason and is often argued to be the result of availability of higher light following disturbance. This result is in conformity with Armesto and Pickett (1985) and Lalfakawma *et al.* (2009) who reported that disturbances have positive impact in maintaining species richness in herbaceous communities. Swindel *et al.* (1983) also observed an increase in the abundance of herbaceous species and diversity after clear–cutting in a forest in the southeastern U.S. The highest density of trees in RF10 stands may be attributed to the presence of large number of stems of low girth class. Grove and Malajczuk (1985) reported that naturally regenerated stands are usually dense during initial stage. As the stands develop into a matured forest, stem density reduces through natural self–thinning (O'Connell, 1985) and thus, the basal area increases.

In most plant communities, regardless of their species richness, the community consists of relatively few dominant species and a large number of other species with low abundance value. However, the latter species are crucial for maintenance of diversity (Leps, 2005). Dominance–diversity curves showed two types of trend which differed in their shape and length. First type extended and moved away from the origin of the graph i.e. the wider curve which was exhibited by the undisturbed grassland sites and tree species in the undisturbed forest stands. This type of curve is due to the increased diversity and even distribution of the species, and implies stability of the community. The second type was a steep curve that converged with the diversity axis in the middle portion. This pattern was exhibited by the most recently disturbed stands, where the curve was characterized by high dominance of a single species i.e. *Castanopsis purpurella* resulting in steep slope of the curve, and only a few species in the community that yielded a short curve. This is in agreement with the findings of other workers (Mishra *et al.*, 2004). However the pattern was reverse for shrub and herb components. The curves exhibited a shift from the second type towards the first type as recovery progressed. Colonization of more species as recovery progressed, thus increasing the diversity indices, was responsible for more equitable distribution and lower dominance in the recovering stands (Connell and Slatyer, 1977). The pattern obtained for plant communities in the grassland ecosystem was similar with the tree component of the forest ecosystem i.e. low equitable and high dominance for disturbed stands, and high equitable and low dominance for the recovering and undisturbed stands.

In general, vegetation characteristics in terms of tree diversity and density increased with recovery age. However, herbs and shrub species richness and density decreased with forest recovery. In grassland the trend was similar with that of tree species of forest ecosystem. As recovery progressed, more equitability distribution was achieved for all the vegetation components in both the ecosystems. Disturbance affected the soil physical properties as indicated by the higher bulk density, water holding capacity and reduced porosity of the soil. However as recovery started, porosity of the soil increased, and WHC and clay contents decreased with recovery age in both forest and grassland ecosystems. In forest ecosystem, all the nutrients declined initially till 5th year of recovery, after which the nutrient levels started to recover as indicated from the values of the stand representing 10th year of recovery. Although, the net recovery action starts after 5th year of recovery but it did not reach the undisturbed condition even after the 10th year of recovery. This suggested that complete recovery of soil nutrients in broad leaved evergreen forests of Cherrapunjee plateau subjected to deforestation would take much more than 10 years. However, in case of grassland ecosystem, nutrient build up was not found as recovery progressed. Most soil chemical characteristics showed a declining trend with recovery age. This result suggested a long term adverse impact of mining related activities on the grassland ecosystem of this region. A further study including more ecosystem attributes might shed light on the factors responsible for the failure of grassland ecosystem to recover in terms of nutrient build up.

CHAPTER 5

ROLE OF SOIL MICROORGANISMS IN CARBON DYNAMICS DURING ECOSYSTEM RECOVERY

INTRODUCTION

The terrestrial ecosystems are an important component of global C cycle and contain about 2100 Gt of C globally (Schulze, 2006). Soil, a major reservoir for C in terrestrial ecosystems, stores over two–third of the terrestrial C (Jobbagy and Jackson, 2000; Amundson, 2001) which is more than twice that vegetation or atmosphere can store (Schlesinger, 1997). Soil organic carbon (SOC), a function of the amount and quality of the C input, is a major and important pool of soil C pool. SOC is a large reservoir that can act as a sink (Lal, 2004), or source for atmospheric CO_2 (Lugo and Brown, 1993) depending on the ecosystem categories viz. forests, grasslands, arable land, and the type of management they are subjected to (Bodlak *et al.*, 2012). Soils of tropical and subtropical ecosystems account for almost 30% of total global soil organic matter (Dalal and Carter, 2000) and thereby make considerable contribution to the global C cycle. Therefore, a relatively small change in the soil C pool can have a large effect on the global C cycle.

Landuse changes substantially alter the soil carbon pool, and impact the quality as well as the carbon sequestration potential of soil (Davis and Condron, 2002; Rudel *et al.*, 2005; Richards *et al.*, 2007). Impact of land use changes on soil system therefore, has been a topic of intensive study during the past two decades. Fearnside (2000) and Mayaux *et al.* (2005) estimated that ongoing deforestation is causing a dramatic reduction of SOC storage capacity of around 212 Mt C year^{-1}. The variation in soil temperature and moisture regimes due to land use changes strongly affects soil C storage as the soil microbial communities and their activities *get al*tered (Post and Kwon, 2000; Guo and Gifford, 2002; Balser and Firestone, 2005; Waldrop and Firestone, 2006). The change in plant composition associated with the land use changes also impacts the rate of carbon storage which is a function of plant cover (Thoms *et al.*, 2010; Weand *et al.*, 2010) and litter quality (De Deyn *et al.*, 2008; Sun *et al.*, 2011). The long–term patterns and dynamics of C are therefore, likely to differ significantly among different types of ecosystems as well as with recovery age.

Studies on recovery of degraded ecosystems were previously focused primarily on soil physical and chemical properties (Froehlich *et al.*, 1985; Kaye and Hart, 1998; Johnston and Crossley, 2002). However, recent research works emphasised more on soil microbial populations (Izquierdo *et al.*, 2005; Jiang *et al.*, 2009) than soil properties as the former play a key role in ecosystem functioning by driving such crucial processes as decomposition of organic materials and nutrient cycling. There are increasing evidences on links between microbial community functions and C cycling (Melillo *et al.*, 2002; Fontaine *et al.*, 2003; Schimel *et al.*, 2007; Paterson *et al.*, 2011; Zhang *et al.*, 2011). Microorganisms play key roles in the decomposition of organic matter, a key process in the cycling of carbon, and the formation and stabilization of soil structure (Loranger–Merciris *et al.*, 2006). Fontaine *et al.* (2003) suggested that the dynamics of SOM are controlled by competition for energy and nutrients between the microbial groups i.e. r–strategists (organisms capable of rapid growth in the presence of labile C) and the specialist K–strategists (slow–growing organisms capable of mineralisation of recalcitrant SOM).

A number of soil microbiological parameters, such as enzyme activities (Izquierdo *et al.*, 2005), and microbial biomass carbon and basal respiration (Doran and Parkin, 1994; Sparling, 1997) have been employed as possible indicators of recovery of C cycle in degraded ecosystems. Soil enzymes related to C dynamics such as β–glucosidase, N–acetylglucosamine, are considered very sensitive to soil disturbances due to their close relationship with microbial biomass, functions and nutrient cycling. β–glucosidase, which releases glucose, provides energy to soil microorganisms and is directly related to soil organic C content (Bandick and Dick, 1999). It is widely abundant, and is synthesized by soil microorganisms in response to the presence of suitable substrates (Turner *et al.*, 2002). β–glucosidase activity can detect changes in soil within relatively short period of time and is relatively stable with seasons (Bandick and Dick, 1999). As soil dehydrogenase plays an essential role in the initial stages of the oxidation of soil organic matter by transferring hydrogen and electrons from substrates to acceptors, it has been widely used to measure total metabolic activity of soil microorganisms (Ross, 1971).

Soil microbial biomass which is an important source of nutrients for plants is frequently correlated with the organic matter content of soils (Pankhurst *et al.*, 1995). Several other researchers have reported a close relationship between soil fertility and microbial biomass (Insam *et al.*, 1991; Brookes, 1995). Basal respiration, measured as carbon dioxide evolution, the major product of aerobic catabolic processes in the C cycle, serves as an indicator of total C turnover. The ratio of basal respiration to microbial biomass i.e. metabolic quotient (qCO_2) is inversely related to the efficiency with which the microbial biomass uses the indigenous substrates (Anderson and Domsch, 1990). Increase in qCO_2 is considered to be an indicator of ecosystem disturbance (Insam and Haselwandter, 1989; Wardle, 1993; Fritze *et al.*, 1997). In the early stages of ecological succession the rate of primary production (P) exceeds the rate of community respiration (R), so that the P/R ratio is greater than 1. As long as P exceeds R, organic matter and biomass (B) will accumulate in the system, resulting in decrease of the ratio P/B and also R/B (Odum 1971). In an analogy to this, Insam and Haselwandter (1989) hypothesised that if total respiration (R) and total biomass (B) in Odum's model are replaced by soil microbial respiration (R_{mic}) and soil microbial biomass (B_{mic}), then the ratio R_{mic}/B_{mic} should also decrease like R/B with time or succession. They used qCO2 as an indicator to monitor ecosystem recovery and observed an increased value during the first two years of recovery followed by a continuous decrease thereafter. Thus, responses of microbial community to disturbance and related processes during recovery period are important parameters to monitor C dynamics in an ecosystem undergoing recovery.

Although a lot of studies have been conducted on the effects of landuse change on soil C dynamics (Ohta *et al.*, 2000; Woomer *et al.*, 2000; Islam *et al.*, 2001; Desjardins *et al.*, 2004; Paul *et al.*, 2008; van der Kamp *et al.*, 2009), studies focussing on contribution of microbial community and its processes to C dynamics during natural recovery of an ecosystem are rare. Therefore, studies on how soil organic carbon and the associated microbial properties relate to the recovery process can give great insights into the understanding of C dynamics in an ecosystem undergoing recovery and help predicting long–term C dynamics. In this chapter, various soil and microbial parameters associated with C dynamics have been investigated. An attempt has been made to identify the potential parameters including the enzyme activities that can act as indicators of ecosystem recovery. Some of the parameters studied are: soil organic matter, qCO_2, microbial biomass, soil biological activities and processes. Some of the hypotheses tested in this chapter are: (1) enzyme activities related to C cycle increase with recovery age, (2) Microbial biomass and activities, and soil biological processes are affected by soil physico–chemical properties during ecosystem recovery.

METHODS

Soil sampling and processing: Methods for soil sampling and processing have been given in detail in chapter 4.

Soil Chemical and Biological analyses

Soil Organic Carbon and Soil Organic Matter: The method for soil organic carbon has been described in detail in chapter 4. Soil organic matter content (SOM) was obtained by multiplying the soil organic carbon content by 1.724, assuming that the SOM contains 58% of carbon.

Soil Microbial Biomass Carbon: Microbial biomass carbon (MBC) was determined by chloroform fumigation extraction method (Vance *et al.*, 1987). Six sub–samples of 10 ± 0.01 g each of fresh sieved (2 mm mesh) soil were drawn from each composite sample, three of them were fumigated by saturating with 10 ml (alcohol–free) chloroform liquid and kept for 24 hours and the remaining three were not fumigated. After fumigation, chloroform was removed from the samples by evaporation. Organic carbon (C) was extracted from both fumigated and non–fumigated samples with 50 ml of 0.5 M K_2SO_4 by shaking at 100 rpm on a shaker for 30 minutes. The extracts were filtered through Whatman filter paper no. 42 and the filtrates were used for the determination of microbial biomass carbon. The organic C in the extracts of fumigated and non–fumigated soil samples was estimated by digesting 4 ml filtered extract with 1 ml of 0.0667 M $K_2Cr_2O_7$ and 5 ml of H_2SO_4 for 30 minutes. The digested sample was titrated with acidified ferrous ammonium sulphate solution using 0.3 ml (3–4 drops) of indicator (o–phenanthroline monohydrate and ferrous sulphate hexahydrate). The MBC was calculated as follows:

$$\mathrm{MBC} = \mathrm{E_C}/K_{\mathrm{EC}}$$

where, $\mathrm{E_C}$ is the difference in extractable C content between fumigated and non–fumigated samples, both expressed as $\mu g\ g^{-1}$ dry soil, and K_{EC} (= 0.38) is the factor used to account for the efficiency of extraction for MBC.

Basal Respiration and metabolic quotient: For measuring basal respiration (BR) 20 g of moist sample were weighed in a vessel and then placed inside a 1 litre jar. A beaker containing 5 ml of 0.5 N NaOH was placed inside the jar along with the soil. The jars were sealed and incubated at 25^0C for 7 days. After 7 days, the CO_2 trapped was determined by titration of the excess NaOH with 0.1 N HCl (Alef, 1995). Basal respiration was calculated using the following formula:

$$\text{Basal respiration (mg } CO_2\ kg^{-1} \text{ soil } d^{-1}) = (B - V) \times N \times E$$

where, B = volume of acid titrate without the sample; V = volume of acid titrate with soil sample;
N = normality of the acid; E = equivalent weight of CO_2

The metabolic quotient was calculated as BR per unit of MBC (Anderson and Domsch, 1993). The microbial quotient (MBC/SOC) was also calculated.

Enzyme activity: Soil dehydrogenase activity was measured by reducing 2, 3, 5,–triphenyl tetrazolium chloride (Casida *et al.*, 1964). 5 g of fresh sieved (2 mm mesh) soil sample was mixed with 50 mg $CaCO_3$ and 1 ml 3% (w/v) 2, 3, 5,–triphenyl tetrazolium chloride (TTC) and incubated for 2 hours at 37°C. The triphenylformazan (TPF) formed due to reduction of TTC by dehydrogenase was extracted with methanol (50 ml). The extracts were filtered through Whatman filter paper no. 1 and absorption was measured at 485 nm using Spectrophotometer (Lambda–35, UV/VIS, Perkin Elmer, USA). Dehydrogenase activity was calculated using the following formula:

$$\text{Dehydrogenase activity } (\mu\text{g TPF g}^{-1}\text{ dry soil 24 hr}^{-1}) = \frac{\text{C x 50}}{\text{W}}$$

where, C = Concentration of TPF (μg) obtained from standard graph; W = Dry weight of soil

β–glucosidase activity was measured following the method outlined by Tabatabai (1982). 2 ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml 50 mM p–nitrophenyl–β–D–glucopyranoside were added to 0.5 g fresh sieved (2 mm mesh) soil and the reaction mixture was incubated at 37°C for 1 hour. Then, 1 ml 0.5 M CaCl2 and 4 ml 0.5 M NaOH were added. After shaking, the mixture was filtered through Whatman filter paper no. 42 and the p–nitrophenol (PNP) in the filtrate was measured spectrophotometrically at 410 nm. β–glucosidase activity was calculated as follows:

$$\beta\text{–glucosidase activity } (\mu\text{g PNP g}^{-1}\text{ dry soil hr}^{-1}) = \frac{\text{C}}{\text{W}}$$

where, C = Concentration of PNP (μg) obtained from standard graph; W = Dry weight of soil

Statistical Analyses

Statistica version 7.0 (Statistical Software Inc. 2001) and origin version 7.0 (Origin Lab Corporation, USA) softwares were used to describe and analyse the data. Appropriate ANOVA models were used to test the variation in soil physico–chemical and biological properties among land uses, recovery ages, and seasons. The data were subjected to tests of normality, homogeneity of variance, and additivity using appropriate tests before undertaking ANOVA. Wherever necessary, the data were log or square root transformed. Multiple stepwise forward regression analysis was carried out to identify the most statistically significant variables of soil physicochemical properties that contribute to the functioning of various biological activities. The correlation between soil nutrients and the related parameters was calculated and tested for statistical significance. The minimum significance level for all statistical tests was at $p<0.05$ level.

RESULTS

Soil organic matter/Soil organic carbon: Soil organic matter (SOM) followed the same trend as soil organic carbon which was described in detail in chapter 4 (Fig. 4.1). In forest ecosystem, SOM was significantly (Tukey's test, $p<0.01$) greater in CF1 stands (7.9%) i.e. immediately after deforestation than the undisturbed stands (6.6%) (Fig. 5.1 and Table 5.3). Both reduced till 5th year of deforestation (RF5: 7.3%), after which SOM started to increase (RF10: 7.9%) indicating that the system recovered after 5th year of disturbance. In grassland ecosystem, due to coal dumping SOM was significantly (Tukey's test, $p<0.00$) greater in MG sites (8.5%) than the recovering RG15 (4.5%) and RG50 (3.9%) sites, and the undisturbed grassland sites (6.7%) (Fig. 5.1 and Table 5.4). As coal dumping was stopped and the recovery started, the SOM values decreased sharply. It varied significantly ($p<0.00$) among recovery ages in both forest and grassland ecosystem (Tables 5.1 and 5.2). SOM was significantly ($p<0.00$) higher in grassland ecosystem than forest ecosystem. It ranged between 6.6 – 7.9% in forest and 3.9 – 8.5% in grassland ecosystem. SOM varied significantly ($p<0.00$) among the seasons and was highest during rainy season and lowest during summer season in both the ecosystems (Fig. 5.1).

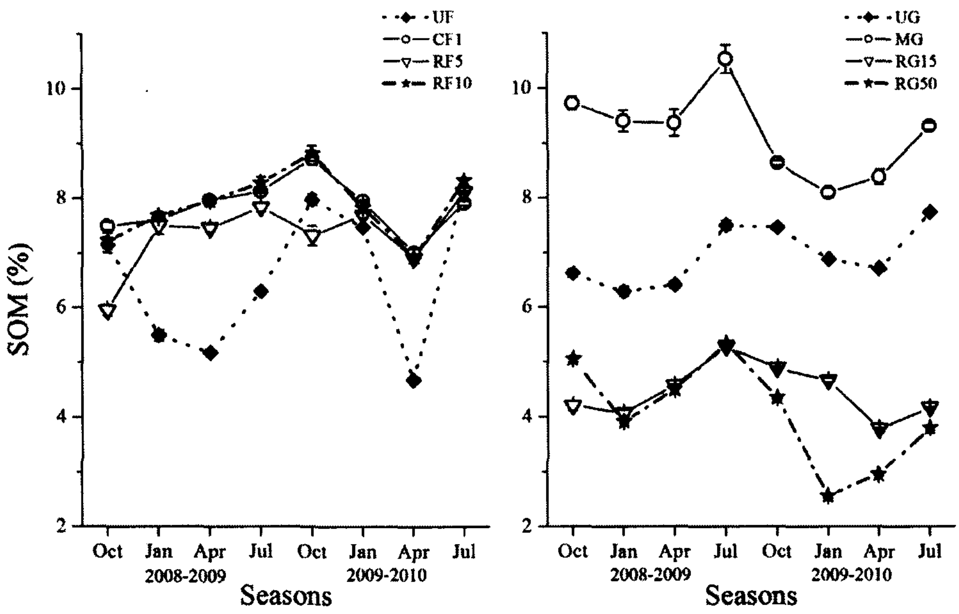

Fig. 5.1: Seasonal variation in soil organic matter in the undisturbed and recovering stands of forest and grassland ecosystems.

Microbial biomass carbon: Soil microbial biomass carbon (MBC) ranged between 324 μg g^{-1} and 663 μg g^{-1} in forest ecosystem and 206 μg g^{-1} and 482 μg g^{-1} in grassland ecosystem. In forest ecosystem, MBC was significantly (Tukey's test, $p<0.05$) lower in CF1 stands i.e. immediately after deforestation than the recovering RF5 and RF10 stands, and the undisturbed forest stands (Fig. 5.2 and Tables 5.1 and 5.3). It increased gradually as recovery progressed. In grassland ecosystem, MBC showed a similar trend as forest ecosystem with an increase as recovery progressed. Mining effected MG sites had significantly (Tukey's test, $p<0.01$) lower amount of MBC than the recovering RG15 and RG50 sites, and the undisturbed grassland sites (Fig. 5.2 and Tables 5.2 and 5.4). MBC was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. MBC varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with highest during winter and lowest during rainy (Fig. 5.2).

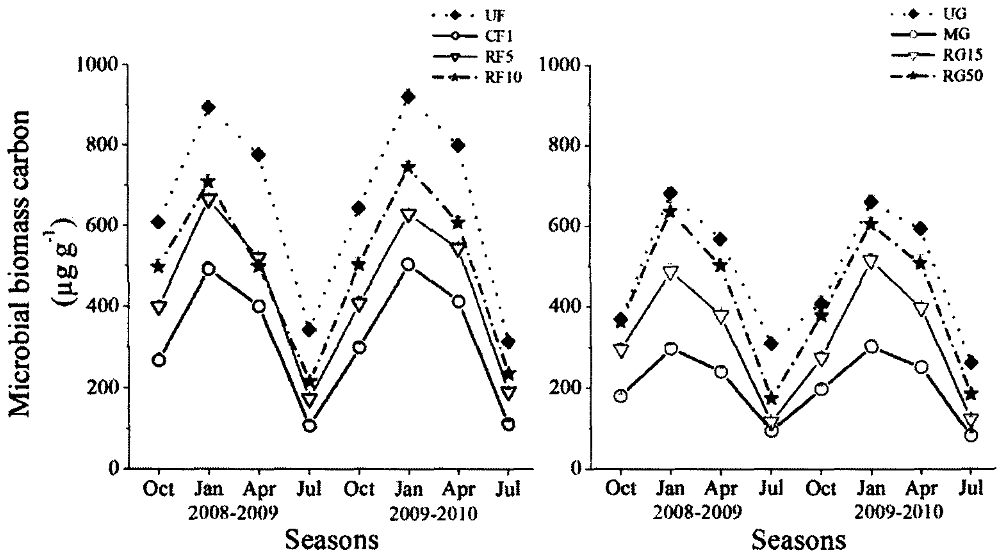

Fig. 5.2: Seasonal variation in microbial biomass carbon in the undisturbed and recovering stands of forest and grassland ecosystems.

MBC/SOC: In forest ecosystem, the MBC/SOC ratio ranged between 0.7 and 1.9% (Table 5.3). The percentage contribution of microbial biomass to soil organic carbon was significantly (Tukey's test, $p<0.05$) lower in CF1 stands than RF5, RF10, and UF stands. As recovery progressed, the contribution of microbial biomass to SOC significantly (Tukey's test, $p<0.05$) increased. In grassland ecosystem, the percentage contribution of microbial biomass to soil organic carbon showed the same trend as in forest ecosystem (Table 5.4). The ratio significantly (Tukey's test, $p<0.00$) increased with recovery age. Soil organic carbon had significantly (Tukey's test, $p<0.00$) lower contribution from MBC in MG sites than RG15, RG50 and UG grassland sites. The ratio ranged between 0.4% and 1.9%.

Basal respiration: In forest ecosystem, basal respiration was significantly (Tukey's test, $p<0.05$) lower in CF1 stands i.e. immediately after disturbance than the recovering RF10 stands and the undisturbed forest stands (Fig. 5.3 and Tables 5.1 and 5.3). Though the respiratory activity did not show any significant change from CF1 to RF5 stands, it increased gradually and significantly (Tukey's test, $p<0.05$) after the 5th year of recovery. In grassland ecosysytem, basal respiration was significantly (Tukey's test, $p<0.05$) lower in the mining affected MG sites than the recovering RG15 and RG50 sites, and the undisturbed grassland sites (Fig. 5.3 and Tables 5.2 and 5.4). It increased significantly (Tukey's test, $p<0.05$) as the coal dumping was stopped and recovery proceeded. Basal respiration was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. It varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystem with highest during rainy season and lowest during winter (Fig. 5.3).

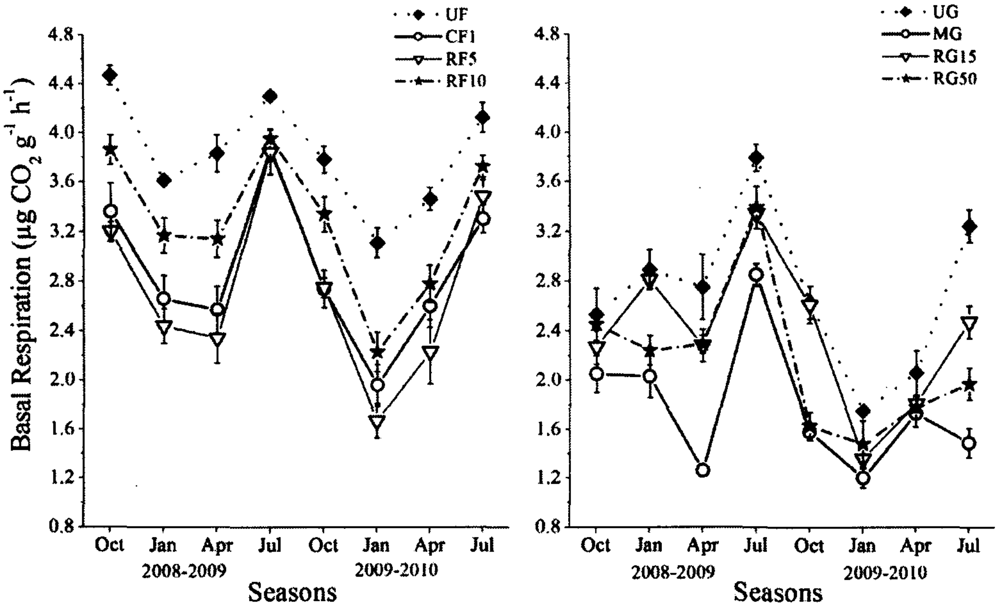

Fig. 5.3: Seasonal variation in basal respiration in the undisturbed and recovering stands of forest and grassland ecosystems.

Metabolic quotient: In forest ecosystem, the metabolic quotient was significantly (Tukey's test, $p<0.05$) higher in the CF1 stands than RF5, RF10 and UF stands (Table 5.3). As recovery proceeded the ratio decreased but it was not significant ($p>0.05$). Metabolic quotient followed a similar trend in grassland ecosystem (Table 5.4). It was significantly (Tukey's test, $p<0.05$) higher in the MG sites than RG50 and UG sites.

Table 5.1: Results of two–way ANOVA to test the effect of recovery age and season on soil chemical and microbiological measures in forest ecosystem.

Parameters	**df**	**Recovery Age**		**Season**	
		F	p	F	P
SOC (%)	3	44.2***	0.00	26.1***	0.00
SOM (%)	3	45.4***	0.00	27.4***	0.00
DHY ($\mu g\ g^{-1}$ soil h^{-1})	3	71.3***	0.00	399.2***	0.00
GLC ($\mu g\ g^{-1}$ soil h^{-1})	3	12.3	0.09	233.8***	0.00
BR ($\mu g\ g^{-1}$ soil h^{-1})	3	57.6***	0.00	72.8***	0.00
MBC ($\mu g\ g^{-1}$ soil)	3	667.4***	0.00	1422.6***	0.00
MBC/SOC (%)	3	376.5***	0.00	487.1***	0.00
$qCO_2^{\#}$	3	75.7***	0.00	512.5***	0.00

SOC – Soil Organic Carbon; SOM – Soil Organic Matter; DHY – Dehydrogenase activity; GLC – beta–Glucosidase; BR – Basal respiration; MBC – Microbial biomass carbon; MBC/SOC – Contribution of Microbial Biomass to Soil Organic Carbon; qCO_2 – Metabolic Quotient ($^{\#}$Unit – ng C–CO_2 mg C_{mic} g^{-1} h^{-1}); *** significant at $p<0.001$

Table 5.2: Results of two–way ANOVA to test the effect of recovery age and season on soil chemical and microbiological measures in grassland ecosystem.

Parameters	**df**	**Recovery Age**		**Season**	
		F	p	F	P
SOC (%)	3	184.1***	0.00	2.7*	0.05
SOM (%)	3	212.4***	0.00	3.8*	0.01
DHY ($\mu g\ g^{-1}$ soil h^{-1})	3	48.8***	0.00	118.4***	0.00
GLC ($\mu g\ g^{-1}$ soil h^{-1})	3	196.5***	0.00	82.5***	0.00
BR ($\mu g\ g^{-1}$ soil h^{-1})	3	22.0***	0.00	22.3***	0.00
MBC ($\mu g\ g^{-1}$ soil)	3	569.5***	0.00	930.2***	0.00
MBC/SOC (%)	3	325.3***	0.00	295.8***	0.00
$qCO_2^{\#}$	3	25.4***	0.00	203.9***	0.00

SOC – Soil Organic Carbon; SOM – Soil Organic Matter; DHY – Dehydrogenase activity; GLC – beta–Glucosidase; BR – Basal respiration; MBC – Microbial biomass carbon; MBC/SOC – Contribution of Microbial Biomass to Soil Organic Carbon; qCO_2 – Metabolic Quotient ($^{\#}$Unit – ng C–CO_2 mg C_{mic} g^{-1} h^{-1}); * and *** significant at $p<0.05$ and 0.001 respectively.

Dehydrogenase activity: In forest ecosystem, dehydrogenase (DHY) activity was significantly (Tukey's test, $p<0.05$) lower in CF1 than UF stands (Fig. 5.4 and Tables 5.1 and 5.3). In grassland ecosystem, the MG sites had significantly (Tukey's test, $p<0.05$) lower activity of DHY than RG50 and UG sites (Fig. 5.4 and Tables 5.2 and 5.4). DHY activity was significantly higher ($p<0.00$) in forest ecosystem than grassland ecosystem. DHY activity varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with its peak during rainy season and trough during winter (Fig. 5.4).

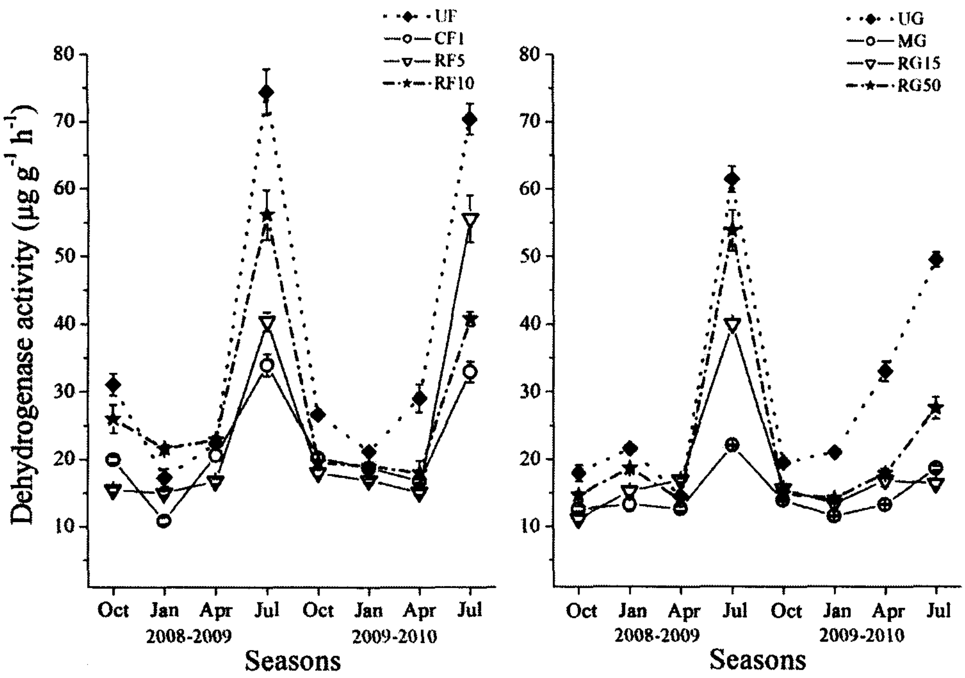

Fig. 5.4: Seasonal variation in dehydrogenase activity in the undisturbed and recovering stands of forest and grassland ecosystems.

β–Glucosiadase activity: In grassland ecosystem, β–Glucosiadase (GLC) activity was significantly (Tukey's test, $p<0.00$) lower in MG than RG15, RG50 and UG sites (Fig. 5.5 and Tables 5.2 and 5.4). It increased gradually and significantly (Tukey's test, $p<0.00$) as recovery followed. The activity was significantly higher ($p<0.00$) in forest ecosystem than grassland ecosystem. GLC activity also followed a similar seasonal trend as DHY activity with its peak during rainy season and trough during winter (Fig. 5.5).

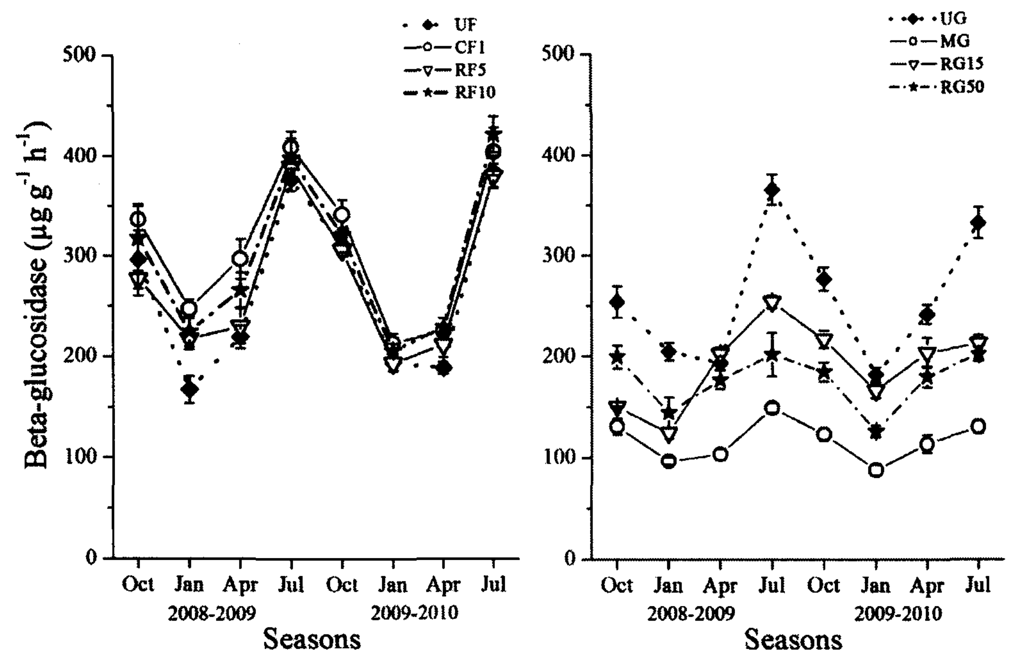

Fig. 5.5: Seasonal variation in beta–glucosidase in the undisturbed and recovering stands of forest and grassland ecosystems.

Table 5.3: Mean values (± standard error) of soil chemical and biological properties in the old growth undisturbed forests (UF) and recovering (CF1, RF5 and RF10) forest stands during the study period (values are the means of 4 samples each at 2 sites measured in 4 seasons over 2 years period, n=64).

	CF1	RF5	RF10	UF
SOC (%)	4.5±0.04^{bc}	4.3±0.05^{b}	4.6±0.05^{c}	3.8±0.04^{a}
SOM (%)	7.9±0.05^{b}	7.3±0.09^{c}	7.9±0.08^{b}	6.6±0.06^{a}
DHY (µg g^{-1} soil h^{-1})	21.8±0.74^{b}	24.2±0.86^{b}	28.1±1.24^{b}	36.6±1.45^{a}
GLC (µg g^{-1} soil h^{-1})	309.0±14.10	275.4±11.83	297.8±15.74	267.0±12.88
BR (µg g^{-1} soil h^{-1})	2.9±0.16^{b}	2.7±0.15^{b}	3.3±0.13^{c}	3.8±0.09^{a}
MBC (µg g^{-1} soil)	324.3±12.55^{b}	441.6±10.71^{c}	502.0±14.70^{c}	663.0±14.90^{a}
MBC/SOC (%)	0.7±0.03^{b}	1.1±0.03^{c}	1.1±0.03^{c}	1.9±0.05^{a}
qCO$_2$*	13.9±1.01^{b}	8.8±0.58^{a}	8.4±0.47^{a}	7.0±0.28^{a}

Mean values (mean ± s.e) sharing same letter within a row do not differ significantly by Tukey test at $p \leq 0.05$

SOC – Soil Organic Carbon; SOM – Soil Organic Matter; DHY – Dehydrogenase activity; GLC – beta Glucosidase activity; BR – Basal Respiration; MBC – Microbial Biomass Carbon; MBC/SOC – Contribution of Microbial Biomass to Soil Organic Carbon; qCO$_2$ – Metabolic Quotient (*Unit – ng C–CO$_2$ mg C$_{mic}$ g^{-1} h^{-1})

Table 5.4: Mean values (± standard error) of soil chemical and biological properties in the undisturbed (UG) and recovering (MG, RG15 and RG50) grassland sites during the study period (values are the means of 4 samples each at 2 sites measured in 4 seasons over 2 years period, n=64).

	MG	RG15	RG50	UG
SOC (%)	4.9 ± 0.07^{b}	2.6 ± 0.03^{c}	2.4 ± 0.03^{c}	3.9 ± 0.04^{a}
SOM (%)	8.5 ± 0.12^{b}	4.5 ± 0.04^{c}	3.9 ± 0.03^{d}	6.7 ± 0.05^{a}
DHY (μg g^{-1} soil h^{-1})	14.8 ± 0.33^{b}	18.2 ± 0.47^{bd}	22.0 ± 0.85^{cd}	29.9 ± 0.83^{a}
GLC (μg g^{-1} soil h^{-1})	116.7 ± 5.99^{b}	190.9 ± 7.35^{c}	176.6 ± 11.19^{c}	256.0 ± 11.11^{a}
BR (μg g^{-1} soil h^{-1})	1.8 ± 0.11^{b}	2.4 ± 0.10^{ac}	2.2 ± 0.12^{c}	2.7 ± 0.15^{a}
MBC (μg g^{-1} soil)	206.3 ± 9.51^{b}	324.1 ± 13.17^{c}	419.6 ± 14.80^{a}	482.3 ± 14.48^{a}
MBC/SOC (%)	0.4 ± 0.02^{b}	1.3 ± 0.06^{a}	1.9 ± 0.07^{c}	1.2 ± 0.04^{a}
qCO_2*	11.4 ± 0.85^{b}	10.7 ± 0.75^{ab}	6.9 ± 0.52^{a}	6.7 ± 0.51^{a}

Mean values (mean ± s.e) sharing same letter within a row do not differ significantly by Tukey test at $p \leq 0.05$

SOC – Soil Organic Carbon; SOM – Soil Organic Matter; DHY – Dehydrogenase activity; GLC – beta Glucosidase activity; BR – Basal Respiration; MBC – Microbial Biomass Carbon; MBC/SOC – Contribution of Microbial Biomass to Soil Organic Carbon; qCO_2 – Metabolic Quotient (*Unit – ng C–CO_2 mg C_{mic} g^{-1} h^{-1}).

Relating soil biological properties with physico–chemical characteristics: The regression models showing the relationship of soil physico–chemical properties and soil microbial properties associated with carbon dynamics were developed using forward stepwise regression analysis (Tables 5.5 and 5.6). In forest ecosystem, dehydrogenase activity was significantly ($p<0.00$) related with soil temperature, porosity, CEC and soil organic carbon while β–glucosidase activity had significant ($p<0.00$) relationship with soil temperature, moisture, soil organic matter and P_{Bray}. Basal respiration was significantly ($p<0.00$) influenced by soil temperature, water holding capacity, soil organic carbon and P_{Bray} while total P, soil temperature, moisture and soil organic matter significantly ($p<0.00$) affected microbial biomass carbon.

In grassland ecosystem, dehydrogenase activity was significantly ($p<0.00$) correlated with soil temperature, moisture, porosity, CEC, soil organic carbon, P_{Bray} and C/N while soil temperature, moisture, clay content, CEC, P_{Bray} and C/N significantly ($p<0.00$) influenced β–glucosidase activity. Basal respiration was significantly ($p<0.00$)

Table 5.5: Regression models based on forward stepwise regression analysis showing the relationship between soil biological properties and soil physical and chemical parameters across the old growth undisturbed forests (UF) and recovering (CF1, RF5 and RF10) forests (n=256). The independent variables of the equations were significant at $p<0.05$ (t–Test).

Dependent Variable (Y)	**Regression Equation**	**Adjusted R^2**	**P value**
Dehydrogenase	Y = – 156.1 + 1.1 x ST + 30.4 x SOC –70.1 x CEC + 1.2 x Porosity – 43.6 x SOM	0.45	<0.00
β–Glucosidase	Y = – 458.0 + 3.0 x SMC + 362.1 x P_{bray} + 153.6 x SOM – 0.3 x TP +5.2 x ST – 3.8 x P_W	0.58	<0.00
Basal Respiration	Y = 3.5+ 6.4 x P_{bray} – 4.1 x BD + 0.08 x ST + 1.5 x SOC – 0.06 x P_W – 2.1 x SOM – 0.002 x TP + 0.01 x WHC	0.64	<0.00
Microbial Biomass Carbon	Y = 1652.7 – 6.9 x SMC – 285.3 x SOM – 22.3 x ST – 14.3 x Silt + 0.4 x TP + 7.1 x P_W	0.79	<0.00

BD – bulk density; WHC – water holding capacity; ST – Soil Temperature; SMC – Soil Moisture Content; CEC – cation exchange capacity; SOC – Soil Organic Carbon; SOM – Soil Organic Matter; TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_W – Water extractable Phosphorus.

Table 5.6: Regression models based on forward stepwise regression analysis showing the relationship between soil biological properties and soil physical and chemical parameters across the undisturbed (UG) and recovering (MG, RG15 and RG50) grasslands (n=256). The independent variables of the equations were significant at $p<0.05$ (t–Test).

Dependent Variable (Y)	**Regression Equation**	**Adjusted R^2**	**P value**
Dehydrogenase	Y = 58.1 + 1.4 x SMC + 0.6 x Porosity – 0.7 x Sand – 97.9 x CEC – 255.2 x P_{Bray} + 3.5 x SOC – 0.1 x TP – 1.1 x P_W – 0.6 x C/N + 0.5 x ST	0.71	<0.00
β–Glucosidase	Y = 239.32 + 8.0 x Clay – 561.6 x CEC + 4.3 x SMC – 1085.5 x P_{Bray} + 5.0 x ST – 2.5 x C/N – 2.3 x Sand	0.74	<0.00
Basal Respiration	Y = 1.9 – 0.1 x P_W + 0.1 x SMC – 6.1 x CEC – 8.2 x P_{Bray} + 0.1 x Clay	0.54	<0.00
Microbial Biomass Carbon	Y = 476.3 – 13.5 x SMC + 13.5 x Silt + 51.1 x pH – 0.4 x C/P + 1378.1 x TKN	0.77	<0.00

ST – Soil Temperature; SMC – Soil Moisture Content; CEC – cation exchange capacity; pH – Soil pH; SOC – Soil Organic Carbon; TKN – Total Kjeldahl Nitrogen; TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_W – Water extractable Phosphorus; C/N – Carbon to Nitrogen ratio; C/P – Carbon to Total phosphorus ratio.

DISCUSSION

Anthropogenic disturbances including land use changes in an ecosystem modify the composition and total amount of soil organic matter, which have a direct effect on the biomass, population size and activities of microbial community (Reeves, 1997). Prolonged changes in microbial biomass and function eventually affect the processes of nutrient transformation that results in either accumulation or reduction of soil organic matter/carbon (Caldwell *et al.*, 1999). Soil organic carbon being a major pool of terrestrial carbon cycle, dynamics of organic carbon in soil, become a major determinant of carbon cycling, particularly during recovery process of ecosystems following perturbations. Microbial biomass, activities and processes, extra–cellular enzyme levels and their activities are important indicators used in the present study to characterize the soil carbon dynamics.

Soil microbial biomass C (MBC) is one of the most commonly analyzed parameters in various studies because it is both a source of labile nutrients and an agent of the transformation and cycling of organic matter in the soil (Sicardi *et al.*, 2004). The sharp decline of MBC after clear–cutting or coal dumping in forest and grassland ecosystems, respectively, is in agreement with the earlier researchers who reported similar reduction in MBC following disturbance (Henrot and Robertson, 1994; Chang *et al.*, 1995; Barbhuiya *et al.*, 2008; Smith *et al.*, 2008). This may be attributed to decrease in the microbial population size as well as lower input of organic matter due to reduced vegetation during post–disturbance period. As recovery progressed, greater accumulation of plant derived organic matter and microbial products (Maithani *et al.*, 1998) led to gradual increase in MBC. This observation was in agreement with Baer *et al.* (2002), McKinley and Wolek (2002) and Chodak *et al.* (2009). The amount of MBC obtained in this study is within the range reported by Kamei (2007) (258–922 $\mu g\ g^{-1}$) for a sub–tropical wet hill forest of north–eastern India, Garcia *et al.* (2002) (265–1426 $\mu g\ g^{-1}$) for land uses such as clear cuts and grazing in mediterranean central Spain, Tan *et al.* (2008) (200–550 $\mu g\ g^{-1}$) in north–eastern British Columbia and Li *et al.* (2010) (402–681 $\mu g\ g^{-1}$) for a Mangolian oak ecosystem in northeastern China.

MBC/SOC ratio describes the contribution of microbial biomass to soil organic carbon, and indicates the availability of labile C, or conversely the fraction of recalcitrant organic matter in the soil (Moscatelli *et al.*, 2005). As microbial populations are largely dependent on the soil labile C as their C source, high MBC/SOC ratio is indicative of soil labile C accumulation and favourable environment for microbial growth, and low ratio indicates poor quality of organic matter (Cheng *et al.*, 2013). In the present study, the low value obtained in the disturbed stands (CF1/MG) indicated reduced availability of labile nutrients to soil microbes and the prevalence of recalcitrant C compounds in the soil C pool. As recovery progressed, the ratio steadily increased and it was highest in the undisturbed stands. This was due to greater substrate availability in the recovering and undisturbed stands. Increased microbial activity either due to greater microbial population size or enhanced activity of the constituent microbial populations could also be the reasons for greater MBC/SOC ratio in the recovering/undisturbed stands. Sparling (1992) and Brookes (1995) argued that the ratio differs among different soils and is greatly influenced by the soil organic matter content. Soil microbial biomass carbon usually comprises 0.7–4% of soil organic C (Anderson and Domsch, 1989; Maithani *et al.*, 1996; Jiang *et al.*, 2009). Thus, the values reported in this study i.e. 0.4–1.9% were within the above range.

Enzyme activities and basal respiration are directly proportional to the size and activity of viable microbial populations. A decline in these functions after disturbance suggests that the viable microbial population either decreased in their size and/or in their functional abilities. Decline in the activities of dehydrogenase and β–glucosidase enzymes was also reported in Central Spain where plant cover declined following a land use change (Garcia *et al.*, 2002). Similar findings were reported by Izquierdo *et al.* (2005) during the recovery of soil quality of a mined area in subtropical Cuba. Dehydrogenase activity is used as a measure of general soil microbial activity (Gil–Sotres *et al.*, 2005; Tan *et al.*, 2008). As dehydrogenases are enzymes that exist only in living cells (Nannipieri *et al.*, 2002), a correlation between microbial biomass and dehydrogenase activity is quite obvious. Lowest dehydrogenase activity was recorded in the recently disturbed CF1 and mining affected MG stands. This may be attributed to the reduction in the microbial population size due to the disturbance. An increasing trend of dehydrogenase activity along recovery process indicated that the microbial populations were also undergoing recovery in terms of size (although relative efficiency of individual microbial population is equally important) and were approaching towards the pre–disturbance condition. Enzyme production is an energy intensive process and microorganisms do this at an expense of their growth and metabolism (Koch, 1985). Immediately following the disturbance, the microbial populations allocated most of their energy towards maintenance of a minimum viable population size rather than enzyme production. Therefore, there was a decline in β–glucosidase activity after disturbance. Soil texture is a major determinant of carbohydrases. For example, Stemmer *et al.* (1998) and Marx *et al.* (2005) concluded that β–glucosidase was more in the coarse sand than the silt and clay fractions. Therefore, in our study, difference in soil texture could be another explanation for the reduced β–glucosidase activity in CF1 and MG stands, as the recently disturbed sites had more clay content than the recovering and undisturbed sites. The greater activity of both the enzymes with recovery age might be due to the enhanced organic matter levels, greater substrate availability and increase in microbial population numbers. The increase in β–glucosidase activity with succession age was consistent with Baldrian *et al.* (2008) and Jiang *et al.* (2009).

The basal respiration (BR) gives an estimation of the activity of heterotrophic soil microbial community and provides information on availability of soil carbon to microorganisms (Ananyeva *et al.*, 2008). Alterations in microbial biomass after disturbance are associated with concurrent changes in microbial respiration (Holden and Treseder, 2013). Therefore, a reduction in microbial biomass might be responsible for the observed decline in BR after disturbance. Also the decrease in respiration was partly due to the reduction in substrate availability. This finding was in agreement with other studies that have measured microbial respiration following disturbance events and found decreases in microbial respiration during post–disturbance period (Czimczik *et al.*, 2006;

Yan *et al.*, 2009; Holden and Treseder, 2013). The subsequent increase in BR with recovery age as observed in this study was consistent with the findings of Baer *et al.* (2002), Chodak *et al.* (2009), Jiang *et al.* (2009), Yan *et al.* (2009) and Holden and Treseder (2013).

The microbial metabolic quotient (qCO_2) has been used as a bioindicator of environmental stress on microbial communities, disturbance and ecosystem development (Anderson and Domsch, 1993). When the soil system is stressed after disturbance, soil microbes need more energy for survival that results in higher qCO_2 (Wardle and Ghani, 1995). The availability of soil organic matter also plays a key role for obtaining higher qCO_2 (Joergensen and Castillo, 2001). Hence, greater qCO_2 in the post disturbance sites was due to increased availability of readily degradable C. This indicated that a relatively large percentage of the substrate is decomposed to meet the energy demand of the microflora (Joergensen and Scheu, 1999; Leiros *et al.*, 2000). As recovery progressed, the prevailing stress on soil microbes gradually subsided resulting in decrease in qCO_2, which was in agreement with Frouz and Novakova (2005) and Jiang *et al.* (2009).

The seasonal variability in soil microbial biomass C is a function of soil environmental factors and physico–chemical properties, and phenology of dominant vegetation components e.g. litter fall (Myers *et al.*, 2001). The lower MBC during rainy season than winter may be explained on the basis of difference in microbial growth and turnover rates during the two seasons. In the rainy season, plant roots provide labile organic carbon for soil microbes through root exudation. During this season, high soil temperature and moisture also favour microbial growth and activity. Therefore, microbes grow actively and have high turnover rates. This in turn, results in lower accumulation of labile organic carbon in their biomass in comparison to winter season. Similar observations were also made by Arunachalam and Arunachalam (2000), Barbhuiya *et al.* (2008).

The enzyme activities were also significantly affected by season. The enzyme activity was strongly dependent on the microbial enzyme production and/or release. The active growth of soil microbial populations during the rainy season due to the favourable conditions of soil moisture and temperature were also responsible for the high enzymatic activity. Garcia *et al.* (1994) found that rainy season enhanced the enzyme activity of soils in the south–east arid region of Spain. Other authors also attributed the increase in microbial activity in forest (Görres *et al.*, 1998; Quilchano and Marañón, 2002), grassland (Banerjee *et al.*, 2000) and in shrubland soils (Sardans *et al.*, 2008) to higher soil moisture contents. Basal respiration also followed a similar trend. The greater need for energy by the actively growing microbial population during rainy season resulted in higher respiratory activity as compared to other seasons. Similar pattern of seasonal variation was observed by Yan *et al.* (2009) in the subtropical forests of Dinghushan Biosphere Reserve and Song *et al.* (2013) in a tropical rainforest in the Xishuangbanna Natural Reserve, China.

Dehydrogenase activity was significantly correlated with soil temperature in both forest and grassland ecosystem. The role of soil temperature in regulating dehydrogenase activity was also reported by Brzezinska *et al.* (2000). Soil moisture content had a significant correlation with dehydrogenase activity only in case of grassland ecosystem. Quilchano and Marañón (2002) had similar finding. Positive correlation of soil water content with dehydrogenase activity was also reported by Nannipieri *et al.* (1990). SOC showed a significant positive relationship with dehydrogenase activity which was consistent with the findings of Leirós *et al.* (2000) and Okur *et al.* (2009). Since soil microbial population size is a function of dehydrogenase activity, it may be concluded that the soil microorganisms in both forest and grassland ecosystems are C–limited. β–glucosidase activity was also positively correlated with soil temperature and moisture in both the ecosystems. This was consistent with the findings of Dilly and Munch (1996) who also observed high β–glucosidase activity during optimum availability of water and high soil temperature. Sardans and Penuelas (2005) pointed out the critical role of water for microbial enzyme activity. They concluded that a 10% reduction in soil moisture was sufficient to reduce the activity of β–glucosidase. As expected, basal respiration showed significant correlation with soil moisture and temperature. This was in consistent with the results of Qiu *et al.* (2005). The significant correlation of basal respiration with SOC and Bray's extractable P in forest ecosystem was in agreement with the findings of Cheng *et al.* (2013). The significant negative correlation of soil temperature with MBC was in agreement with Maithani *et al.* (1996). MBC showed a significant positive correlation with total P and extractable P in forest ecosystem and with total N in grassland ecosystem. Cheng *et al.* (2013) had obtained a similar relationship of MBC with N and P. Liu *et al.* (2012) also reported a strong positive correlation between MBC and total N. Therefore, it may be concluded that the microbial populations are limited by P in forest ecosystem and N in grassland ecosystem.

Thus, soil microbes played a key role in the recovery of both the ecosystems by influencing the cycling of carbon in soil. Disturbances such as deforestation and mining related activities reduced the biomass and activity of soil microbial populations. Low basal respiration after disturbance indicated low microbial activity in the soil and suggested slow decomposition of organic matter. Greater metabolic quotient (qCO_2) during post–disturbance period indicated enhanced stress on microorganisms, low C utilization efficiency, and high energy demand for microbial biomass maintenance. All the microbial parameters including enzyme activities improved as recovery progressed. During the recovery period, irrespective of recovery ages, basal respiration and enzyme activity were highest during rainy season and lowest during winter. Microbial turnover rate was highest during rainy season and therefore, the microbial biomass carbon had an opposite seasonal trend than the other microbial parameters showing highest values during winter season.

The results of this chapter showed that soil organic carbon, basal respiration and microbial biomass carbon could be sensitive indicators of reduction of ecological stress and changes in carbon dynamics as recovery progresses both in forest and grassland ecosystem. Further, dehydrogenase activity and β–glucosidase activity were important indicators only in grassland ecosystem.

CHAPTER 6

PHOSPHORUS DYNAMICS IN DEGRADED ECOSYSTEMS AND ROLE OF PHOSPHORUS SOLUBILISING BACTERIA IN ECOSYSTEM RECOVERY

INTRODUCTION

Phosphorus (P), next to nitrogen, is a critical factor for plant growth due to its limited availability in soluble forms in soil. In subtropical and tropical ecosystems, soil P is one of the most important elements limiting plant growth (Cramer, 2010) as the primary production in these ecosystems is considered to be P limited rather than N–limited (Walker and Syers, 1976; Vitousek *et al.*, 2010). P that can be taken up by plants occurs in a very small fraction in the soil solution as orthophosphate anions, predominantly $H_2PO_4^-$ and HPO_4^{2-}. The low levels of P are due to leaching losses of P over time (Vitousek, 2004), adsorption by the soil matrix (Whitelaw, 2000), and precipitation by aluminium (Al^{3+}) and iron (Fe^{3+}) ions as recalcitrant complexes with low solubility (Vitousek and Sanford, 1986; Maguire *et al.*, 2001). A large proportion of P in soil is present in the organic forms, as much as 50% in soils with high organic matter content (Barber, 1984). However, most of it is rendered insoluble as a result of forming complexes with iron, aluminium and calcium (Alexander, 1977) while the remaining portion also needs to be mineralized to become available to plants (Stutter *et al.*, 2012).

The availability of P for plant growth depends on complex geochemical and biochemical processes (Walker and Syers, 1976). The fluctuation in P fractions, particularly soil P transformation process, is considered an important factor in controlling P availability and ultimately in influencing plant nutrition (Eviner *et al.*, 2006). Although the dynamics of P is governed primarily by the physico–chemical processes of sorption–desorption and precipitation–dissolution in most soils (Sample *et al.*, 1980), the biological processes of remineralization of immobilized P within the microbial biomass and mineralization of non–microbial organic P play very crucial role in replenishing phosphate ions in the soil solution (Frossard *et al.*, 2000).

P inputs and outputs may be affected by land use change. This in turn, would result in an altered P balance and changes in P forms (Ross *et al.*, 1999). Land use change affects soil P transformation by modifying soil physical, chemical and biological properties, especially soil microbial processes (Chen *et al.*, 2008). A clear understanding of the microbial processes of immobilization and remobilization/mineralization of P will improve our understanding of P dynamics during recovery stages as microbes have the ability to rapidly immobilize considerable amounts of P when labile C is readily available (Bünemann *et al.*, 2004). The microbial biomass phosphorous (MB–P) accounts for 2–10% of total soil phosphorous (Chen *et al.*, 2003b; Agbenin and Adeniyi, 2005). The rapid turnover of phosphorous in microbial biomass may contribute significantly to available P pool and is available for plant uptake. Also the microbial immobilization of inorganic P protects the phosphorous from physico–chemical fixation (Chauhan *et al.*, 1981; Oberson *et al.*, 2001).

Soil microorganisms play a key role in facilitating P transformations through mineralization of P from organic sources, synthesis and release of organic P, and solubilization of barely soluble P forms (Oberson *et al.*, 2001). Phosphatases, originating from microbes as well as root exudates, enhance P availability to ecosystems by catalyzing the hydrolysis of ester bonds between phosphate and carbon compounds in organic substrates (Turner and Haygarth, 2005). In P–poor soils both plants and microbes acquire P largely via the production of extracellular phosphatases and the subsequent mineralization of the organic P into bio–available inorganic P (Pi) forms (McGill and Cole, 1981; Dilly and Nanniperi, 2001; Olander and Vitousek, 2000). These enzymes are therefore thoroughly studied as they play important role in maintaining and controlling the rate of P cycling in an ecosystem, and their activity reflects the capacity of organic P mineralization potential in soils (Chen, 2003; Criquet *et al.*, 2004; Huang *et al.*, 2011). Among the phosphatases, acid phosphatase is predominant in acidic soils. Thus, a detailed knowledge of the vital factors affecting P cycling and transformation is important for understanding the dynamics of P availability and mineralization in soil.

Plants growing in P–limited soils have evolved a wide range of P acquisition strategies to overcome the persistent P–stress. For example, plants harbour a variety of microbes capable of converting the insoluble phosphatic compounds into soluble forms in soil and promote the proliferation of such microorganisms by increased rhizodeposition (release of C compounds from living plant roots into the surrounding soil) (Gyaneshwar *et al.*, 1998), form mycorrhizal association for P acquisition through mycorrhizal symbioses (Bolan, 1991; Richardson *et al.*, 2009). A large number of soil microbial species demonstrate the *in vitro* ability to solubilise inorganic P (Richardson *et al.*, 2009) and therefore, play a key role in the mobilisation of soil P into plant–available forms.

Phosphate solubilising microorganisms are ubiquitous, though their numbers vary from soil to soil. Several soil bacteria, as well as fungi, e.g. *Burkholderia, Bacillus, Pseudomonas, Streptomyces, Aspergillus, Paenibacillus* and *Trichoderma* spp., are known to dissolve inorganic phosphorus (Kucey *et al.*, 1989; Kim *et al.*, 2005; Vassilev *et al.*, 2006; Hamdali *et al.*, 2008; Kim *et al.*, 2008). Phosphate solubilising bacteria constitute 1 to 50% and fungi 0.1 to 0.5% of the total respective populations in soil (Chen *et al.*, 2006). Fungal isolates in general, exhibit greater P–solubilizing ability than bacteria in both liquid and solid media (Banik and Dey, 1982; Kucey, 1983). However in soil, PSB generally out–number P solubilizing fungi by 2–150 fold (Banik and Dey, 1982; Kucey, 1983; Kucey *et al.*, 1989).

PSB have proved to be economically sound alternative to the more expensive superphosphates and promises a greater agronomic utility (Khan *et al.*, 2007; Xiao *et al.*, 2009). Therefore, several studies have focussed on studies relating to the inoculation of

PSB into the soil to increase the availability of P to plants (Sundara *et al.*, 2002; Dey *et al.*, 2004; Gupta *et al.*, 2012). However, the establishment and performance of PSB is largely hampered by environmental variables and the inocula developed from a particular soil fail to function as efficiently in soils having different properties (Rodríguez and Fraga, 1999). Therefore, knowledge of the PSB population native to a particular region and their solubilization efficiencies would be valuable for addressing the P–deficiency.

Changes in microbial community composition and functioning modify ecosystem processes and functions (Gallo *et al.*, 2004; Compant *et al.*, 2010). Since the association of microbial communities with P cycling is well known (Chen *et al.*, 2003a; Chen *et al.*, 2008; Bünemann *et al.*, 2012), any alteration to microbial community composition due to perturbations would affect P–dynamics both during the disturbance period (Steenwerth *et al.*, 2005; Ratcliff *et al.*, 2006) as well as during the subsequent recovery phase (Zornoza *et al.*, 2009; Andersen *et al.*, 2010). However, studies on P dynamics during ecosystem recovery are scarce (Huang *et al.*, 2013). This is particularly true for mining affected landscapes, as no earlier studies are available.

In the humid subtropical regions of north–east India where the soils are highly weathered and P– limited, the present study was undertaken with an objective to:

1. Understand P–dynamics in degraded terrestrial ecosystems, viz., grassland and forest.
2. Study P –dynamics during different stages of ecosystem recovery.
3. PSB colonization during different stages of recovery.
4. Evaluate relative efficiency of isolated PSBs at different stages of recovery.

METHODS

Phosphorus dynamics was studied through estimation of total and available P content in soil, microbial biomass P, P mineralization rate and phosphatase activity. PSB colonization was studied by isolation and identification of PSB from various recovery stages and their relative efficiency was evaluated through plate assay, *in vitro* studies in liquid NBRIP medium and crop application.

Soil sampling and processing: Methods for soil sampling and processing have been given in detail in chapter 4.

P dynamics

Total and Available Phosphorus: The method for total phosphorus (P) has been described in detail in chapter 4. Available P was estimated as Bray and Kurtz P–1 extractable P and water extractable P (P_w). For determination of Bray and Kurtz P–1 extractable P (Sims, 2000), 2 g air dried, sieved (0.2 mm mesh) soil was subjected to extraction with 20 ml of Bray and Kurtz P–1 extracting solution (0.025 M HCl in 0.03 M NH_4F) by shaking at 200 rpm on a shaker for 30 minutes at room temperature (24–27 °C). The suspension was filtered through Whatman filter paper no. 42 and inorganic orthophosphate (PO_4^{3-}) in the filtrate was measured using ammonium–molybdate blue method (Allen *et al*., 1974). Five ml aliquot was taken and mixed with 2 ml of ammonium molybdate solution and 2 ml of stannous chloride reagent. The solution is mixed well and made upto 50 ml by adding distilled water. One reagent blank and standard solutions were prepared in similar way without adding soil samples. Transmittance was recorded at 700 nm after 30 minutes using spectrophotometer (Lambda–35, UV/VIS, Perkin Elmer, USA). Bray and Kurtz P–1 extractable P was calculated as follows:

$$\text{Bray and Kurtz P–1 extractable P (mg P kg}^{-1}\text{ soil)} = \frac{\text{C x 0.02}}{0.002}$$

where, C = Concentration of P (mg) obtained from standard graph

P_w was extracted using the method of Zhou *et al.* (2001). One gram of air dried, sieved (0.2 mm mesh) soil in 25 ml of distilled water was agitated at 150 rpm for 2 hours at 25 °C. The mixture was filtered and PO_4^{3-} was determined as mentioned above. The inorganic orthophosphate was calculated using the formula as follows:

$$P\ (\%) = \frac{\text{C x Solution volume}}{\text{10 x Aliquot x Sample weight}}$$

where, C = Concentration of P (mg) obtained from standard graph

Soil Microbial Biomass Phosphorus: Microbial biomass phosphorus (MBP) was determined by chloroform fumigation extraction method (Brookes *et al.*, 1982). Six sub–samples of 10 ± 0.01 g each of fresh sieved (2 mm mesh) soil were drawn from each composite sample, three of them were fumigated by saturating with 10 ml (alcohol–free) chloroform liquid and kept for 24 hours and the remaining three were not fumigated. After fumigation, chloroform was removed from the samples by evaporation. Inorganic orthophosphate (PO_4^{3-}) was extracted from both fumigated and non–fumigated samples with 50 ml of 0.5 M $NaHCO_3$ by shaking at 100 rpm on a shaker for 30 minutes. The extracts were filtered through Whatman filter paper no. 42 and the filtrates were analysed for PO_4^{3-} using ammonium–molybdate blue method (Allen *et al.*, 1974). The MBP was calculated using the following formula:

$$MBP = E_P/K_{EP}$$

where, E_P is the difference in extractable P content between fumigated and non–fumigated samples, both expressed as μg g^{-1} dry soil, and K_{EP} (= 0.40) is the factor used to account for the efficiency of extraction for MBP.

Phosphorus mineralization: P mineralization was studied in situ by buried bags technique (Eno, 1960) on monthly basis for two years. At each sampling, paired soil cores were collected from the upper soil layer (0–10 cm). One of the cores was sealed in sterilized polyethylene bags after removing roots and larger organic debris, and reinserted in soil at 0–10 cm depth. The other pair was brought to laboratory and sieved through a 2 mm mesh sieve. Initial moisture content and PO_4^{3-} concentration were determined within 24 hours of sample collection following the method outlined in Allen *et al.* (1974). After one month, the buried bags were retrieved from each plot and soil samples were analysed for final moisture content and PO_4^{3-} concentration. The increase in the concentration of PO_4^{3-} during the field exposure is referred to as P mineralization. Values of one month per season viz. October for autumn, January for winter, April for spring and July for rainy were used for plotting the graph.

Enzyme activity: Phosphatase activity was determined according to Tabatabai (1982). 4 ml of 0.5 M sodium acetate buffer (pH 5.5) and 1 ml of 115mM p–nitrophenyl phosphate were added to 1 g fresh sieved (2 mm mesh) soil and the reaction mixture was incubated at 37 °C for 1 hour. Then, 1 ml 0.5 M CaC12 and 4 ml 0.5 M NaOH were added. After shaking, the mixture was filtered through Whatman filter paper no. 42 and the p–nitrophenol (PNP) in the filtrate was measured spectrophotometrically at 410 nm. Phosphatase activity was calculated as follows:

$$\text{Phosphatase activity } (\mu\text{g PNP g}^{-1} \text{ dry soil hr}^{-1}) = \frac{C}{W}$$

where, C = Concentration of PNP (μg) obtained from standard graph
W = Dry weight of soil

Isolation of phosphate solubilising bacteria

The PSB populations were isolated using National Botanical Research Institute's phosphate growth medium "NBRIP–BPB" (Nautiyal, 1999), a selective medium containing (g l^{-1}) glucose (10), $Ca_3(PO_4)_2$ (5), $MgCl_2.6H_2O$ (5), $MgSO_4.7H_2O$ (0.25), KCl (0.2), $(NH_4)_2SO_4$ (0.1), bromophenol blue (0.025) and adjusted to pH 7.0 before autoclaving. $Ca_3(PO_4)_2$ (TCP) was the sole P source for selectively screening the bacteria which have the ability to release inorganic phosphate from TCP. Bromophenol blue (BPB) is a blue coloured dye that decolorizes owing to drop in pH of the medium. Each soil sample was homogenously suspended in sterile saline solution (0.85% sodium chloride) for bacterial isolation. A series of 10–fold dilutions were prepared and 100 μl aliquot of 10^2 diluted samples were spread on NBRIP–BPB agar plates. The plates were incubated at 30^0C for 3 days. To avoid fungal contamination amphotericin B at a final concentration of 2 μg ml^{-1} was added to the medium before plating. The reliability of the halo based technique is often questioned as many isolates that did not produce any visible halo on agar plates could solubilise various types of insoluble inorganic phosphates in liquid medium (Louw and Webley, 1959; Gupta *et al.*, 1994). Therefore, morphologically distinct colonies growing on the selective medium and decolorizing the medium were picked, purified and grown repeatedly on that selective medium to ensure that they are capable of solubilising TCP.

Identification of PSB

The selected PSB isolates were identified through morphological, phenotypic and biochemical characterization as well as using 16s rDNA sequence.

Morphological, Phenotypic and Biochemical characterization

The growth, morphological, phenotypic and biochemical characteristics and relationship to oxygen were studied following the methods detailed in Aneja (2003). Phenotypic characteristics were studied by staining behaviour (Gram staining, negative staining, capsule staining). Physiological and metabolic characteristics were determined by performing a series of biochemical tests.

DNA extraction

DNA was extracted from the pure cultures of PSB isolates following the phenol/chloroform/isoamyl alcohol method (Sambrook *et al.*, 1989) with minor modification. Briefly, 50 ml of freshly grown overnight culture was centrifuged at 7000 rpm for 10 min. The pellet was resuspended in 5 ml of 25% sucrose Tris HCl (50 mM, pH 8.0) solution and 2ml EDTA (0.25 mM, pH 8.0), and mixed gently to obtain a homogeneous suspension. The suspension was kept on ice for 15 min. This was followed by addition of 1 ml freshly prepared lysozyme solution (10mg/ml) and incubated at 37°C for 60 min. To this 1 ml of SDS (20% w/v) solution was added, mixed gently and then incubated at 65°C for 30 min. This was followed by RNase (20µl, 5mg/ml) treatment and incubation at 37°C for 20 min. Equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was then added to the samples and vortexed gently. This was subjected to centrifugation at 12000 rpm for 25 min at 4°C and the upper aqueous phase was aspirated out. To this an equal volume of chloroform/isoamyl alcohol (24:1) was added, mixed by gentle vortexing and centrifuged at 12000 rpm for 20 min at 4°C. This step was repeated twice. The clear aqueous phase was precipitated using double volume of chilled ethanol and 3 M sodium acetate (1%) at pH 5.0. Samples were left overnight at –20°C (Vestfrost deep freezer, Blue Star, India) to allow DNA precipitation. Precipitated DNA pellet was collected by centrifugation at 12000 rpm for 20 min at 4°C. Pellet was washed with 70% ethanol twice and air dried followed by suspension in 100 µl of TE buffer (Tris–HCl 10 mM l^{-1}, EDTA 1 mM l^{-1}, pH 8.0). The DNA sample so prepared was qualitatively checked on 0.8% agarose gel and stored at –20°C for further work.

PCR amplification of 16S rRNA gene

Amplification of *16S rRNA* gene was performed from the genomic DNA of the isolates using universal primers 27F (5'–AGAGTTTGATCCTGGCTCAG–3') and 1492R (5'–GGTTACCTT GTTACGACTT–3') (Espejo *et al.*, 1998), commercially synthesized from Sigma Chemical Co., USA. The PCR amplification was run in an ABI 9700 thermal cycler (Applied Biosystems, Singapore). The reaction mixture (30 µl) contained 1 µl (200 ng) of template DNA solution, 60 nM of primer, 1X *Taq* DNA polymerase buffer, 3 U of *Taq* DNA polymerase (Merck), 0.3 mM of each dNTP, and 1.5 mM $MgCl_2$. The template was substituted with sterile milliQ water in the negative control sample. Thermal cycler profile was as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles each consisting of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C and a final extension of 15 min at 72°C. Amplification of PCR product of the proper size was confirmed by electrophoresis through a 1.5% agarose gel in 0.5× TBE buffer at 6 V/cm for 30 min, stained with ethidium bromide and visualised with Gel Doc EZ Imager (Biorad, Biorad Laboratories India Pvt. Ltd.).

Sequencing of 16S rDNA and phylogenetic analysis

The PCR products were lyophilized (Scanvac coolsafe vacuum concentrator, Labogene, Denmark) and subjected to purification and sequencing (Macrogen Inc., Korea). The sequences obtained were manually corrected by deleting the unambiguous nucleotides from 3' and 5' ends. The sequences were then screened against those in the NCBI GenBank database using the nucleotide BLAST program. To perform molecular phylogenetic analyses, reference 16S rRNA gene sequences required for comparison were downloaded from the database. All the sequences were aligned using multiple sequence alignment program Clustal W (Larkin *et al.*, 2007). The aligned sequences were then checked for gaps manually and saved as molecular evolutionary genetics analysis (MEGA) format in software MEGA version 5.05. The pair wise evolutionary distances were computed using the Kimura 2–parameter model (Kimura, 1980). Bootstrap confidence values were obtained with 100 resamplings. The multiple distance matrix obtained from the bootstrapped data set was then used to construct phylogenetic trees by the unweighted pair–group method using arithmetic averages (UPGMA) and neighbour–joining (NJ) method (Saitou and Nei, 1987) using MEGA 5.05 package (Tamura *et al.*, 2011).

Determination of phosphate solubilising efficiency

Phosphate solubilising activities of each isolate was assayed by spotting a small portion of a freshly grown colony of each isolate using sterile toothpicks on top of NBRIP agar plates. The plates were incubated at 30^0C for a week. P solubilization efficiency index (z/n) was obtained by multiplying the ratio between the diameter of the halo (z) and the diameter of the colony (n) by 100 (Nguyen *et al.*, 1992). The kinetics of TCP solubilisation mediated by each of the isolates was further monitored in liquid NBRIP medium. 100 ml of NBRIP broth (without BPB) was inoculated with 1% (v/v) freshly grown inoculum (prepared by growing the isolate in the same medium). The flasks containing inoculated media were incubated at 30^0C on an OS2 incubator orbital shaker (Bangalore Genei India Pvt. Ltd.) at medium speed (100 rpm). An aliquot was taken aseptically at different times (12h, 24h and 48h) to determine the pH and soluble inorganic phosphate (Pi) concentration. pH was measured immediately after the sampling using a digital pH meter (Professional Meter, PP–20, Sartorius). For soluble Pi determination, a sub– sample of the aliquot was centrifuged at 10000 rpm for 5 minutes to sediment the bacterial cells. Soluble Pi concentration was measured using ammonium–molybdate blue method (Allen *et al.*, 1974). Five ml of cell free supernatant was taken and mixed with 2 ml of ammonium molybdate solution and 2 ml of stannous chloride reagent. The solution was mixed well and made upto 50 ml by adding distilled water. Transmittance was recorded at 700 nm after 30 minutes using spectrophotometer (Lambda–35, UV/VIS, Perkin Elmer, USA). Pi concentration was calculated using the following formula:

$$\text{Pi (mg l}^{-1}\text{)} = \frac{\text{C x }10^3}{\text{aliquot}}$$

where, C = Concentration of P (mg) obtained from standard graph

Assessment of phosphate solubilization in pot experiment

Six most efficient species occurring in undisturbed and recovered stands based on their P solubilization efficiency in liquid media were selected for pot experiment. Ethanol disinfected plastic pots were filled with 4 kg of sterilized soil and sand mixture (2:1). Maize (*Zea mays* var. Vivek QPM 9) was selected due to its fast growth, and short life cycle (85–90 days). Seeds were surface sterilized prior to germination with sodium hypochlorite (1.2%), and then washed and kept wetted overnight to provide suitable moisture for germination. They were sown next day and inoculated with 1 ml of a microbial culture. The experiment included two different doses of inoculum: 10^8 CFU ml^{-1}, and 10^9 CFU ml^{-1} and two different sets of treatments: (1) soil + PSB species and (2) soil + PSB species + TCP (@ 0.3 g kg^{-1}, added to the soil before sowing the seeds).

Uninoculated pots were also set up as control. All the treatments and doses were performed in triplicates. The plants were harvested twice: 60 days after planting i.e. germination (DAP) and 90 DAP. Plant height, total leaf area, plant dry weight, plant total P content and un–utilized P in the respective pot were evaluated after each harvest. Phosphorus solubilising efficiency (PSE) in crop application was determined following the formula:

$$\text{PSE} = \text{Total P content in plant} + \text{un–utilized P in soil} - \text{initial soil P}$$

Statistical Analyses

Statistica version 7.0 (Statistical Software Inc. 2001) and origin version 7.0 (Origin Lab Corporation, USA) softwares were used to describe and analyse the data. Appropriate ANOVA models were used to test the variation in soil physicochemical and biological properties among land uses, recovery ages, and seasons. The data were subjected to tests of normality, homogeneity of variance, and additivity using appropriate tests before undertaking ANOVA. Wherever necessary, the data were log or square root transformed. Multiple stepwise forward regression analysis was carried out to identify the most statistically significant variables of soil physicochemical properties that contribute to various biological activities. The correlation between soil nutrients and the related parameters was calculated and tested for statistical significance. The minimum significance level for all statistical tests was at $p<0.05$ level.

RESULTS

Phosphorus Dynamics

Bray's extractable phosphorus and water extractable phosphorus: Bray's extractable P (P_{Bray}) ranged between 0.03 and 0.07 μg g^{-1} in forest ecosystem, and 0.03 and 0.12 μg g^{-1} in grassland ecosystem. In forest ecosystem, P_{Bray} was significantly (Tukey's test, $p<0.01$) lower in CF1 stands i.e. immediately after deforestation than the recovering RF10, and undisturbed forest stands (Fig. 6.1 and Tables 6.1 and 6.3). As recovery proceeded, it increased significantly (Tukey's test, $p<0.01$). In grassland ecosystem, P_{Bray} followed the same trend as forest ecosystem showing a significant (Tukey's test, $p<0.00$) increase as recovery progressed (Fig. 6.1 and Tables 6.2 and 6.4). The undisturbed grassland and mining affected MG sites had significantly (Tukey's test, $p<0.00$) lower P_{Bray} than the recovering RG15 and RG50 sites. P_{Bray} was not significantly different ($p>0.05$) between the two land uses. It varied significantly ($p<0.00$) among the seasons, and was highest during rainy/autumn seasons and lowest during winter (Fig. 6.1).

Although the variation in water extractable P was not significant ($p>0.05$) among recovery ages in either of the land uses, it varied significantly ($p<0.05$) among the seasons (Fig. 6.2 and Tables 6.1, 6.2, 6.3 and 6.4). It was highest during autumn and lowest during winter/spring seasons (Fig. 6.2).

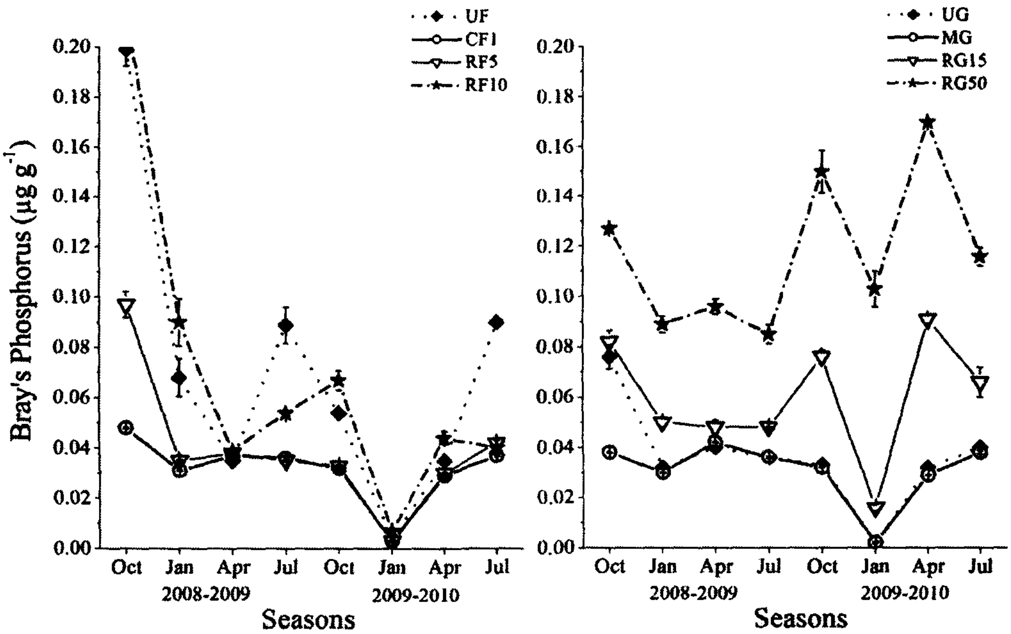

Fig. 6.1: Seasonal variation in Bray Kurtz extractable phosphorus in the undisturbed and recovering stands of forest and grassland ecosystems.

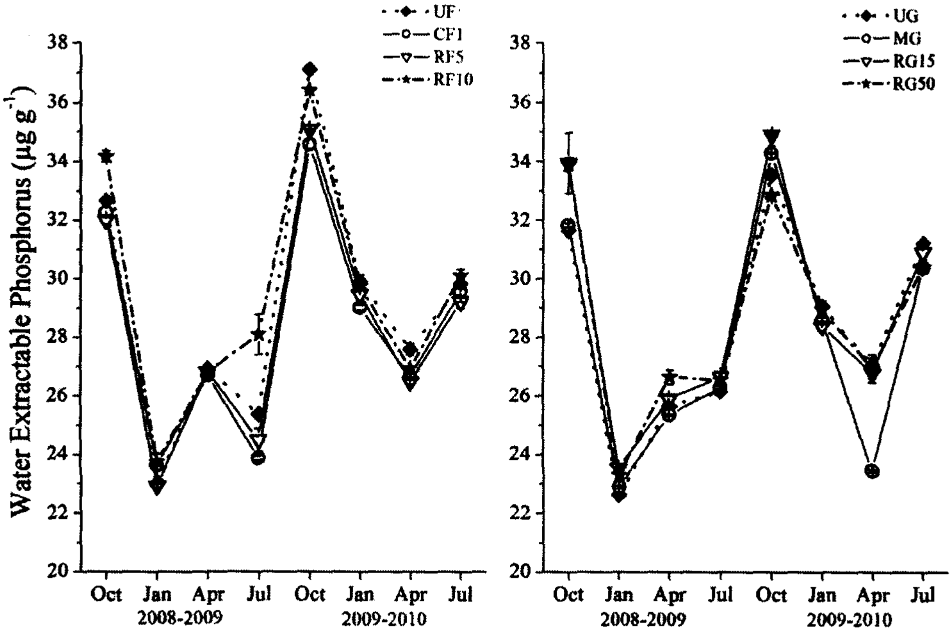

Fig. 6.2: Seasonal variation in water extractable phosphorus in the undisturbed and recovering stands of forest and grassland ecosystems.

Table 6.1: Results of two–way ANOVA to test the effect of recovery age and season on soil chemical and microbiological measures in the forest ecosystem.

Parameters	**df**	**Recovery Age**		**Season**	
		F	p	F	p
TP	3	39.9***	0.00	17.5***	0.00
P_{Bray}	3	15.6***	0.00	30.4***	0.00
P_{Water}	3	1.1	0.34	15.9***	0.00
APase	3	14.1***	0.00	70.2***	0.00
P_{min}	3	72.8	0.72	8423.5***	0.00
MBP	3	690.4***	0.00	1077.2***	0.00
MBP/TP	3	194.3***	0.00	87.0***	0.00

TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_W – Water extractable Phosphorus; APase – Acid phosphatase; P_{min} – Net phosphorus mineralization; MBP – Microbial biomass phosphorus; MBP/TP – Contribution of Microbial Biomass to Total phosphorus; *** significant at $p<0.001$

Table 6.2: Results of two–way ANOVA to test the effect of recovery age and season on soil chemical and microbiological measures in the grassland ecosystem.

Parameters	**df**	**Recovery Age**		**Season**	
		F	p	F	p
TP	3	57.1***	0.00	17.3***	0.00
P_{Bray}	3	212.5***	0.00	34.0***	0.00
P_{Water}	3	1.2	0.30	8.4***	0.00
APase	3	16.4***	0.00	72.5***	0.00
Pmin	3	102.1	0.27	3800.7***	0.00
MBP	3	98.0***	0.00	195.0***	0.00
MBP/TP	3	169.3***	0.00	44.1***	0.00

TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_W – Water extractable Phosphorus; APase – Acid phosphatase; P_{min} – Net phosphorus mineralization; MBP – Microbial biomass phosphorus; MBP/TP – Contribution of Microbial Biomass to Total phosphorus; *** significant at $p<0.001$

Total phosphorus: The patterns of variation in total phosphorus (TP) among different recovery ages and seasons in the forest and grassland ecosystems have been described in detail in chapter 4 (Fig. 4.5). TP varied between 217.6 and 363.8 μg g^{-1} in forest ecosystem, and 153.1 and 297.2 μg g^{-1} in grassland ecosystem. It was significantly (Tukey's test, $p<0.01$) greater in the disturbed stands than the recovering stands in both the ecosystems. In forest ecosystem, TP content declined initially till 5th year of deforestation and increased thereafter as recovery followed. TP varied significantly ($p<0.00$) among the seasons, and was highest during autumn and lowest during summer/rainy seasons.

Microbial biomass phosphorus: Soil microbial biomass phosphorus (MBP) ranged between 27 μg g^{-1} and 42 μg g^{-1} in forest ecosystem and 23 μg g^{-1} and 30 μg g^{-1} in grassland ecosystem. MBP in forest ecosystem was significantly (Tukey's test, $p<0.05$) lower in CF1 stands than RF5, RF10 and UF stands (Fig. 6.3 and Tables 6.1 and 6.3). As recovery progressed, it significantly (Tukey's test, $p<0.05$) increased. MBP followed similar trend in grassland ecosystem with significant (Tukey's test, $p<0.01$) increase along recovery ages (Fig. 6.3 and Tables 6.2 and 6.4). MG sites had significantly (Tukey's test, $p<0.01$) lower amount of MBP than RG15, RG50 and UG sites. MBP was significantly higher ($p<0.00$) in forest ecosystem than grassland ecosystem. It exhibited a significant ($p<0.00$) variation among seasons in both forest and grassland ecosystem, and was highest during winter season and lowest during rainy (Fig. 6.3).

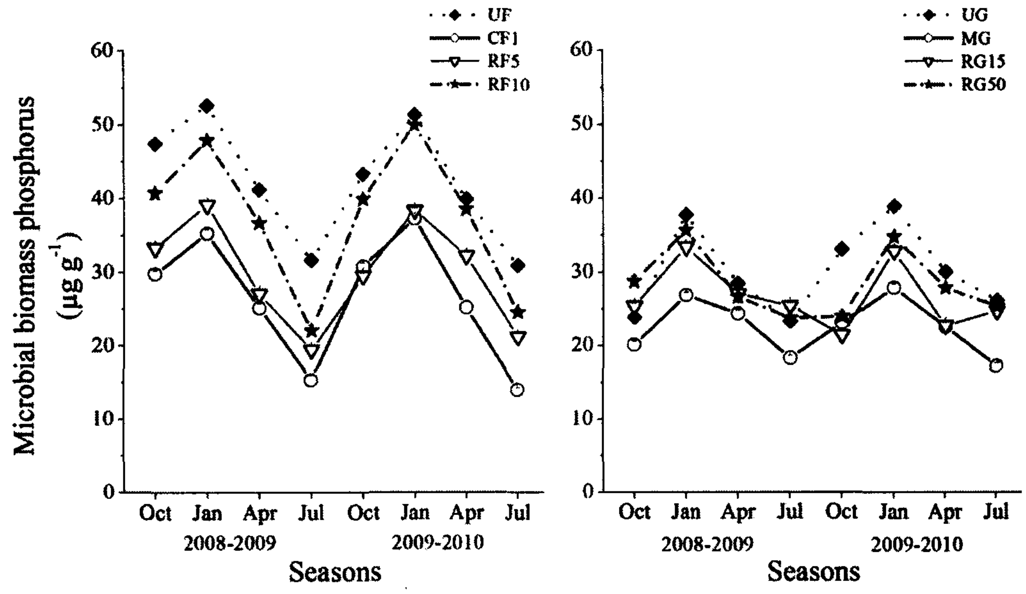

Fig. 6.3: Seasonal variation in microbial biomass phosphorus in undisturbed and recovering stands of forest and grassland ecosystems.

In forest ecosystem, the ratio of MBP/TP showed values between 7.8% and 21.4% (Table 6.3). The percentage contribution of microbial biomass to TP was significantly (Tukey's test, $p<0.00$) reduced in CF1 stands than RF10 and UF stands. As recovery progressed, the contribution of microbial biomass significantly (Tukey's test, $p<0.00$) increased. In grassland ecosystem, the percentage contribution of microbial biomass to TP showed the same trend as in forest ecosystem (Table 6.4). The MBP/TP ratio significantly (Tukey's test, $p<0.05$) increased with recovery age. TP had significantly (Tukey's test, $p<0.05$) lower contribution from MBP in MG sites than RG15, RG50 and UG sites in grassland ecosystem. The ratio ranged between 8.0% and 19.4%.

Table 6.3: Mean values (± standard error) of soil chemical and biological properties in the old growth undisturbed forests (UF) and recovering (CF1, RF5 and RF10) forests during the study period (values are the means of 4 samples each at 2 sites measured in 4 seasons over 2 years period, n=64).

	CF1	RF5	RF10	UF
TP ($\mu g\ g^{-1}$)	363.8 ± 11.93^{b}	308.2 ± 12.91^{c}	328.9 ± 11.27^{c}	217.6 ± 14.47^{a}
P_{bray} ($\mu g\ g^{-1}$)	0.03 ± 0.000^{b}	0.04 ± 0.001^{b}	0.07 ± 0.003^{a}	0.07 ± 0.003^{a}
P_{W} ($\mu g\ g^{-1}$)	28.3 ± 0.17	28.3 ± 0.15	29.5 ± 0.18	29.0 ± 0.10
Apase ($\mu g\ g^{-1}h^{-1}$)	311.6 ± 0.53^{b}	278.2 ± 0.34^{a}	274.5 ± 0.80^{a}	279.5 ± 0.51^{a}
P_{min} ($\mu g\ g^{-1}month^{-1}$)	7.8 ± 0.21	9.3 ± 0.18	9.9 ± 0.20	10.3 ± 0.20
MBP ($\mu g\ g^{-1}$)	26.6 ± 0.54^{b}	30.0 ± 0.55^{b}	37.6 ± 0.60^{c}	42.3 ± 0.51^{a}
MBP/TP (%)	7.8 ± 0.32^{b}	10.4 ± 0.46^{bc}	11.8 ± 0.54^{c}	21.4 ± 1.40^{a}

Mean values (mean ± s.e) sharing same letter within a row do not differ significantly by Tukey test at $p \leq 0.05$

TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_{W} – Water extractable Phosphorus; APase – Acid Phosphatase activity; P_{min} – Net Phosphorus Mineralization; MBP – Microbial Biomass Phosphorus; MBP/TP – Contribution of Microbial Biomass to Total phosphorus.

Table 6.4: Mean values (± standard error) of soil chemical and biological properties of the undisturbed (UG) and recovering (MG, RG15 and RG50) grasslands during the study period (values are the means of 4 samples each at 2 sites measured in 4 seasons over 2 years period, n=64).

	MG	RG15	RG50	UG
TP ($\mu g\ g^{-1}$)	290.8 ± 10.32^{a}	172.1 ± 8.48^{b}	153.0 ± 8.17^{b}	297.2 ± 10.02^{a}
P_{bray} ($\mu g\ g^{-1}$)	0.03 ± 0.000^{a}	0.06 ± 0.003^{b}	0.12 ± 0.004^{c}	0.04 ± 0.001^{a}
P_{W} ($\mu g\ g^{-1}$)	27.9 ± 0.05	28.9 ± 0.26	27.5 ± 0.14	28.4 ± 0.12
Apase ($\mu g\ g^{-1}h^{-1}$)	287.4 ± 0.93^{a}	244.9 ± 0.37^{b}	263.4 ± 0.52	274.1 ± 0.37^{a}
P_{min} ($\mu g\ g^{-1}month^{-1}$)	4.7 ± 0.28	7.3 ± 0.17	6.3 ± 0.17	7.7 ± 0.23
MBP ($\mu g\ g^{-1}$)	22.6 ± 0.50^{b}	26.6 ± 0.56^{c}	28.4 ± 0.55^{ac}	30.2 ± 0.47^{a}
MBP/TP (%)	8.0 ± 0.30^{a}	16.3 ± 1.03^{b}	19.4 ± 1.22^{c}	10.4 ± 0.48^{d}

Mean values (mean ± s.e) sharing same letter within a column do not differ significantly by Tukey test at $p \leq 0.05$

TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_{W} – Water extractable Phosphorus; APase – Acid Phosphatase activity; P_{min} – Net Phosphorus Mineralization; MBP – Microbial Biomass Phosphorus; MBP/TP – Contribution of Microbial Biomass to Total phosphorus.

Phosphorus mineralization: Phosphorus mineralization was significantly higher ($p<0.01$) in forest ecosystem than grassland ecosystem. Although it did not vary significantly ($p>0.05$) among the recovery ages in either of the land uses (Tables 6.1 and 6.2), there was a significant ($p<0.00$) variation among the seasons in both forest and grassland ecosystem (Fig. 6.4). It was highest during rainy season and lowest during winter with negative values.

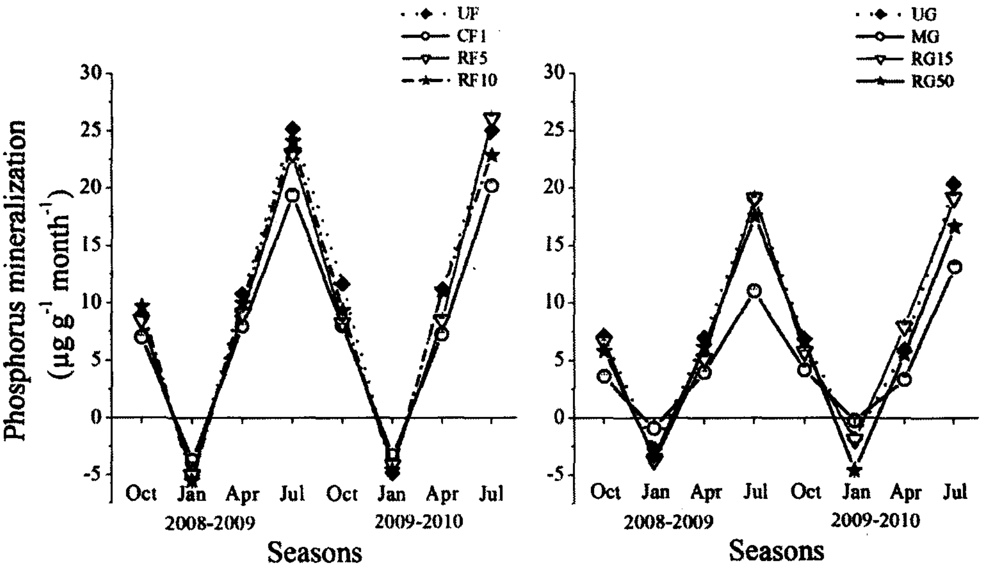

Fig. 6.4: Seasonal variation in net phosphorus mineralization in undisturbed and recovering stands of forest and grassland ecosystems.

Acid phosphatase activity: In forest ecosystem, acid phosphatase (APase) activity was significantly (Tukey's test, $p<0.01$) greater in CF1 stands than RF5, RF10 and UF stands (Fig. 6.5 and Tables 6.1 and 6.3). The activity significantly increased immediately after deforestation and gradually reduced till 5th year of recovery, after which it started increasing indicating that the net recovery action initiated after 5th year of recovery and continued thereafter as evident from the values of RF10. In grassland ecosystem, APase activity significantly (Tukey's test, $p<0.05$) increased as recovery progressed (Fig. 6.5 and Tables 6.2 and 6.4). MG sites did not show significant ($p>0.05$) variation with the UG sites. APase activity was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. It varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems, and showed highest activity during rainy season and lowest during spring (Fig. 6.5).

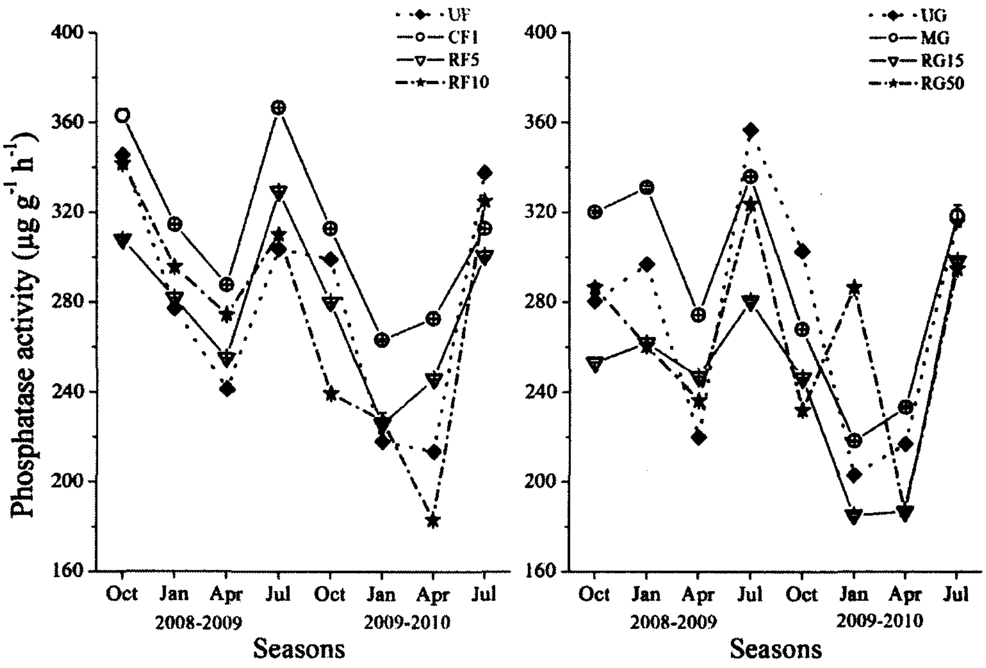

Fig. 6.5: Seasonal variation in acid phosphatase activity in the undisturbed and recovering stands of forest and grassland ecosystems.

Relationships with soil physico–chemical variables: The regression models showing the relationship of soil physico–chemical properties and soil microbial properties associated with P dynamics were developed using forward stepwise regression analysis (Tables 6.5 and 6.6). In forest ecosystem, microbial biomass P was significantly ($p<0.00$) influenced by total P, soil temperature, moisture, soil organic matter and C/N. While Acid phosphatase activity was significantly ($p<0.00$) correlated with soil moisture, C/P, soil organic matter, total nitrogen, P_W and P_{Bray}, phosphorus mineralization was significantly ($p<0.00$) related to soil temperature, moisture, soil organic matter and total P.

In grassland ecosystem, microbial biomass P was significantly ($p<0.00$) influenced by soil temperature, moisture, soil organic matter and CEC. Acid phosphatase activity showed significant ($p<0.00$) correlation with soil moisture, C/N, P_W and P_{Bray}. Soil temperature, moisture, and P_{Bray} were significantly ($p<0.00$) related with phosphorus mineralization.

Table 6.5: Regression models based on forward stepwise regression analysis showing the relationship between soil biological properties and soil physical and chemical parameters across the old growth undisturbed forests (UF) and recovering (CF1, RF5 and RF10) forests (n=256). The independent variables of the equations were significant at $p<0.05$ (t–Test).

Dependent Variable (Y)	Regression Equation	Adjusted R^2	P value
Acid Phosphatase	$Y = 179.5 - 497.8 \times P_{Bray} + 106.6 \times TKN + 2.5 \times SMC - 4.9 \times P_W + 0.2 \times C/P + 61.1 \times SOC - 83.2 \times SOM - 167.4 \times CEC + 2.6 \times Clay$	0.73	<0.00
Net Phosphorus Mineralization	$Y = -38.2 + 1.5 \times ST - 0.1 \times WHC - 0.03 \times TP - 20.0 \times BD + 0.2 \times SMC + 9.9 \times SOM$	0.82	<0.00
Microbial Biomass Phosphorus	$Y = 99.0 - 0.3 \times SMC - 4.7 \times SOM - 1.2 \times ST - 18.4 \times BD - 0.7 \times Silt + 0.02 \times TP - 0.4 \times C/N$	0.83	<0.00

BD – bulk density; WHC – water holding capacity; ST – Soil Temperature; CEC – cation exchange capacity; SMC – Soil Moisture Content; SOC – Soil Organic Carbon; SOM – Soil Organic Matter; TKN – Total Kjeldahl Nitrogen; TP – Total Phosphorus; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_W – Water extractable Phosphorus; C/N – Carbon to Nitrogen ratio; C/P – Carbon to Total phosphorus ratio.

Table 6.6: Regression models based on forward stepwise regression analysis showing the relationship between soil biological properties and soil physical and chemical parameters across the undisturbed (UG) and recovering (MG, RG15 and RG50) grasslands (n=256). The independent variables of the equations were significant at $p<0.05$ (t–Test).

Dependent Variable (Y)	Regression Equation	Adjusted R^2	P value
Acid Phosphatase	$Y = 420.8 + 4.8 \times SMC - 6.5 \times P_W - 2.5 \times C/N - 130.8 \times CEC - 333.3 \times P_{Bray}$	0.47	<0.00
Net Phosphorus Mineralization	$Y = -38.5 + 0.6 \times ST + 0.8 \times SMC + 0.3 \times Porosity - 35.2 \times CEC - 103.4 \times P_{Bray}$	0.73	<0.00
Microbial Biomass Phosphorus	$Y = 35.9 - 0.5 \times ST - 0.3 \times SMC + 0.4 \times Silt - 11.6 \times CEC + 2.1 \times SOC - 3.2 \times SOM$	0.77	<0.00

ST – Soil Temperature; SMC – Soil Moisture Content; CEC – cation exchange capacity; SOC – Soil Organic Carbon; SOM – Soil Organic Matter; P_{Bray} – Bray–Kurtz Extractable Phosphorus; P_W – Water extractable Phosphorus; C/N – Carbon to Nitrogen ratio.

Role of phosphate solubilising bacteria

Isolation and Identification of Phosphate solubilising bacteria: The population load of Phosphate solubilising bacterial (PSB) significantly ($p<0.00$) reduced following disturbance in both the forest and grassland ecosystems (Fig. 6.6). In forest ecosystem, PSB population was significantly (Tukey's test, $p<0.05$) lower in CF1 stands (16×10^4 CFU g^{-1} soil) i.e. immediately after deforestation than the recovering RF10 stands (24×10^4 CFU g^{-1} soil) and the undisturbed stands (29×10^4 CFU g^{-1} soil). As recovery progressed, the population size increased. Similar trend was found in grassland ecosystem showing an increase in population size with progress in recovery. Mining effected MG sites (13×10^4 CFU g^{-1} soil) had significantly (Tukey's test, $p<0.05$) lower population size of PSB than the recovering RG15 (22 x 10^4 CFU g^{-1} soil) and RG50 (21 x 10^4 CFU g^{-1} soil) sites, and the undisturbed grassland sites (27 x 10^4 CFU g^{-1} soil). PSB population varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with highest during rainy season and lowest during winter.

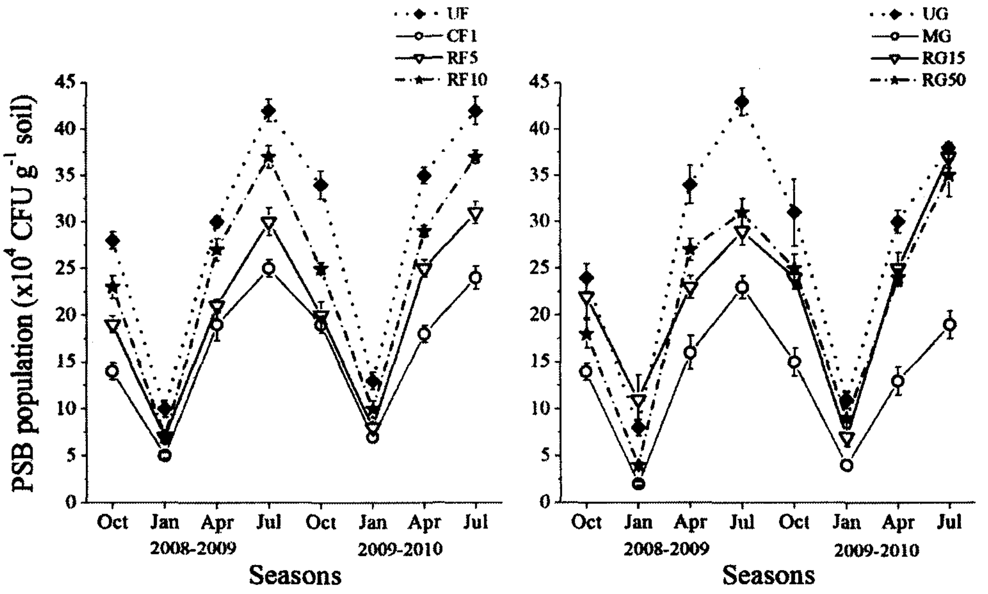

Fig. 6.6: Seasonal variation in population load of phosphorus solubilising bacteria in the undisturbed and recovering forest stands and grassland sites.

Table 6.7: Results of two–way ANOVA to test the variance in PSB population due to recovery age and season in the forest and grassland ecosystem.

	df	recovery age		season	
		F	p	F	p
PSB forest ecosystem	3	8.0***	0.00	82.1***	0.00
PSB grassland ecosystem	3	8.4***	0.000	64.5***	0.000

PSB – Phosphorus solubilising bacteria population; *** significant at $p<0.001$

The screening strategy employed during this research work enabled identification of 47 isolates of PSB from both forest and grassland ecosystems. About 94% of the PSB isolates were gram negative while the remaining were gram positive. All the PSB isolates were unicellular rods, motile and aerobic. Bacterial colonies were circular/irregular/rhizoid and the surface was either smooth or rough. The colony colour was white/off white/yellow/beige (Table 6.8). The isolates did not ferment glucose, sucrose and mannitol. All the PSB isolates were negative for hydrogen sulphide production and indole production whereas 85% of PSB isolates tested positive for catalase activity, 94% for oxidase activity, 28% for nitrate reduction, 17% for starch hydrolysis, 9% for methyl red, 21% for Voges–proskauer and 77% for citrate utilization (Table 6.9). Nucleotide sequencing of PCR amplified 16S rRNA genes and sequence comparison with available data in the GenBank using BLAST algorithm indicated that the isolates were closely related to various species of *Burkholderia*, *Pseudomonas koreensis*, *P. moraviensis*, *Agrobacterium rhizogenes*, *Bacillus amyloliquefaciens* and *Brevibacillus agri* (Table 6.10). Based on the neighbour joining and UPGMA phylogenetic tree constructed with the 16S rDNA similarity (%), the PSB isolates were affiliated to five genera: 39 isolates were similar to the species of *Burkholderia* genus, 3 were similar to the species of *Pseudomonas* genus, 2 isolates each were closely related to the species of *Agrobacterium* genus, and *Bacillus* genus and one isolate was a member of the genus *Brevibacillus* (Fig. 6.9).

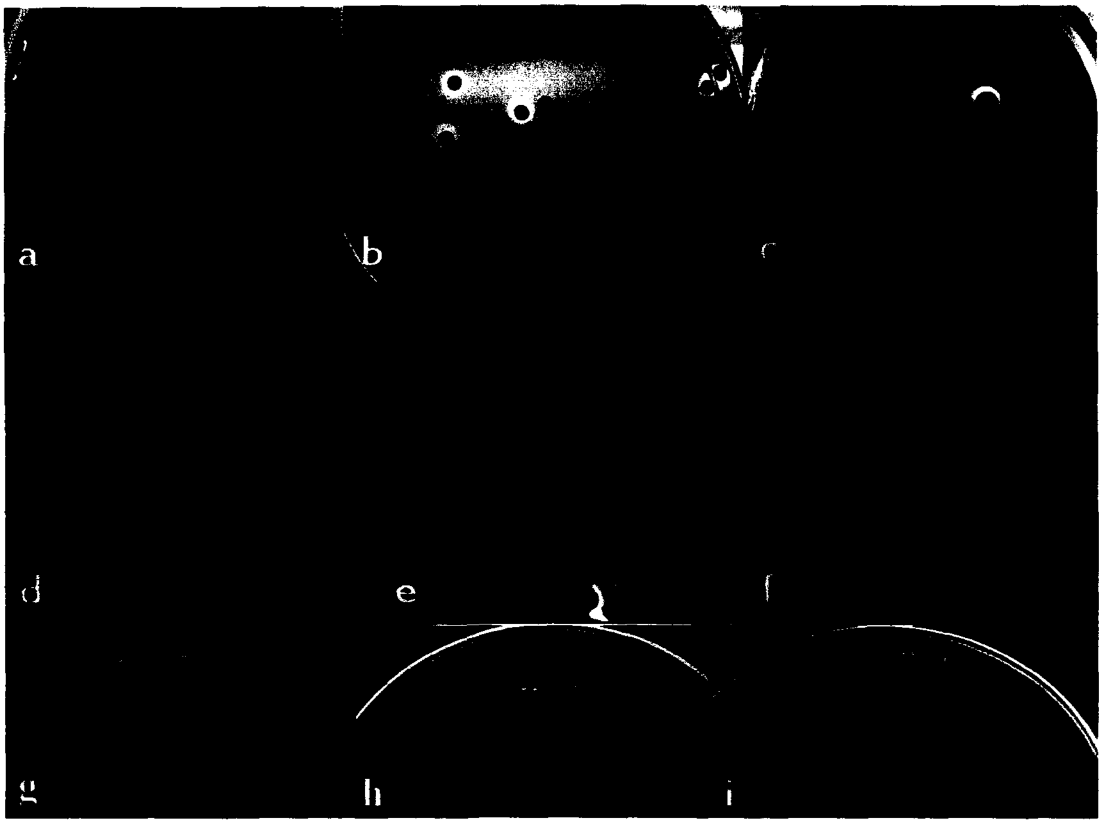

Fig. 6.7: (a) Control NBRIP plate containing pH indicator dye bromophenol blue (BPB); (b) and (c) Growth of PSB on NBRIP medium during initial screening; (d) and (e) Plate assay for P solubilization index (%) i.e. z/n on Pikovaskaya medium; (f) Plate assay for P solubilization index (%) i.e. z/n on NBRIP medium; (g), (h) and (i) Dye decolorization by PSB isolates on NBRIP medium with BPB.

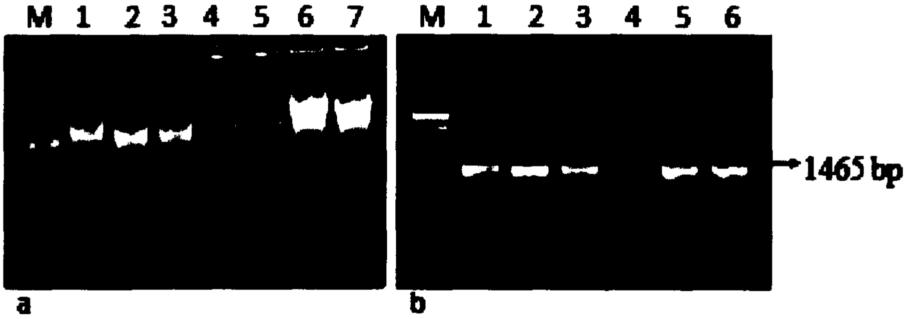

Fig. 6.8: (a) Genomic DNA extraction (b) PCR amplicon of 1465 bp.

Table 6.8: Phenotypic characteristics of PSB isolates from the forest and grassland ecosystems.

Isolate	Colony Morphology						Gram stain	Shape	Motility	O_2 Needs	Capsule
	Form	Elevation	Margin	Colour	Surface	Texture					
LU2RA101	I	wUm	U	Y	S	M, Cy	–	Rods	+	aerobic	+
LU2RA102	C	R	E	B	S	M, Cy	–	Small rods	+	aerobic	+
LU2RA103	I	F	U	OW	S	Cy	–	Small rods	+	aerobic	+
LU2RA104	I	F	Fi	Y	Ro	D	–	Rods	+	aerobic	–
LU2RA105	C	F	Cu	Y	S	Cy	–	Small rods	+	aerobic	–
LU2RA107	C	F	E	W	S, G	M	–	Small rods	+	aerobic	–
LU2RA201	I	Um	Sc	OW	Wr	Cy	–	Small rods	+	aerobic	–
LU2RA202	C	R	U	Y	S, G	Cy	–	Rods	+	aerobic	–
LU2RA203	C	R	U	Y	S, G	Cy	–	Rods	+	aerobic	–
LU2RA205	I	F	U	Y	S, G	Cy	–	Small rods	+	aerobic	–
LU2RA301	C	wUm	U	Y	S	Cy	–	Small rods	+	aerobic	–
LU2RA302	Rh	F	Fi	W	Ro	M	+	Rods	+	aerobic	+
LU2RA303	C	R	Sc	OW	Ro	Cy	–	Small rods	+	aerobic	–
LU2RA304	I	F	Sc	OW	S	Cy	–	Rods	+	aerobic	–
LU2RA305	I	F	U	Y	Wr	V	–	Rods	+	aerobic	–
LU2RA306	C	F	E	W	S	V	–	Small rods	+	aerobic	–
LU2RA307	I	F	U	W	S, G	M	–	Small rods	+	aerobic	+
LU2RA308	I	F	Cu	W	S, G	V	+	Small rods	+	aerobic	+
LU2RA401	I	F	Sc	OW	S	Cy	–	Small rods	+	aerobic	+
LU2RA402	I	F	U	Y	S	Cy	–	Rods	+	aerobic	+
LU2RA403	I	F	wU	Y	S	Cy	–	Small rods	+	aerobic	–
LU2RA404	C	Um	E	Y	S, G	Cy	–	Small rods	+	aerobic	+
LU2RA405	C	F	E	OW	S, G	M	–	Rods	+	aerobic	+
LU1RA101	I	F	Sc	Y	S	Cy	–	Rods	+	aerobic	–
LU1RA102	I	Um	U	Y	S	Cy	–	Rods	+	aerobic	–
LU1RA103	C	Cx	E	OW	S, G	Cy	–	Rods	+	aerobic	–

LU1RA104	C	Cx	E	Y	S, G	M	–	Small rods	+	aerobic	+
LU1RA105	C	R	E	OW	S, G	M, V	–	Rods	+	aerobic	+
LU1RA106	C	F	E	Y	S	Cy	–	Rods	+	aerobic	–
LU1RA107	I	Cr	U	Y	S, G	M, Cy	–	Rods	+	aerobic	+
LU1RA108	I	Cx	L	B	S, G	Cy	–	Rods	+	aerobic	+
LU1RA201	I	F	wU	Y	S	Cy	–	Small rods	+	aerobic	+
LU1RA202	C	F	E	W	S, G	Cy	+	Rods	+	aerobic	–
LU1RA203	I	F	U	B	S, G	V	–	Small rods	+	aerobic	+
LU1RA301	C	Cx	E	OW	S, G	Cy	–	Small rods	+	aerobic	–
LU1RA303	C	R	E	Y	S, G	V	–	Small rods	+	aerobic	–
LU1RA304	C	F	E	OW	S, G	Cy	–	Rods	+	aerobic	+
LU1RA305	I	F	Sc	B	S	Cy	–	Rods	+	aerobic	–
LU1RA306	I	F	Cu	W	S, G	Cy	–	Small rods	+	aerobic	+
LU1RA307	I	F	U	B	S	Cy	–	Rods	+	aerobic	+
LU1RA401	C	Cx	E	Y	S, G	Cy	–	Rods	+	aerobic	+
LU1RA402	I	F	E	W	S	V	–	Small rods	+	aerobic	+
LU1RA403	C	Cr	E	OW	S, G	Cy	–	Rods	+	aerobic	+
LU1RA404	C	R	E	OW	S	Cy	–	Rods	+	aerobic	+
LU1RA405	I	F	U	Y	S	Cy	–	Rods	+	aerobic	–
LU1RA406	C	F	U	Y	S	V	–	Rods	+	aerobic	+
LU1RA407	C	F	Sc	Y	S	V	–	Rods	+	aerobic	+

Form: I – Irregular, C – Circular, Rh – Rhizoid; **Elevation**: wUm/Um – weakly Umbonate/Umbonate, R – Raised, F – Flat, Cx – Convex, Cr – Crateriform; **Margin**: E – Entire, wU/U – weakly Undulate/Undulate, Fi – Filamentous, Cu – Curled, Sc – Scalloped, L – Lobate; **Colour**: W – White, OW – Off white, Y – Yellow, B – Beige; **Surface**: S – Smooth, G – Glistening, Ro – Rough, Wr – Wrinkled; **Texture**: V – Viscous, Cy – Creamy, M – Moist, D – Dry.

Table 6.9: Biochemical characteristics of PSB isolates from the forest and grassland ecosystems.

Isolate	Glu. ferm.	Suc. ferm.	Mann. ferm.	Litmus Milk	H2S prod.	Nitrate red.	Catalase	Oxidase	Starch hydro.	Indole prod.	MR test	VP test	Citrate utili.
LU2RA101	–	–	–	Acid production	–	–	+	+	–	–	+	–	+
LU2RA102	–	–	–	Acid,reduction, curd form.	–	+	+	+	–	–	+	–	+
LU2RA103	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA104	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA105	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA107	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	–
LU2RA201	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	–	–
LU2RA202	–	–	–	Acid production	–	+	+	+	–	–	–	+	–
LU2RA203	–	–	–	Acid production	–	+	+	+	–	–	–	+	–
LU2RA205	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA301	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA302	–	–	–	Alkaline reaction	–	+	+	–	–	–	+	–	+
LU2RA303	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA304	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA305	–	–	–	Acid production	–	–	+	+	–	–	–	–	+
LU2RA306	–	–	–	Alkaline reaction	–	+	–	+	–	–	–	–	+
LU2RA307	–	–	–	Alkaline reaction	–	–	–	+	–	–	–	–	+
LU2RA308	–	–	–	Alkaline reaction	–	+	+	–	–	–	–	–	–
LU2RA401	–	–	–	Alkaline reaction	–	+	+	+	–	–	–	–	+
LU2RA402	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA403	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU2RA404	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	–	+
LU2RA405	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	+	+
LU1RA101	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	–	+

LU1RA102	–	–	–	Alkaline reaction	–	+	+	+	–	–	–	–	+
LU1RA103	–	–	–	Alkaline reaction	–	+	–	+	–	–	–	+	–
LU1RA104	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	+	+
LU1RA105	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	–
LU1RA106	–	–	–	Alkaline reaction	–	+	+	+	–	–	–	–	–
LU1RA107	–	–	–	Alkaline reaction	–	–	–	+	–	–	–	+	–
LU1RA108	–	–	–	Proteolysis of casein	–	+	–	+	–	–	–	–	+
LU1RA201	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	–	+
LU1RA202	–	–	–	Alkaline reaction	–	–	+	–	–	–	–	–	–
LU1RA203	–	–	–	Proteolysis of casein	–	+	+	+	–	–	–	–	+
LU1RA301	–	–	–	Alkaline reaction	–	–	–	+	–	–	–	–	+
LU1RA303	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU1RA304	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	–	+
LU1RA305	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU1RA306	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	+	+
LU1RA307	–	–	–	Acid production	–	–	+	+	–	–	–	–	+
LU1RA401	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	+	+
LU1RA402	–	–	–	Alkaline reaction	–	–	–	+	+	–	–	–	+
LU1RA403	–	–	–	Alkaline reaction	–	+	+	+	–	–	–	–	+
LU1RA404	–	–	–	Alkaline reaction	–	–	+	+	+	–	–	+	+
LU1RA405	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	–	+
LU1RA406	–	–	–	Acid,reduction, curd form.	–	–	+	+	–	–	+	–	+
LU1RA407	–	–	–	Alkaline reaction	–	–	+	+	–	–	–	+	–

'+'Tested positive/utilised as substrate; '–' Tested negative/non–utilised as substrate
Glu. ferm.– Glucose fermentation; Suc. ferm.– Sucrose fermentation; Mann. ferm.– Mannitol fermentation; H_2S prod.– Hydrogen sulphide production; Nitrate red.– Nitrate reduction; Starch hydro.– Starch hydrolysis; Indole prod.– Indole production; MR: Methyl red; VP– Voges–proskauer; Citrate utili.– Citrate utilization.

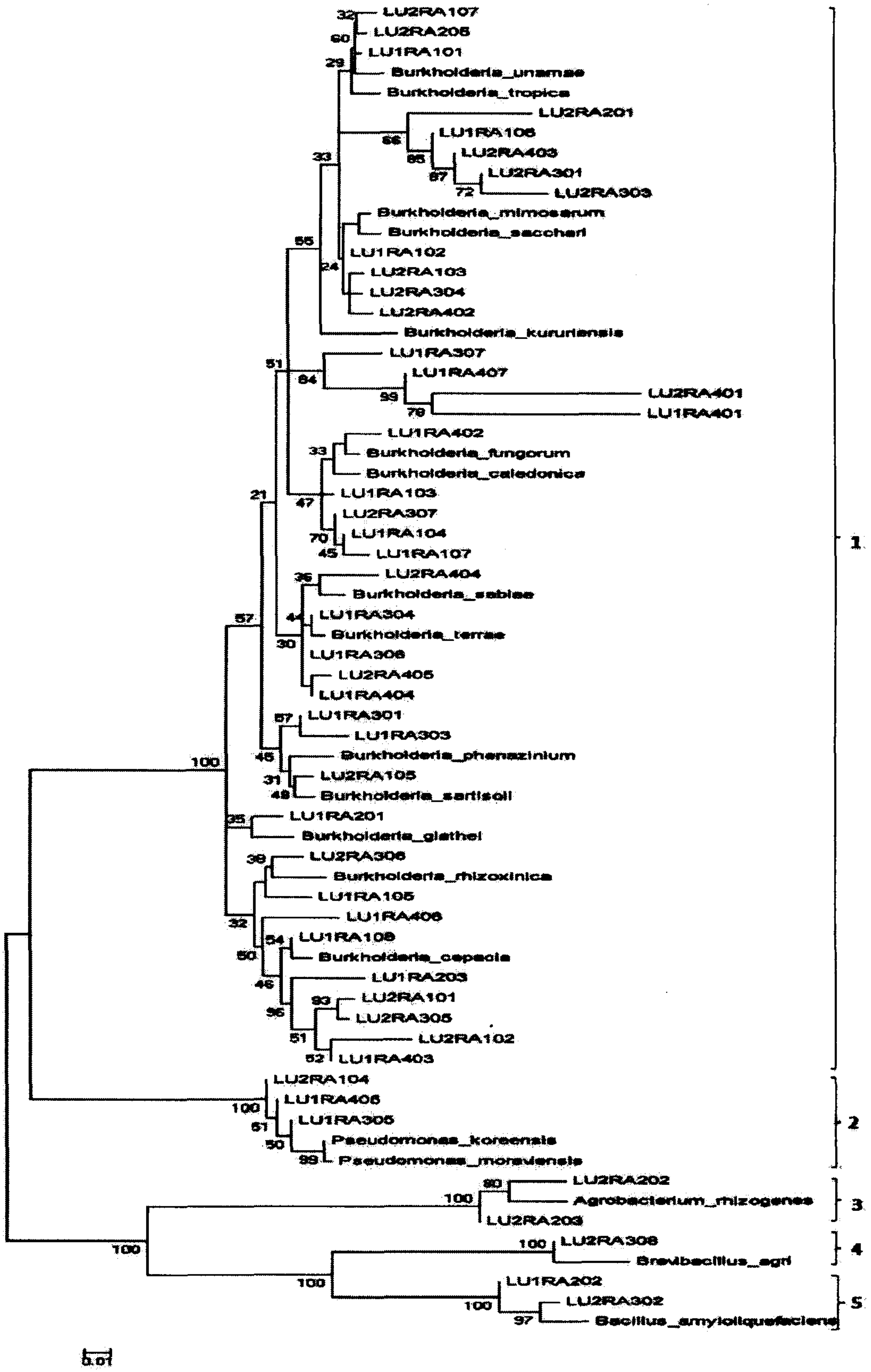

Fig. 6.9: Phylogenetic analyses of phosphate solubilising bacterial isolates based on 16SrDNA sequence. Multiple sequence alignment was done by CLUSTAL W. Distances (distance options according to the Kimura–2 parameter model) and clustering with neighbour–joining method was performed in MEGA 5.05 (Tamura *et al.*, 2011). Bootstrap values are based on 100 replications.

Table 6.10: Identification of PSB isolates by 16S rDNA sequencing.

Isolates	Closest relative		
	Species (strain)	**Accession no.**	**% Identity**
LU2RA101	*Burkholderia cepacia* (LMG 14294)	NR_041719.1	99
LU2RA102	*Burkholderia glumae* (P 1–22–1)	NR_029211.1	99
LU2RA103	*Burkholderia sacchari* (IPT10)	NR_025097.1	97
LU2RA104	*Pseudomonas moraviensis* (CCM 7280)	NR_043314.1	96
LU2RA105	*Burkholderia sartisoli* (RP007)	NR_041709.1	98
LU2RA107	*Burkholderia unamae* (MTl–641)	NR_027569.1	97
LU2RA201	*Burkholderia unamae* (MTl–641)	NR_027569.1	95
LU2RA202	*Agrobacterium rhizogenes* (IAM 14222)	NR_025832.1	95
LU2RA203	*Agrobacterium rhizogenes* (IAM 14222)	NR_025832.1	94
LU2RA205	*Burkholderia tropica* (Ppe8)	NR_028965.1	98
LU2RA301	*Burkholderia mimosarum* (PAS44)	NR_043167.1	96
LU2RA302	*Bacillus amyloliquefaciens* FZB42 (FZB42)	NR_075005.1	97
LU2RA303	*Burkholderia mimosarum* (PAS44)	NR_043167.1	96
LU2RA304	*Burkholderia mimosarum* (PAS44)	NR_043167.1	97
LU2RA305	*Burkholderia cepacia* (LMG 14294)	NR_041719.1	96
LU2RA306	*Burkholderia arboris* (R–24201)	NR_042634.1	96
LU2RA307	*Burkholderia fungorum* (LMG 16225)	NR_025058.1	97
LU2RA308	*Brevibacillus agri* (DSM 6348)	NR_040983.1	98
LU2RA401	*Burkholderia nodosa* (Br3437	NR_043181.1	97
LU2RA402	*Burkholderia mimosarum* (PAS44)	NR_043167.1	97
LU2RA403	*Burkholderia mimosarum* (PAS44)	NR_043167.1	97
LU2RA404	*Burkholderia sabiae* (Br3407)	NR_043180.1	97
LU2RA405	*Burkholderia terrae* (KMY02)	NR_041287.1	98
LU1RA101	*Burkholderia tropica* (Ppe8)	NR_028965.1	99
LU1RA102	*Burkholderia mimosarum* (PAS44)	NR_043167.1	98
LU1RA103	*Burkholderia phenoliruptrix* (AC1100)	NR_042901.1	98
LU1RA104	*Burkholderia fungorum* (LMG 16225)	NR_025058.1	98
LU1RA105	*Burkholderia glathei* (N15)	NR_037065.1	98
LU1RA106	*Burkholderia sacchari* (IPT10)	NR_025097.1	98
LU1RA107	*Burkholderia caledonica* (LMG 19076)	NR_025057.1	98
LU1RA108	*Burkholderia arboris* (R–24201)	NR_042634.1	98
LU1RA201	*Burkholderia ambifaria* AMMD (AMMD)	NR_074687.1	96
LU1RA202	*Bacillus amyloliquefaciens* (NBRC 15535)	NR_041455.1	98
LU1RA203	*Burkholderia arboris* (R–24201)	NR_042634.1	96
LU1RA301	*Burkholderia phenazinium* (A 1)	NR_029212.1	98
LU1RA303	*Burkholderia phenazinium* (A 1)	NR_029212.1	98
LU1RA304	*Burkholderia terrae* (KMY02)	NR_041287.1	97
LU1RA305	*Pseudomonas koreensis* (Ps 9–14)	NR_025228.1	97
LU1RA306	*Burkholderia terrae* (KMY02)	NR_041287.1	97
LU1RA307	*Burkholderia bryophila* (LMG 23644)	NR_042593.1	97
LU1RA401	*Burkholderia phytofirmans* PsJN (PsJN)	NR_042931.1	95
LU1RA402	*Burkholderia fungorum* (LMG 16225)	NR_025058.1	98
LU1RA403	*Burkholderia arboris* (R–24201)	NR_042634.1	96
LU1RA404	*Burkholderia terrae* (KMY02)	NR_041287.1	98
LU1RA405	*Pseudomonas koreensis* (Ps 9–14)	NR_025228.1	98
LU1RA406	*Burkholderia cepacia* (717)	NR_029209.1	98
LU1RA407	*Burkholderia phytofirmans* PsJN (PsJN)	NR_042931.1	99

Table 6.11: Phosphorus solubilising bacteria present at different stages of recovery in the forest and grassland ecosystem ('+' present and '–' absent).

Species	MG	RG15	RG60	UG	CF1	RF5	RF10	UF
Agrobacterium rhizogenes (IAM 14222)	+	–	–	–	–	–	–	–
Bacillus amyloliquefaciens (NBRC 15535)	–	–	–	–	+	–	–	–
Bacillus amyloliquefaciens FZB42 (FZB42)	–	+	–	–	–	–	–	–
Brevibacillus agri (DSM 6348)	–	+	–	–	–	–	–	–
Burkholderia ambifaria AMMD (AMMD)	–	–	–	–	+	–	–	–
Burkholderia arboris (R–24201)	–	+	–	–	+	–	+	+
Burkholderia bryophila (LMG 23644)	–	–	–	–	–	+	–	–
Burkholderia caledonica (LMG 19076)	–	–	–	–	–	–	–	+
Burkholderia cepacia (717)	–	–	–	–	–	–	+	–
Burkholderia cepacia (LMG 14294)	–	+	–	+	–	–	–	–
Burkholderia fungorum (LMG 16225)	–	+	–	–	–	–	+	+
Burkholderia glathei (N15)	–	–	–	–	–	–	–	+
Burkholderia glumae (P 1–22–1)	–	–	–	+	–	–	–	–
Burkholderia mimosarum (PAS44)	–	+	+	–	–	–	–	+
Burkholderia nodosa (Br3437)	–	–	+	–	–	–	–	–
Burkholderia phenazinium (A 1)	–	–	–	–	–	+	–	–
Burkholderia phenoliruptrix (AC1100)	–	–	–	–	–	–	–	+
Burkholderia phytofirmans PsJN (PsJN)	–	–	–	–	–	–	+	–
Burkholderia sabiae (Br3407)	–	–	+	–	–	–	–	–
Burkholderia sacchari (IPT10)	–	–	–	+	–	–	–	+
Burkholderia sartisoli (RP007)	–	–	–	+	–	–	–	–
Burkholderia terrae (KMY02)	–	–	+	–	–	+	+	–
Burkholderia tropica (Ppe8)	+	–	–	–	–	–	–	+
Burkholderia unamae (MTl–641)	+	–	–	+	–	–	–	–
Pseudomonas koreensis (Ps 9–14)	–	–	–	–	–	+	+	–
Pseudomonas moraviensis (CCM 7280)	–	–	–	+	–	–	–	–
Total	**3**	**6**	**4**	**6**	**3**	**4**	**6**	**8**

In forest ecosystem, CF1 stands had 3 species of PSB of which 2 species viz., *Bacillus amyloliquefaciens* and *Burkholderia ambifaria* were exclusive to these stands. Four species were found in RF5 stands, of which 2 species viz., *Burkholderia bryophila* and *Burkholderia phenazinium* were confined only to these stands. RF10 and UF stands had 6 and 8 species, respectively of which two species viz., *Burkholderia cepacia* and *Burkholderia phytofirmans* were exclusively found in RF10 and 6 species viz., *Burkholderia caledonica, Burkholderia glathei, Burkholderia mimosarum, Burkholderia phenoliruptrix, Burkholderia sacchari* and *Burkholderia tropica* were found only in UF (Tables 6.11 and 6.12).

Table 6.12: Species composition of PSB at different stages of recovery in the forest ecosystem. The species unique to a particular stage of recovery age are given in bold letters.

Site	PSB
CF1	***Bacillus amyloliquefaciens* (NBRC 15535)**
	***Burkholderia ambifaria* AMMD (AMMD)**
	Burkholderia arboris (R–24201)
RF5	***Burkholderia bryophila* (LMG 23644)**
	***Burkholderia phenazinium* (A 1)**
	Burkholderia terrae (KMY02)
	Pseudomonas koreensis (Ps 9–14)
RF10	*Burkholderia arboris* (R–24201)
	***Burkholderia cepacia* (717)**
	Burkholderia fungorum (LMG 16225)
	***Burkholderia phytofirmans* PsJN (PsJN)**
	Burkholderia terrae (KMY02)
	Pseudomonas koreensis (Ps 9–14)
UF	*Burkholderia arboris* (R–24201)
	***Burkholderia caledonica* (LMG 19076)**
	Burkholderia fungorum (LMG 16225)
	***Burkholderia glathei* (N15)**
	***Burkholderia mimosarum* (PAS44)**
	***Burkholderia phenoliruptrix* (AC1100)**
	***Burkholderia sacchari* (IPT10)**
	***Burkholderia tropica* (Ppe8)**

In grassland ecosystem, MG stands had 3 species of PSB of which 2 species viz., *Agrobacterium rhizogenes* and *Burkholderia tropica* were exclusive to these stands. Out of the 6 species found in the RG15 stands, 4 species viz., *Bacillus amyloliquefaciens, Brevibacillus agri*, *Burkholderia arboris* and *Burkholderia fungorum* were confined only to these stands. RG50 and UG stands had 4 and 6 species, respectively out of which 3 species viz., *Burkholderia nodosa*, *Burkholderia sabiae* and *Burkholderia terrae* were exclusively found in RG50 and 4 species viz., *Burkholderia glumae*, *Burkholderia sacchari*, *Burkholderia sartisoli* and *Pseudomonas moraviensis* were found only in UG (Tables 6.11 and 6.13).

Table 6.13: Species composition of PSB at different stages of recovery in the grassland ecosystem. The species unique to a particular stage of recovery age are given in bold letters.

Site	PSB
MG	***Agrobacterium rhizogenes* (IAM 14222)**
	***Burkholderia tropica* (Ppe8)**
	Burkholderia unamae (MTl–641)
RG15	***Bacillus amyloliquefaciens* FZB42 (FZB42)**
	***Brevibacillus agri* (DSM 6348)**
	***Burkholderia arboris* (R–24201)**
	Burkholderia cepacia (LMG 14294)
	***Burkholderia fungorum* (LMG 16225)**
	Burkholderia mimosarum (PAS44)
RG50	*Burkholderia mimosarum* (PAS44)
	***Burkholderia nodosa* (Br3437)**
	***Burkholderia sabiae* (Br3407)**
	***Burkholderia terrae* (KMY02)**
UG	*Burkholderia cepacia* (LMG 14294)
	***Burkholderia glumae* (P 1–22–1)**
	***Burkholderia sacchari* (IPT10)**
	***Burkholderia sartisoli* (RP007)**
	Burkholderia unamae (MTl–641)
	***Pseudomonas moraviensis* (CCM 7280)**

Determination of Phosphate solubilising efficiency:

All the 47 isolates decolourised the medium while only 9 isolates exhibited a clear halo around the colonies during plate assay on NBRIP agar medium supplemented with tri–calcium phosphate. The solubilization efficiency index (z/n ratio) of these 9 isolates was calculated following Nguyen *et al.* (1992) (Table 6.14). Phosphate solubilization by all the 47 isolates was therefore, further confirmed by monitoring pH drop (initial pH 7.0) and soluble phosphorus (P) content in liquid NBRIP culture medium over a time period of 72 hours. All the isolates exhibited varying degree of P solubilisation (Table 6.14). Maximum P solubilisation was detected after 12 hours for 4 isolates, 24 hours for 24 isolates and 48 hours for 19 isolates. In all cases, P solubilisation was concomitant with a significant pH decrease (Table 6.15). In case of forest ecosystem, isolate LU1RA108 (*Burkholderia arboris*) from UF showed highest P solubilization followed by LU1RA101 (*Burkholderia tropica*) from UF stands, LU1RA406 (*Burkholderia cepacia*) from RG10 and LU1RA307 (*Burkholderia bryophila*) from RF5 stands. From grassland ecosystem, isolate LU2RA201 (*Burkholderia unamae*) from MG sites showed highest P solubilization followed by LU2RA205 (*Burkholderia tropica*) from MG and isolate LU2RA102 (*Burkholderia glumae*) from UG sites. All these above isolates which solubilised greater amounts of P in liquid culture medium as compared to other isolates, also exhibited clear halos during plate assay except isolate LU2RA205 (*Burkholderia tropica*) from MG sites in grassland ecosystem which failed to show halo formation despite solubilising a substantial quantity of P in the liquid NBRIP medium.

Table 6.14: Phosphorus solubilization efficiency of the isolates at different time periods of growth in NBRIP broth and tri–calcium phosphate solubilization by PSB isolates in NBRIP agar. (Values are mean of three replicates and highest soluble P is in bold for each isolate).

Isolate	Stand	Species	Soluble P (mg L^{-1})			[illegible]
			12 hr	24hr	48hr	
LU1RA108	UF	*Burkholderia arboris* (R–24201)	25.1±0.01	**29.9±0.01**	23.3±0.20	183±11.9
LU2RA201	MG	*Burkholderia unamae* (MTl–641)	25.1±0.12	**27.4±0.22**	21.8±0.07	115±0.9
LU1RA101	UF	*Burkholderia tropica* (Ppe8)	24.8±0.17	**26.9±0.01**	21.9±0.10	134±2.7
LU2RA205	MG	*Burkholderia tropica* (Ppe8)	25.1±0.01	**26.4±0.10**	23.3±0.01	–
LU2RA107	UG	*Burkholderia unamae* (MTl–641)	24.0±0.12	**26.0±0.01**	20.7±0.20	124±5.5
LU1RA406	RF10	*Burkholderia cepacia* (717)	24.3±0.03	**26.0±0.08**	22.3±0.13	152±4.0
LU2RA102	UG	*Burkholderia glumae* (P 1–22–1)	23.2±0.22	**25.3±0.01**	17.8±0.07	120±0.0
LU1RA307	RF5	*Burkholderia bryophila* (LMG 23644)	23.3±0.00	23.8±0.01	**24.9±0.01**	153±6.7
LU1RA404	RF10	*Burkholderia terrae* (KMY02)	21.6±0.01	22.7±0.00	**24.7±0.08**	–
LU1RA403	RF10	*Burkholderia arboris* (R–24201)	22.6±0.01	**24.0±0.09**	23.4±0.04	–
LU2RA302	RG15	*Bacillus amyloliquefaciens* FZB42 (FZB42)	22.1±0.07	**24.0±0.06**	20.6±0.04	141±1.8
LU1RA203	CF1	*Burkholderia arboris* (R–24201)	23.3±0.06	**23.8±0.08**	22.9±0.01	129±10.8
LU1RA405	RF10	*Pseudomonas koreensis* (Ps 9–14)	21.8±0.02	22.7±0.09	**23.6±0.22**	–
LU2RA101	UG	*Burkholderia cepacia* (LMG 14294)	22.3±0.01	**23.4±0.02**	18.9±0.05	–
LU1RA301	RF5	*Burkholderia phenazinium* (A 1)	22.1±0.11	**23.2±0.00**	20.7±0.04	–
LU1RA102	UF	*Burkholderia mimosarum* (PAS44)	**23.1±0.13**	22.4±0.11	20.7±0.06	–
LU1RA402	RF10	*Burkholderia fungorum* (LMG 16225)	17.6±0.00	**22.8±0.01**	19.0±0.18	–
LU1RA107	UF	*Burkholderia caledonica* (LMG 19076)	19.9±0.01	**22.8±0.15**	20.3±0.06	–
LU2RA203	MG	*Agrobacterium rhizogenes* (IAM 14222)	18.9±0.01	19.7±0.01	**22.7±0.01**	–
LU2RA104	UG	*Pseudomonas moraviensis* (CCM 7280)	**22.7±0.00**	21.3±0.00	19.6±0.02	–
LU1RA106	UF	*Burkholderia sacchari* (IPT10)	19.4±0.07	**22.7±0.00**	20.3±0.18	–
LU2RA307	RG15	*Burkholderia fungorum* (LMG 16225)	18.5±0.31	19.4±0.01	**22.4±0.01**	–
LU1RA401	RF10	*Burkholderia phytofirmans* PsJN (PsJN)	**22.3±0.06**	21.4±0.10	20.7±0.11	–
LU2RA305	RG15	*Burkholderia cepacia* (LMG 14294)	21.3±0.01	**22.2±0.09**	20.0±0.01	–

LU2RA105	UG	*Burkholderia sartisoli* (RP007)	19.2±0.01	**21.8±0.02**	20.8±0.01	–
LU1RA305	RF5	*Pseudomonas koreensis* (Ps 9–14)	21.3±0.05	**21.7±0.11**	20.6±0.00	–
LU2RA303	RG15	*Burkholderia mimosarum* (PAS44)	19.4±0.06	20.2±0.02	**21.4±0.01**	–
LU2RA202	MG	*Agrobacterium rhizogenes* (IAM 14222)	19.1±0.01	20.4±0.02	**21.3±0.07**	–
LU1RA201	CF1	*Burkholderia ambifaria* AMMD (AMMD)	19.6±0.01	**21.2±0.11**	19.1±0.01	–
LU2RA401	RG50	*Burkholderia nodosa* (Br3437)	18.0±0.07	18.9±0.03	**21.1±0.04**	–
LU2RA403	RG50	*Burkholderia mimosarum* (PAS44)	18.9±0.03	**20.8±0.01**	16.8±0.18	–
LU2RA103	UG	*Burkholderia sacchari* (IPT10)	18.4±0.01	19.5±0.02	**20.8±0.06**	–
LU2RA402	RG50	*Burkholderia mimosarum* (PAS44)	18.4±0.02	**20.8±0.05**	19.6±0.17	–
LU1RA306	RF5	*Burkholderia terrae* (KMY02)	18.7±0.16	**20.3±0.16**	20.1±0.02	–
LU2RA405	RG50	*Burkholderia terrae* (KMY02)	19.2±0.03	20.1±0.39	**20.3±0.05**	–
LU1RA304	RF5	*Burkholderia terrae* (KMY02)	17.0±0.03	19.3±0.01	**20.2±0.10**	–
LU2RA404	RG50	*Burkholderia sabiae* (Br3407)	19.1±0.16	19.6±0.13	**20.0±0.09**	–
LU1RA407	RF10	*Burkholderia phytofirmans* PsJN (PsJN)	18.3±0.08	18.8±0.01	**20.0±0.07**	–
LU1RA105	UF	*Burkholderia glathei* (N15)	19.1±0.02	**20.0±0.00**	19.1±0.01	–
LU2RA308	RG15	*Brevibacillus agri* (DSM 6348)	18.6±0.09	18.8±0.09	**19.9±0.02**	–
LU1RA103	UF	*Burkholderia phenoliruptrix* (AC1100)	18.9±0.01	19.2±0.00	**19.9±0.08**	–
LU1RA303	RF5	*Burkholderia phenazinium* (A 1)	18.5±0.02	19.0±0.06	**19.9±0.18**	–
LU2RA306	RG15	*Burkholderia arboris* (R–24201)	17.0±0.00	18.3±0.06	**19.7±0.09**	–
LU1RA104	UF	*Burkholderia fungorum* (LMG 16225)	**19.6±0.04**	19.5±0.01	18.7±0.02	–
LU1RA202	DF1	*Bacillus amyloliquefaciens* (NBRC 15535)	18.9±0.01	18.8±0.12	**19.4±0.24**	–
LU2RA301	RG15	*Burkholderia mimosarum* (PAS44)	18.5±0.02	18.9±0.05	**19.2±0.01**	–
LU2RA304	RG15	*Burkholderia mimosarum* (PAS44)	17.8±0.10	**19.2±0.30**	18.9±0.13	–

*z/n – Solubilization efficiency index (%), determined as the proportion of the solubilization halo (z) to the colony diameter (n) on NBRIP agar.

Table 6.15: Reduction in pH of NBRIP broth associated with the microbial phosphate solubilization (Values are mean of three replicates).

Species	pH		
	12hr	24hr	[illegible]
Burkholderia arboris (R–24201)	4.11±0.02	**3.56±0.01**	5.52±0.03
Burkholderia unamae (MTl–641)	4.09±0.02	**3.59±0.03**	5.66±0.02
Burkholderia tropica (Ppe8)	4.00±0.04	**3.84±0.02**	5.62±0.02
Burkholderia tropica (Ppe8)	3.73±0.02	**3.41±0.03**	5.77±0.02
Burkholderia unamae (MTl–641)	4.16±0.02	**3.53±0.03**	5.51±0.02
Burkholderia cepacia (717)	4.01±0.03	**3.86±0.02**	5.30±0.03
Burkholderia glumae (P 1–22–1)	4.13±0.02	**3.82±0.03**	5.87±0.02
Burkholderia bryophila (LMG 23644)	4.77±0.04	4.50±0.02	**4.05±0.02**
Burkholderia terrae (KMY02)	4.97±0.01	4.60±0.03	**4.06±0.02**
Burkholderia arboris (R–24201)	4.46±0.02	**4.26±0.02**	4.33±0.02
Bacillus amyloliquefaciens FZB42 (FZB42)	4.12±0.02	**4.03±0.02**	5.32±0.03
Burkholderia arboris (R–24201)	4.30±0.03	**4.25±0.02**	4.54±0.02
Pseudomonas koreensis (Ps 9–14)	4.84±0.02	4.66±0.02	**4.29±0.04**
Burkholderia cepacia (LMG 14294)	4.19±0.05	**4.14±0.02**	6.13±0.01
Burkholderia phenazinium (A 1)	5.06±0.03	**4.59±0.03**	6.00±0.04
Burkholderia mimosarum (PAS44)	**4.74±0.02**	4.84±0.03	5.47±0.04
Burkholderia fungorum (LMG 16225)	6.75±0.02	**4.52±0.03**	6.49±0.03
Burkholderia caledonica (LMG 19076)	5.73±0.03	**4.84±0.02**	5.21±0.03
Agrobacterium rhizogenes (IAM 14222)	6.57±0.02	6.21±0.03	**4.50±0.05**

Pseudomonas moraviensis (CCM 7280)	**4.48±0.01**	4.88±0.02	5.41±0.02
Burkholderia sacchari (IPT10)	6.04±0.03	**4.60±0.02**	5.19±0.03
Burkholderia fungorum (LMG 16225)	6.68±0.03	6.07±0.02	**4.69±0.03**
Burkholderia phytofirmans PsJN (PsJN)	**4.70±0.02**	4.87±0.01	5.37±0.02
Burkholderia cepacia (LMG 14294)	4.71±0.03	**4.25±0.02**	5.91±0.03
Burkholderia sartisoli (RP007)	6.29±0.03	**5.05±0.02**	5.45±0.01
Pseudomonas koreensis (Ps 9–14)	5.02±0.03	**4.92±0.02**	5.34±0.02
Burkholderia mimosarum (PAS44)	6.18±0.02	6.00±0.05	**5.20±0.03**
Agrobacterium rhizogenes (IAM 14222)	5.87±0.02	5.66±0.02	**4.76±0.01**
Burkholderia ambifaria AMMD (AMMD)	6.42±0.02	**5.06±0.03**	6.52±0.03
Burkholderia nodosa (Br3437)	6.79±0.03	6.50±0.02	**4.92±0.04**
Burkholderia mimosarum (PAS44)	6.49±0.03	**5.36±0.02**	6.93±0.03
Burkholderia sacchari (IPT10)	6.3±0.02	6.29±0.01	**5.33±0.03**
Burkholderia mimosarum (PAS44)	6.57±0.04	**5.37±0.02**	6.24±0.03
Burkholderia terrae (KMY02)	6.16±0.03	**6.05±0.02**	5.22±0.02
Burkholderia terrae (KMY02)	6.31±0.03	6.28±0.03	**6.06±0.03**
Burkholderia terrae (KMY02)	6.41±0.02	6.03±0.02	**5.75±0.01**
Burkholderia sabiae (Br3407)	6.51±0.02	6.15±0.02	**6.06±0.01**
Burkholderia phytofirmans PsJN (PsJN)	6.65±0.02	6.22±0.01	**5.18±0.02**
Burkholderia glathei (N15)	6.32±0.01	**6.11±0.03**	6.32±0.03
Brevibacillus agri (DSM 6348)	6.28±0.03	6.28±0.05	**6.06±0.02**
Burkholderia phenoliruptrix (AC1100)	6.29±0.02	6.21±0.02	**6.00±0.03**
Burkholderia phenazinium (A 1)	6.52±0.03	6.40±0.02	**6.05±0.02**
Burkholderia arboris (R–24201)	6.37±0.02	6.13±0.03	**5.19±0.05**
Burkholderia fungorum (LMG 16225)	**6.01±0.03**	6.09±0.01	6.43±0.02
Bacillus amyloliquefaciens (NBRC 15535)	6.53±0.01	6.57±0.02	**6.31±0.03**
Burkholderia mimosarum (PAS44)	6.47±0.03	6.21±0.03	**6.03±0.03**
Burkholderia mimosarum (PAS44)	6.48±0.03	**6.04±0.02**	6.09±0.03

Assessment of phosphate solubilization in pot experiment:

Dose effects were not significant ($p>0.05$) on any of the measured parameters viz., plant height, plant dry weight, total leaf area and phosphorus solubilization efficiency while TCP addition had significant stimulatory ($p<0.00$) affect on all the parameters (Table 6.16). All growth parameters except total leaf area significantly ($p<0.00$) varied between the two growth stages viz., vegetative and reproductive stages. PSB application had a significant ($p<0.00$) positive impact on all the parameters measured except plant height. It significantly ($p<0.00$) increased plant dry weight, total leaf area and phosphorus solubilization efficiency as compared to the control plants (Table 6.16). In case of plant dry weight, only inoculation with *Burkholderia cepacia* (isolate LU1RA406 from RF10) showed significant (Tukey's test, $p<0.05$) increase over the control plants (178.8% increase over control without TCP and 295.8% with TCP) (Fig. 6.13). For total leaf area, maximum stimulatory effects were obtained by *Burkholderia cepacia* (isolate LU1RA406 from RF10) (141.0% without TCP and 154.6% with TCP) followed by *Burkholderia tropica* (isolate LU2RA205 from MG) (212.8% without TCP and 266.7% with TCP) (Fig. 6.14). Phosphorus solubilization efficiency (PSE) was significantly (Tukey's test, $p<0.00$) increased by *Burkholderia cepacia* (isolate LU1RA406 from RF10) and *Burkholderia tropica* (isolate LU2RA205 from MG) over the control plant. *Burkholderia cepacia* (isolate LU1RA406 from RF10) exhibited maximum PSE (Table 6.17).

Fig. 6.10: Assessing Phosphorus solubilising efficiency through crop application (5 days after planting).

Fig. 6.11: Assessing Phosphorus solubilising efficiency through crop application (90 days after planting).

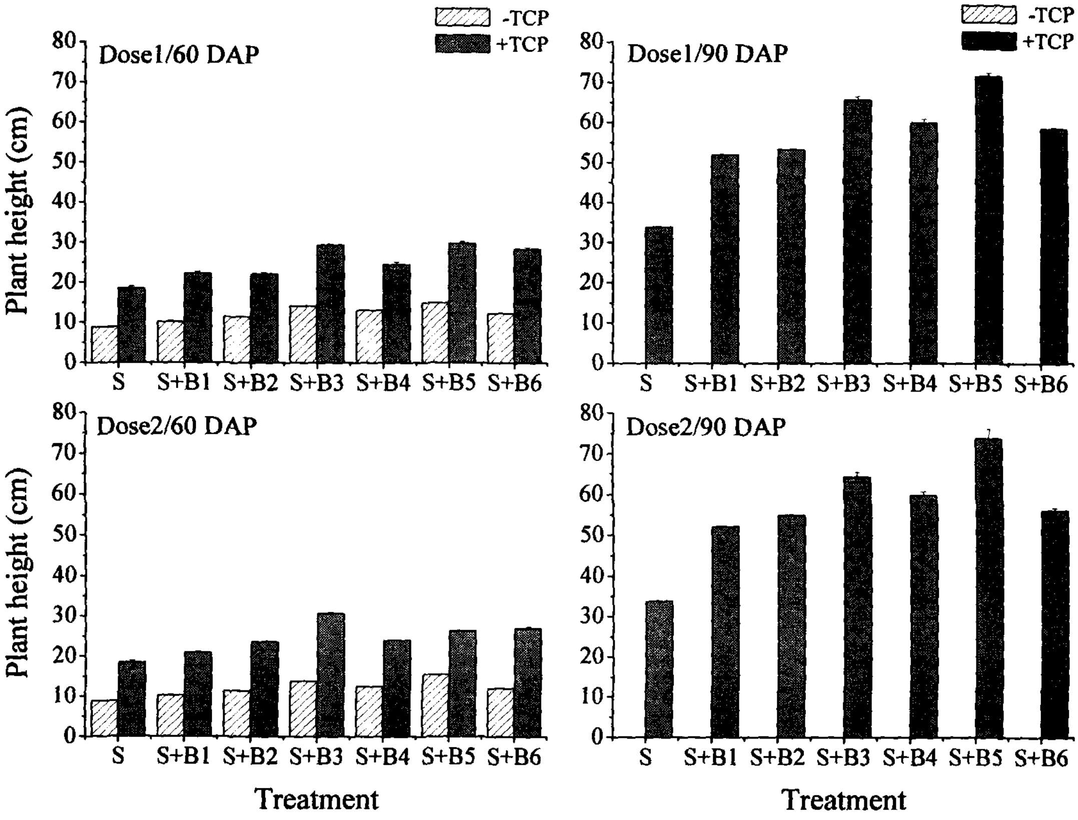

Fig. 6.12: Effect of PSB treatment, varying dose of PSB inoculum, presence/absence of TCP and time of harvest on plant height.

S – sterilized soil; B1 – *Burkholderia unamae* (isolate LU2RA107 form UG); B2 – *Burkholderia unamae* (isolate LU2RA201 from MG); B3 – *Burkholderia tropica* (isolate LU2RA205 from MG); B4 – *Burkholderia bryophila* (isolate LU1RA307 from RF5); B5 – *Burkholderia cepacia* (isolate LU1RA406 from RF10); B6 – *Burkholderia arboris* (isolate LU1RA108 from UF); DAP – Days after planting; TCP – tri–calcium phosphate

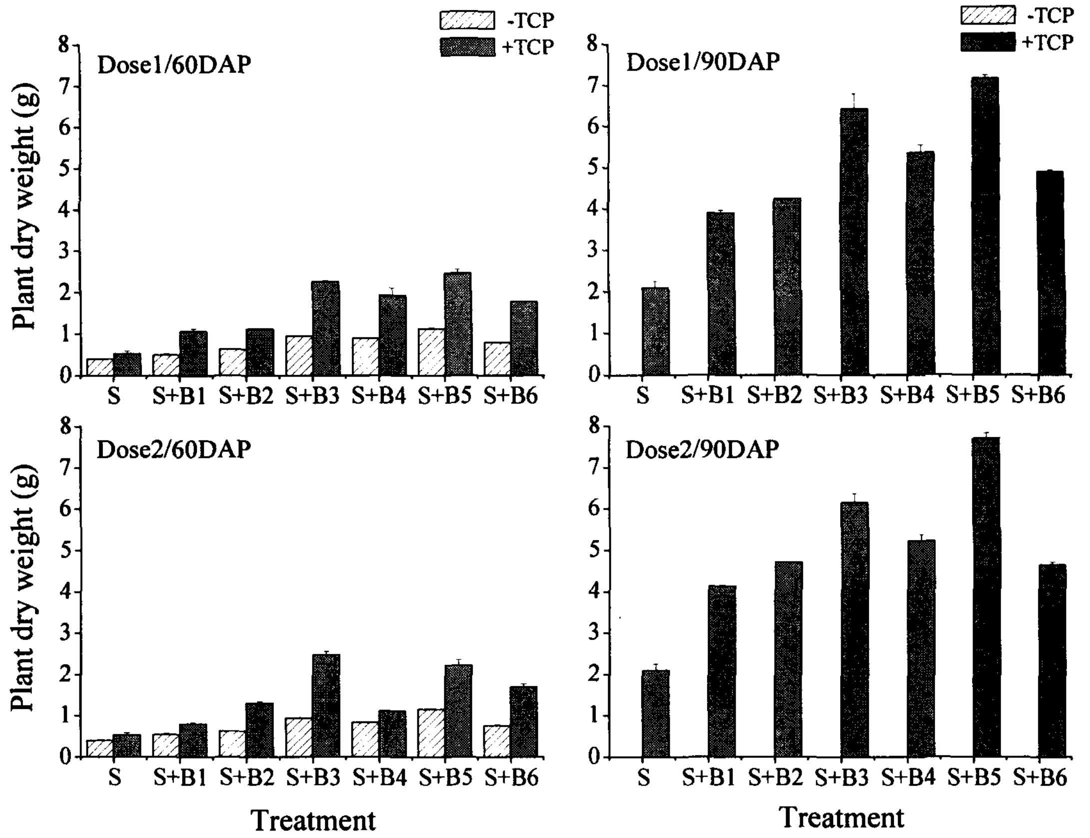

Fig. 6.13: Effect of PSB treatment, varying dose of PSB inoculum, presence/absence of TCP and time of harvest on plant dry weight.

S – sterilized soil; B1 – *Burkholderia unamae* (isolate LU2RA107 form UG); B2 – *Burkholderia unamae* (isolate LU2RA201 from MG); B3 – *Burkholderia tropica* (isolate LU2RA205 from MG); B4 – *Burkholderia bryophila* (isolate LU1RA307 from RF5); B5 – *Burkholderia cepacia* (isolate LU1RA406 from RF10); B6 – *Burkholderia arboris* (isolate LU1RA108 from UF); DAP – Days after planting; TCP – tri–calcium phosphate

Table 6.16: Results of ANOVA to test the effect of inoculum dose, plant growth stage, inoculum treatment and TCP on plant height, plant dry weight, total leaf area and phosphorus solubilization efficiency.

	df	Plant height (cm)		Plant dry weight (g)		Total leaf area (cm^2)		PSE	
		F	p	F	p	F	p	F	p
Dose	1	0.7	0.40	0.6	0.44	0.2	0.69	0.05	0.82
Growth stage	1	5395.9***	0.00	3156.1***	0.00	1.2	0.27	3039.0***	0.00
Treatment	6	1.00	0.43	445.3***	0.00	168.9***	0.00	1273.0***	0.00
TCP	1	66140.8***	0.00	14680.9***	0.00	9312.6***	0.00	19552.3***	0.00

TCP – tri calcium Phosphate; PSE – Phosphorus solubilization efficiency; *** significant at $p<0.001$

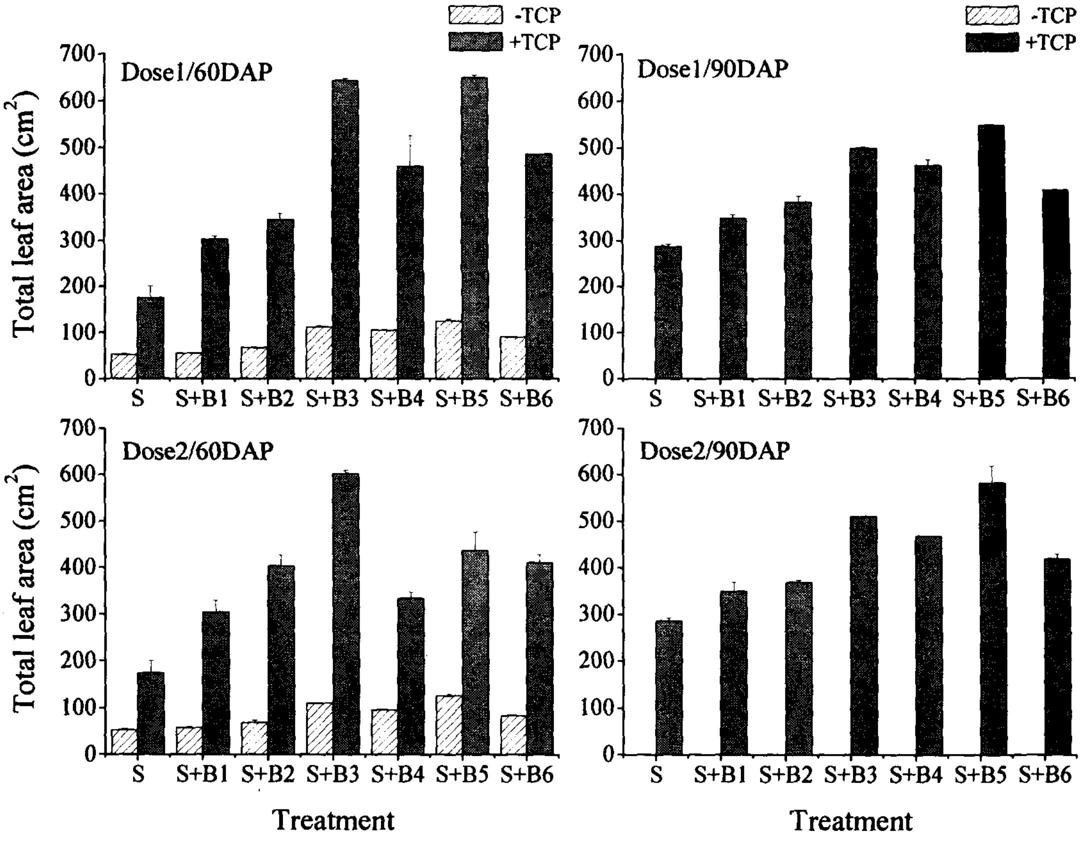

Fig. 6.14: Effect of PSB treatment, varying dose of PSB inoculum, presence/absence of TCP and time of harvest on total leaf area.

S – sterilized soil; B1 – *Burkholderia unamae* (isolate LU2RA107 form UG); B2 – *Burkholderia unamae* (isolate LU2RA201 from MG); B3 – *Burkholderia tropica* (isolate LU2RA205 from MG); B4 – *Burkholderia bryophila* (isolate LU1RA307 from RF5); B5 – *Burkholderia cepacia* (isolate LU1RA406 from RF10); B6 – *Burkholderia arboris* (isolate LU1RA108 from UF); DAP – Days after planting; TCP – tri–calcium phosphate

Table 6.17: Phosphorus solubilization efficiency (PSE) based on crop application experiment of six most efficient PSB species.

Treatment	**Dose1**			**Dose2**		
	60 DAP		**90 DAP**	**60 DAP**		**90 DAP**
	–TCP	+TCP	+TCP	– TCP	+TCP	+TCP
S	0.3±0.00	0.8±0.06	3.4±0.11	0.3±0.00	0.8±0.06	3.4±0.11
S + *Burkholderia unamae*	0.9±0.03	2.2±0.19	8.3±0.16	1.0±0.03	1.5±0.01	8.5±0.17
S + *Burkholderia unamae*	1.2±0.03	2.6±0.03	9.6±0.03	1.2±0.03	3.1±0.09	10.4±0.05
S + *Burkholderia tropica*	2.0±0.04	8.6±0.05	17.7±0.77	2.0±0.04	9.6±0.43	16.8±0.47
S + *Burkholderia bryophila*	1.8±0.01	5.7±0.62	13.4±0.46	1.7±0.03	3.4±0.07	13.0±0.32
S + *Burkholderia cepacia*	**2.6±0.10**	**10.1±0.41**	**25.1±0.42**	**2.7±0.07**	**9.1±0.45**	**23.5±0.09**
S + *Burkholderia arboris*	1.6±0.03	5.0±0.01	11.6±0.15	1.5±0.01	4.7±0.28	11.0±0.06

S – Sterilized soil; DAP – Days after planting; TCP – tri calcium phosphate

DISCUSSION

Phosphorus dynamics during ecosystem recovery

The decline in extractable P after disturbance may be partly attributed to the weathering of soil due to removal of vegetation cover. Highly weathered soils have been reported to be associated with high P–sorption capacities (Henry and Smith, 2002) leading to low availability of P for plants. In addition, the significantly low extractable P in DF1 and MG sites might be due to the higher soil temperature in these sites. Higher soil temperature has been found to reduce P solubility by increasing the rate of P immobilization (van Meerteren *et al.*, 2007) and P sorption by the soil matrix (Sah and Mikkelsen, 1986). Roberts *et al.* (1988) reported that extractable P decreased in the early stages of mine soil genesis following rapid Fe oxidation and possible plant and microbial uptake. P availability increased with the progress of recovery as the soil conditions improved and temperature reduced due to vegetation recovery. Also, increased microbial activities and improvement of biological processes with recovery age might be responsible for the greater availability of P. The range of Bray's extractable P reported in the present study was lower than that reported by Pandey and Srivastava (2009) (0.05–2.08 $\mu g\ g^{-1}$). This might be due to the lower pH in this study. Phosphate uptake on metal hydroxide in soils generally increases with decreasing pH (Arai and Sparks, 2007).

The decline of MBP after disturbance is in agreement with other researchers (Srivastava, 1992; Tan *et al.*, 2008). This declining trend may be attributed to reduced microbial population owing to the lowered pH after disturbance. Decline of soil pH in acidic soils can decrease microbial biomass (Aciego Pietri and Brookes, 2008). Rousk *et al.* (2009) found that a largely acidic soil pH disfavoured bacterial biomass and it declined as pH value dropped below 4.5. Also the increased bulk density after disturbance was most likely responsible for the decline in microbial biomass as it reduced soil porosity and affected aeration (Tan *et al.*, 2005). The amount of microbial biomass P obtained in this study was comparable with Brookes *et al.* (1984) (5–67 μg g^{-1}), Arunachalam and Arunachalam (2000) (23–46 μg g^{-1}), Barbhuiya *et al.* (2004) (19–37 μg g^{-1}) and Tan *et al.* (2008) (20–40 μg g^{-1}).

Microorganisms have the capacity to act both as a sink and a source of nutrient resources. During the mineralization process, a substantial fraction of P in the decomposing residues is immobilized in the microbial biomass as part of their cellular constituents, and then released upon microorganism's death (Jonasson *et al.*, 1999). The percentage contribution of microbial biomass P to total P in the present study was higher than the values reported by Maithani *et al.* (1996) (2.8–7.9%), and Yang *et al.* (2010) (2.6–5.5%). This suggests that microbial communities in the present study sites have evolved a more complex system of substrate–use efficiency with the heterogeneous input of organic matter (Ralte, 2004), enabling them to immobilise a greater portion of P in their biomass. This higher rate of immobilization of P in the microbial biomass observed in the present study might be a possible reason for the very low available P in these ecosystems.

Sinsabaugh and Moorhead (1997) demonstrated that the synthesis of extracellular enzymes is subject to regulation by induction and repression/derepression mechanisms, which are linked to nutrient availability in the surrounding environment. Phosphatase is an inducible enzyme and can therefore, vary with the demand for P by plant roots and soil microbes (Juma and Tabatabai, 1978; Chen *et al.*, 2008). The decreased concentration of available P during post disturbance period induced synthesis and release of phosphatase enzyme resulting in greater activity in DF1 stand than the recovering and the undisturbed stands. In addition, the greater SOC content in this stand might be another reason for the enhanced activity, as acid phosphatase activity is largely dependent on soil properties such as SOC (Speir and Ross, 1978). However, the increased phosphatase activity in DF1 stand does not correspond to a higher biomass. This suggests that the increased activity is not due to enhanced microbial production of phosphatase enzyme. Rather it might be attributed to increased specific activity of phosphatase enzyme due the prevailing stress or secretion by plant roots as phosphatase can be produced by both microbes and plant roots (Johnson *et al.*, 1999). Haussling and Marschner (1989) and Izquierdo *et al.* (2005) also pointed out that increased phosphatase activity in the rhizosphere may be due to an induced secretion by the plant roots. The decline in activity during the initial years of recovery as evident from DF5 values might be attributed to reduced amount of organic matter and poor substrate quality. The DF5 stand lacks any natural litter layer and is left mainly with the coarse woody residues from the slash generated after deforestation, as the fine residues were decomposed during the early years of degradation. The woody residues contain larger and recalcitrant macromolecules that are more resistant to decay and therefore, decomposition takes several years (Hyvonen *et al.*, 2000). After 5th year of recovery the activity increased showing net recovery. This finding was consistent with Allison *et al.* (2007), Jiang *et al.* (2009) and Huang *et al.* (2011). These workers reported that acid phosphatase activity was closely linked to forest successional stages, with enhanced values in the later stages of forest succession. Baldrian *et al.* (2008) reported an increase in acid phosphatase activity with recovery age in grassland ecosystems which had been degraded by mining activities.

The higher extractable P during wet season than the dry season might be attributed to the enhanced water filled pore space due to rainfall (Pandey and Srivastava, 2009) which restricted the diffusion of oxygen from the atmosphere to the soils (Smith and Tiedje, 1979) thus, creating anaerobic condition in soils. This anaerobic condition reduces the redox potential of soil promoting Fe reduction and the concomitant release of Fe–bound P (Chacon *et al.*, 2006; Pandey and Srivastava, 2009; Liptzin and Silver, 2009). Weber *et al.* (2006) reported links between soil redox potential, iron cycling and P availability.

Microbial biomass P followed a similar seasonal trend as microbial biomass C discussed in chapter 5. The lower microbial biomass P during wet season than the dry period corroborates with Arunachalam and Arunachalam (2000), Singh and Yadava (2006) and Kamei (2007). The low microbial biomass P during the rainy season could be due to high turnover rate of microorganism and peak growth of vegetation during the wet periods, whereas during the dry period plant residues continue to decompose but plant uptake as well as microbial turnover is slowed down. This results in immobilisation of the excess P into the microbial biomass.

The seasonal influence on phosphatase activity was similar to the trend shown by the other two enzymes viz., dehydrogenase and β–glucosidase studied in relation to carbon cycle in chapter 5. During wet season, plant growth is very fast and immense as well as microbial proliferation. Also leaching losses become high during the wet season due to heavy rainfall. Therefore, the greater demand for P nutrition is met by increasing phosphates activity. This is in agreement with Grierson and Adams (2000) and Huang *et al.* (2011). Sardans and Penuelas (2005) reported a decrease in acid phosphatase activity during the dry season and suggested that water plays a critical role for enzyme activity. Acid phosphatase activity was significantly correlated with soil moisture in both the ecosystems. This result is in agreement with other reports in the literature, showing that acid phosphatase activity is well correlated with soil water availability (Kramer and Green, 2000; Sardans and Penuelas, 2005; Baldrian *et al.*, 2010; Ushio *et al.*, 2010). Soil moisture has been reported as a key factor that controls P availability in soils as it has been found to affect mineralization processes, influence P demand for plant growth and impact microbial activity (Grierson and Adams, 2000; Sardans *et al.*, 2007). Huang *et al.* (2011) also observed that acid phosphatase activity was depressed by no precipitation. Significant positive effects of SOC, and total N on acid phosphatase activity observed in the present study was consistent with Ushio *et al.* (2010) and Cheng *et al.* (2013).

Treseder and Vitousek (2001) suggested that phosphatase production requires substantial quantities of N. A negative correlation between the inorganic P fractions (water extractable P and Bray's extractable P) and acid phosphatase activity was observed which was consistent with other researchers (Wright and Reddy, 2001; Allison and Vitousek, 2005; Allison *et al.*, 2007). This is due to the inhibition of acid phosphatase activity by available P (negative feedback regulation). MBP was positively correlated with total P ($p<0.00$) in forest ecosystem and with SOC in grassland ecosystem. Maithani *et al.* (1996) reported positive correlation between MBP and SOC. The significant positive correlation between microbial biomass P and total P indicates that it could be used as an indicator of ecosystem recovery. In conclusion, microbial biomass played a crucial role in the present study as it acted as a transient sink of labile P. The activity of acid phosphatase increased with recovery age in both the ecosystems.

Role of phosphate solubilising bacteria

The present study used a combination of approaches viz., morphological, biochemical and 16S rRNA gene sequences for the identification of PSB from undisturbed and recovering stands of forest and grassland ecosystems. The phosphate solubilization potential of PSB species was evaluated through *in vitro* experiments as well as crop application. The decrease in the population size and diversity of PSB after disturbance was in agreement with Liu *et al.* (2000), who reported decrease of soil microbial diversity with disturbance. *Burkholderia* was the dominant genus involved in P solubilization in both the ecosystems. Recent studies have found a high abundance and growth of *Burkholderia* in acidic soils (Curtis *et al.*, 2002; Belova *et al.*, 2006). This could be one of the reasons of *Burkholderia* abundance in the present study as the soils in both the ecosystems are acidic.

In spite of growing in selective NBRIP medium, only a few isolates formed a halo around the colony. On the other hand, all the isolates growing on NBRIP medium reduced pH suggesting that pH reduction assay is a far superior tool to screen PSB. Isolate LU2RA205 (*Burkholderia tropica*) from MG sites in the grassland ecosystem that failed to produce a clear halo during agar plate assay demonstrated substantial P solubilization (4th highest) in liquid NBRIP medium. This was also argued by Gupta *et al.* (1994) who reported that many isolates not producing any visible halo on agar plates were indeed able to solubilize different types of insoluble phosphates in liquid medium.

Results of in vitro study to monitor the solubilization activities on liquid NBRIP medium demonstrated that 8.5% isolates displayed highest P solubilization activity after 12 hours, 51.1% after 24 hours and 40.4% after 48 hours. The difference in time required for the highest P solubilisation activity by different species was due to differential growth rate exhibited by the concerned species as well as their pH reduction ability through secretion of organic acids. Kumar *et al.* (2010) obtained a linear relationship between soluble phosphorus level in the supernatant and growth of culture. Perez *et al.* (2007) also argued that acidification is one of the main strategies followed by the PSB for P solubilization. However, further experimentation is required to confirm the hypothesis of organic acid secretion and other mechanisms used by these PSB isolates to solubilise P.

Crop application of selected PSB showed positive effects on the growth of maize plants. Inoculation with PSB made more soluble phosphates available to the growing plants and therefore, was the reason for improved growth of maize plants in pot experiment. Many bacteria (Rodriguez and Fraga, 1999) are able to improve plant growth by solubilizing sparingly soluble inorganic phosphates in the soil. Although pot experiment confirmed the potential of the isolates to promote plant growth, further field trials are necessary to recommend them as biofertilizers. This is because of the fact that the competence of PSB in the natural field will depend on a number of factors such as nutrient limitation, competition with indigenous microbes as well as precipitation of soluble phosphorus with cations present in the soil (Rodríguez and Fraga, 1999).

The isolate LU1RA108 (*Burkholderia arboris*) from UF stands solubilised more P during *in vitro* study than the isolates from recovering stands of forest ecosystem. However, crop application experiment i.e. stressed pot environment represented by low nutrient availability vis–a–vis nutrient rich *in vitro* condition, showed that the isolate LU1RA406 (*Burkholderia cepacia*) from the recovering RF10 stands exhibited greater P solubilization efficiency than isolate LU1RA108 (*Burkholderia arboris*) from UF stands. This suggests that the PSB species with inherent high solubilising efficiency in *in vitro* conditions failed to retain the similar efficiency when exposed to stress. In contrast, species with intermediate level of efficiency in *in vitro* conditions excels in the stressed conditions. In case of grassland ecosystem, isolate LU2RA201 (*Burkholderia unamae*) from MG sites solubilised highest amount of P during *in vitro* study while isolate LU2RA205 (*Burkholderia tropica*) from MG sites showed greater P solubilization efficiency over isolate LU2RA107 (*Burkholderia unamae*) from UG sites. This suggested that stress originating from disturbance events such as mining related activities, resulted in occurrence of more efficient strains of PSB in disturbed ecosystems. This finding was consistent with Kumar *et al.* (2010) who reported enhanced phosphorus solubilising efficiency of Enterobacter species isolated from an agricultural farm under salt and temperature stress.

Therefore, it may be concluded that the species of *Burkholderia* genus in the soil microbial community have been playing an important role in P dynamics during ecosystem recovery process as they are abundant (39 out of 47 species) and most efficient species. The occurrence of most efficient PSB species in the recovering forest stands that represent a stressed environment in terms of low available P suggests that the PSB community responds to such stress through occurrence of efficient strains. Such a response is key to achieve resiliency of the ecosystem in terms of P availability. In contrast, the most efficient species in case of grassland ecosystem were found in the mining affected grassland site. Also, the presence of certain species exclusive to a particular recovery age and absence of any common species among the stands suggest that PSB community in general is not a resilient community because they are dependent on age of recovery.

CHAPTER 7
GENERAL DISCUSSION

Notwithstanding the constraints of chronosequence studies (Nyberg *et al.*, 2012), the present study clearly demonstrated that the land use changes in two ecosystems of Cherrapunjee plateau viz., forest and grassland induced substantial changes in microbial communities in terms of density and diversity, and also affected their dynamics and activities. Such changes in microbial communities had a direct impact on ecosystem functioning and ecosystem stability. As the microbial communities, plant communities, nutrient availability and ecosystem processes are interlinked in an ecosystem, changes in any one of these components exerted a significant influence on the others. Ecosystem recovery quantified by examining changes in microbial community structure and functions, profiling the ensuing C and P regimes, and monitoring the changes in vegetation structure and soil properties over time, revealed that microbes did play a major role in both the ecosystems during post–disturbance recovery process. As stress resulting from ecosystem degradation reduced with recovery age, the functional diversity and activity of the soil microbial communities increased. The microbes influenced the recovery process by impacting various ecosystem processes and functions through their activities such as enzyme activities, mineralization and immobilization.

Microbial community structure and functions related to C–dynamics

Disturbances in the form of deforestation in the forest ecosystem, and mining related activities in the grassland ecosystem modified the total amount of soil organic matter, which had a direct effect on the biomass, population size and activities of microbial community. Microbial biomass, activities and processes, extra–cellular enzyme levels (dehydrogenase and β–glucosidase) and their activities are important indicators used in the present study to characterize the soil carbon dynamics. A decline in the biological properties associated with C cycle viz., enzyme activity, microbial biomass carbon, MBC/SOC ratio and basal respiration was observed following disturbance. This was attributed to decrease in the microbial population size as well as lower input of organic matter due to reduced vegetation during post–disturbance period. As recovery progressed, these parameters gradually increased due to greater accumulation of plant derived organic matter and microbial products (Maithani *et al.*, 1998), greater substrate availability and increase in microbial population numbers (Reeves, 1997).

MBC/SOC ratio that describes the contribution of microbial biomass to soil organic carbon, and also indicates the availability of labile C, and the fraction of recalcitrant organic matter in the soil, increased as recovery progressed and indicated accumulation of labile C in soil and favourable environment for microbial growth. As microbial populations are largely dependent on the soil labile C as their C source, low MBC/SOC ratio after disturbance was indicative of poor quality of organic matter (Cheng *et al.*, 2013).

Since enzyme activities and basal respiration are directly proportional to the size and activity of viable microbial populations, a decline in these functions after disturbance suggests that the viable microbial population either decreased in their size and/or in their functional abilities. A decline in β–glucosidase and dehydrogenase activity after disturbance indicated that the microbial populations allocated most of their energy towards maintenance of a minimum viable population size rather than enzyme production (Koch, 1985). Differences in soil texture could be another explanation for the reduced β–glucosidase activity in CF1 and MG stands, as the recently disturbed sites had more clay content than the recovering and undisturbed sites. Stemmer *et al.* (1998) and Marx *et al.* (2005) concluded that β–glucosidase was more in the coarse sand than the silt and clay fractions.

Basal respiration (BR) that gives an estimation of the activity of heterotrophic soil microbial community and provides information on availability of soil carbon to microorganisms (Ananyeva *et al.*, 2008), declined after disturbance due to the decrease in microbial population. BR increased gradually with recovery age. The microbial metabolic quotient (qCO_2), a bioindicator of ecosystem development as well as of environmental stress on microbial communities, had greater values in the post disturbance sites. This was due to increased availability of readily degradable C indicating that a relatively large percentage of the substrate was decomposed to meet the energy demand of the microflora (Joergensen and Scheu, 1999; Leiros *et al.*, 2000). As recovery progressed, the prevailing stress on soil microbes gradually subsided resulting in decreased qCO_2.

Microbial biomass carbon, enzyme activities and basal respiration exhibited a clear seasonality in both the ecosystems. MBC was highest during winter season and lowest during rainy season. Similar observations were also made by Arunachalam and Arunachalam (2000), Barbhuiya *et al.* (2008). In contrast, enzyme activities and basal respiration were highest during rainy season and lowest during winter. Several authors have attributed this trend to enhanced availability of water in the soil during rainy season (Görres *et al.*, 1998; Banerjee *et al.*, 2000; Quilchano and Maranon, 2002; Sardans *et al.*, 2008).

Among the soil biological parameters studied, dehydrogenase activity, glucosidase activity and basal respiration showed significant correlation with soil moisture and temperature. SOC showed a significant positive relationship with dehydrogenase activity which was consistent with the findings of Leiros *et al.* (2000) and Okur *et al.* (2009). The significant correlation of basal respiration with SOC and Bray's extractable P in forest ecosystem was in agreement with the findings of Cheng *et al.* (2013). MBC showed a significant positive correlation with total P and extractable P in forest ecosystem and with

TKN in grassland ecosystem. Cheng *et al.* (2013) had obtained a similar relationship of MBC with N and P. Liu *et al.* (2012) also reported a strong positive correlation between MBC and total N. Therefore, it may be concluded that the microbial populations were limited by P in forest ecosystem and by N in grassland ecosystem.

Microbial community structure and functions related to P–dynamics

While total P increased immediately after the disturbance both in forest and grassland ecosystems, extractable P declined. The decline in extractable P after disturbance was partly attributed to weathering of soil due to removal of vegetation cover. Highly weathered soils have been reported to be associated with high P–sorption capacities (Henry and Smith, 2002) leading to low availability of P to the plants. Microbial biomass played a crucial role in P dynamics as it acted as a transient sink of labile P. During the mineralization process, a substantial fraction of P in the decomposing residues was immobilized in the microbial biomass as part of their cellular constituents, and then released upon microorganism's death (Jonasson *et al.*, 1999). The percentage contribution of microbial biomass P to total P (7–21%) was higher than the values reported by Maithani *et al.* (1996) (2.8–7.9%), and Yang *et al.* (2010) (2.6–5.5%). This suggests that microbial communities in the present study sites have evolved a more complex system of substrate–use efficiency with the heterogeneous input of organic matter (Ralte, 2004), enabling them to immobilise a greater portion of P in their biomass. This higher rate of immobilization of P in the microbial biomass observed in the present study might be a possible reason for the very low available P in these ecosystems.

The decline in phosphatase activity during the initial years of recovery was attributed to reduced amount of organic matter and poor substrate quality as the stands lacked natural litter layer and contained more fraction of the coarse woody residues from the slash generated after deforestation. Phosphatase is an inducible enzyme and its activity is driven by P demand from plants and soil microbes (Juma and Tabatabai, 1978;

Chen *et al.*, 2008). After 5th year of recovery the activity increased showing net recovery. This finding was consistent with Allison *et al.* (2007), Jiang *et al.* (2009) and Huang *et al.* (2011). These workers reported that acid phosphatase activity was closely linked to forest successional stages, with enhanced values in the later stages of forest succession. Baldrian *et al.* (2008) reported an increase in acid phosphatase activity with recovery age in grassland ecosystems which had been degraded by mining activities.

The extractable P was higher during wet season than the dry seasons, which was attributed to the enhanced water filled pore space due to rainfall (Pandey and Srivastava, 2009) that restricted the diffusion of oxygen from the atmosphere to the soils (Smith and Tiedje, 1979) creating anaerobic condition in soils. This anaerobic condition reduced the redox potential of soil promoting Fe reduction and the concomitant release of Fe–bound P (Chacon *et al.*, 2006; Pandey and Srivastava, 2009; Liptzin and Silver, 2009). The lower microbial biomass P during wet season than the dry period corroborates with Arunachalam and Arunachalam (2000), Singh and Yadava (2006) and Kamei (2007). Acid phosphatase activity was highest during rainy season and lowest during dry season. This seasonal trend was in conformity with the findings of Sardans and Penuelas (2005), who reported a decrease in acid phosphatase activity during the dry season and suggested that water plays a critical role for enzyme activity.

Soil moisture seemed to be a key factor that controlled P availability in soils as evident from the significant correlation between acid phosphatase activity and soil moisture. Several researchers found acid phosphatase activity to be correlated with soil water availability (Kramer and Green, 2000; Sardans and Penuelas, 2005; Baldrian *et al.*, 2010; Ushio *et al.*, 2010). Significant positive effects of SOC, and total N on acid phosphatase activity observed in the present study was consistent with Ushio *et al.* (2010) and Cheng *et al.* (2013). Treseder and Vitousek (2001) suggested that phosphatase production requires substantial quantities of N. A negative correlation between the inorganic P fractions (water extractable P and Bray's extractable P) and acid phosphatase activity was observed which was consistent with other researchers (Wright and Reddy, 2001; Allison and Vitousek, 2005; Allison *et al.*, 2007). This is due to the inhibition of acid phosphatase activity by available P (negative feedback regulation). MBP was positively correlated with total P ($p<0.00$) in forest ecosystem and with SOC in grassland ecosystem. Maithani *et al.* (1996) reported positive correlation between MBP and SOC. The significant positive correlation between microbial biomass P and total P indicates that it could be used as an indicator of ecosystem recovery.

Phosphorus solubilising bacterial diversity during ecosystem recovery, and their relative efficiency

A combination of approaches viz., morphological, biochemical and 16S rRNA gene sequences were used for the identification of PSB from undisturbed and recovering stands of forest and grassland ecosystems. A total of 47 PSB isolates were obtained after screening which were identified. The phosphate solubilization potential of PSB species was evaluated through *in vitro* experiments as well as crop application.

The decrease in the population size and diversity of PSB after disturbance was in agreement with Liu *et al.* (2000), who reported decrease in soil microbial diversity with disturbance. *Burkholderia* was the dominant genus involved in P solubilization in both the ecosystems. Recent studies have found a high abundance and growth of *Burkholderia* in acidic soils (Curtis *et al.*, 2002; Belova *et al.*, 2006). This could be one of the reasons of *Burkholderia* abundance in the present study as the soils in both the ecosystems are acidic. In spite of growing in selective NBRIP medium, only a few isolates formed a halo around the colony. On the other hand, all the isolates growing on NBRIP medium reduced pH suggesting that pH reduction assay is a far superior tool to screen PSB.

Crop application of selected PSB showed positive effects on the growth of maize plants. Inoculation with PSB made more soluble phosphates available to the growing plants and therefore, was the reason for improved growth of maize plants in pot experiment. The PSB species with inherent high solubilising efficiency in *in vitro* conditions failed to retain the similar efficiency when exposed to stress in terms of reduced nutrients presented by the pot experiment. In contrast, species with intermediate level of efficiency in *in vitro* conditions excelled in the stressed conditions. The stress originating from disturbance events such as mining related activities, resulted in occurrence of the most efficient strains of PSB e.g. *Burkholderia tropica* in the disturbed grassland ecosystem indicating the positive response of microbes to high intensity disturbances.

Recovering stands varying in recovery age and the undisturbed stands had different PSB species composition. New groups of PSBs colonised each stand and no species was common to all the recovering/undisturbed stands. This pattern of species composition during different recovery ages indicates that PSB as a community exhibits a microbial succession model that is very close to the "Relay floristic model" (Egler, 1954) as exhibited by higher plants during the succession process. However, this analogy needs further experimental confirmation.

Factors affecting the distribution of PSB during ecosystem recovery

The disturbance events resulted in reduction in PSB populations from 16 x 10^4 CFU g^{-1} soil to 29 x 10^4 CFU g^{-1} soil in forest ecosystem and 13 x 10^4 CFU g^{-1} soil to 27 x 10^4 CFU g^{-1} soil in grassland ecosystem. Nüsslein and Tiedje (1999) also reported reduced soil bacterial populations in pastures that was created by clearing the undisturbed forest.

The soil physico–chemical and biological properties responsible for differential PSB species composition during different recovery stages in each ecosystem were identified through Canonical Correspondence Analysis (CCA) considering the wide variation in climatic and edaphic environmental conditions in the undisturbed and recovering stands. The relationship between the PSB composition and plant species composition was also examined using CCA. Although both soil characteristics and tree composition explained relatively less proportion of the total variability in PSB composition, there was a significant association of PSBs with some plant species and soil parameters. *Castanopsis tribuloides*, *Quercus glauca*, *Elaeocarpus lanceifolius and Neolitsea cassia* were important tree species in forest ecosystem influencing PSB distribution. The PSB species *Burkholderia arboris* (BBar) showed association with two tree species viz., *Castanopsis tribuloides* (TCt) and *Quercus glauca* (TQg), PSB species *Burkholderia fungorum* (BBf) was influenced by *Elaeocarpus lanceifolius* (TEl) and *Castanopsis purpurella* (TCp), and the PSBs *Burkholderia phytofirmans* (BBpy) and *Burkholderia cepacia* (BBce) showed association with *Symplocos glomerata* (TSg) and *Neolitsea cassia* (TNc) tree species (Fig. 7.1). In grassland ecosystem, *Arundinella khasiana*, *Cyanotis vaga*, *Eriocaulon cristatum* and *Themeda intermedia* were important plant species influencing PSB distribution. Only two species of PSB viz., *Burkholderia unamae* (BBu) and *Burkholderia mimosarum* (BBm) showed association with the grass species *Arundinella khasiana* (GAk) and *Cyanotis vaga* (GCv) (Fig. 7.2). Microbial community composition has been reported to be influenced by plant species composition by earlier workers (Kourtev *et al.*, 2002; Garbeva *et al.*, 2006). While working in post–mining afforested sites, Snajdr *et al.* (2013) recently concluded that the dominant tree species contribute significantly to the variation in the microbial community composition.

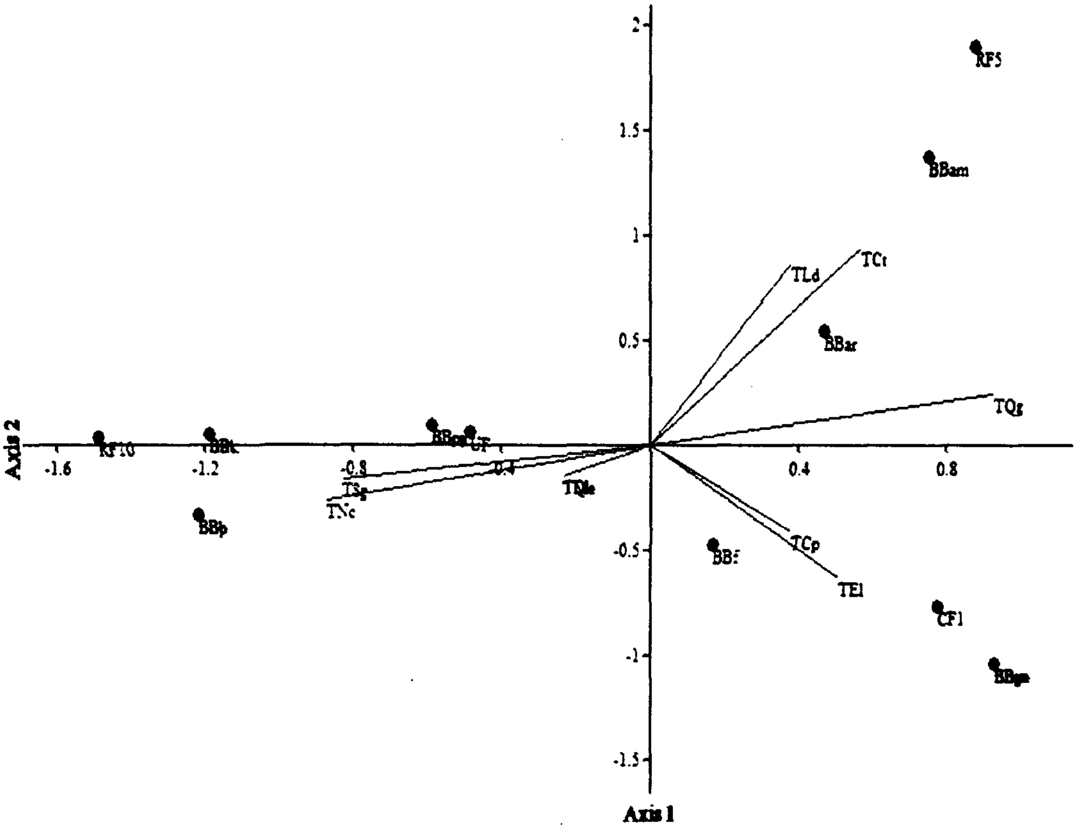

Fig. 7.1: Canonical correspondence analysis of PSB species (blue dots) and four dominant trees species from each stand of forest ecosystem.

(**Tree species: TCp** – *Castanopsis purpurella*; **TCt** – *Castanopsis tribuloides*; **TEl** – *Elaeocarpus lanceifolius*; **TLd** – *Lithocarpus dealbatus*; **TMe** – *Myrica esculenta*; **TNc** – *Neolitsea cassia*; **TQg** – *Quercus glauca*; **TQk** – *Quercus kamroopii*; **TSg** – *Symplocos glomerata*).

(**PSB species: BBa** – *Bacillus amyloliquefaciens*; **BBam** – *Burkholderia ambifaria*; **BBar** – *B. arboris*; **BBb** – *B. bryophila*; **BBc** – *B. caledonica*; **BBce** – *B. cepacia*; **BBf** – *B. fungorum*; **BBg** – *B. glathei*; **BBm** – *B. mimosarum*; **BBp** – *B. phenazinium*; **BBpe** – *B. phenoliruptrix*; **BBpy** – *B. phytofirmans*; **BBs** – *B. sacchari*; **BBt** – *B. terrae*; **BBtr** – *B. tropica*; **BPk** – *Pseudomonas koreensis*).

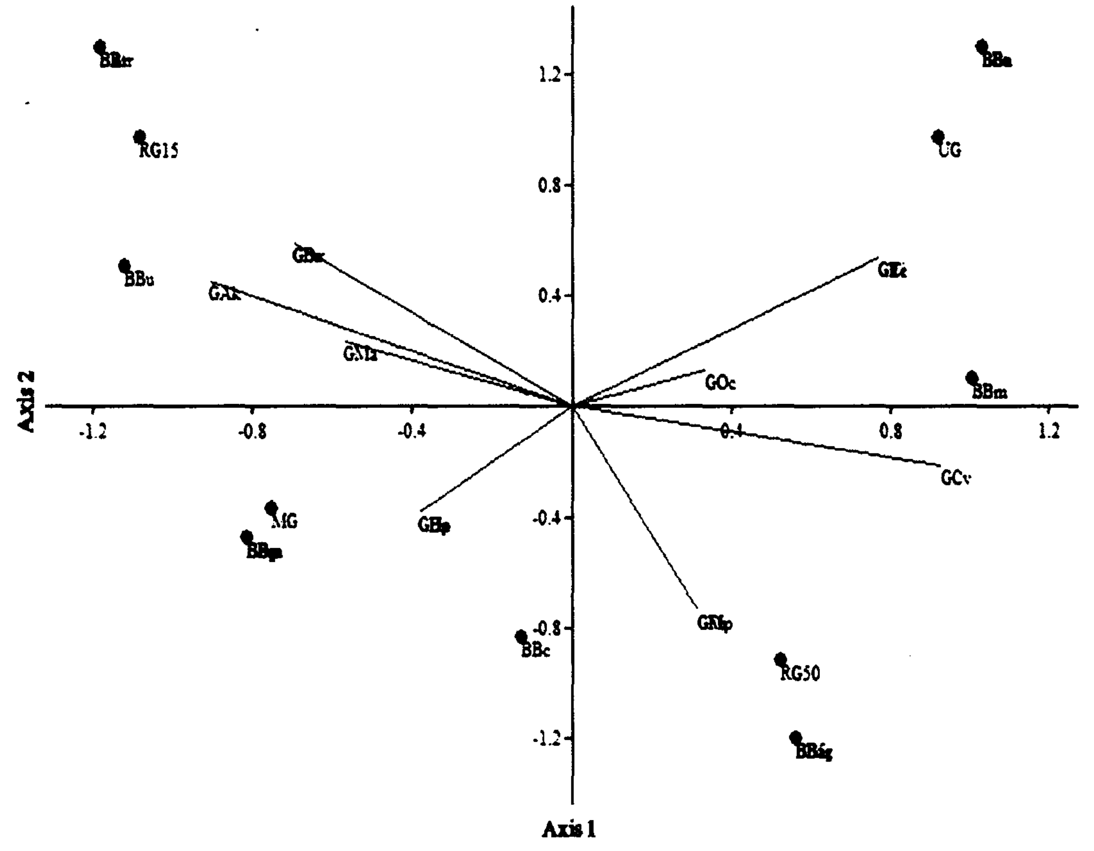

Fig. 7.2: Canonical correspondence analysis of PSB species (blue dots) and four dominant plant species from each stand of grassland ecosystem.

(**Plant species: GAk** – *Arundinella khasiana*; **GBa** – *Borreria articularis*; **GCv** – *Cyanotis vaga*; **GDp** – *Drosera peltata*; **GDv** – *Digitaria violascens*; **GEc** – *Eriocaulon cristatum*; **GFh** – *Fimbristylis hookeriana*; **GHa** – *Hypoxis aurea*; **GMa** – *Ischaemum hirtum*; **GMp** – *Mazus pumilus*; **GOc** – *Osbeckia capitata*; **GTi** – *Themeda intermedia*).

(**PSB species: BAr** – *Agrobacterium rhizogenes*; **BBa** – *Bacillus amyloliquefaciens*; **BBag** – *Brevibacillus agri*; **BBar** – *Burkholderia arboris*; **BBc** – *B. cepacia*; **BBf** – *B. fungorum*; **BBg** – *B. glumae*; **BBm** – *B. mimosarum*; **BBn** – *B. nodosa*; **BBs** – *B. sabiae*; **BBsa** – *B. sacchari*; **BBsr** – *B. sartisoli*; **BBt** – *B. terrae*; **BBtr** – *B. tropica*; **BBu** – *B. unamae*; **BPm** – *Pseudomonas moraviensis*).

Among the edaphic factors, soil temperature, soil moisture, acid phosphatase activity, water extractable phosphorus and phosphorus mineralization were important for the distribution of PSB species among the different recovering stands. Two PSB species viz., *Burkholderia phytofirmans* (BBpy) and *Burkholderia phenoliruptrix* (BBpe) showed strong association with porosity (Po), microbial biomass carbon (MBC) and phosphorus (MBP). A third species viz., *Burkholderia fungorum* (BBf) was associated with total nitrogen (TKN) (Fig. 7.3). A few earlier workers had also reported the role of above factors in regulating the populations of microbial communities in different ecosystems.

Conforming to our findings, Brockett *et al.* (2012) through their study in seven matured and undisturbed forests in British Columbia and Alberta also concluded that soil moisture was the most important factor that influenced microbial community structure. Similarly, Ruppel *et al.* (2007) reported that prokaryotic diversity and soil N availability were interrelated. Mander *et al.* (2012) found that the incidence of PS phenotype in the bacterial populations was closely linked to soil P status.

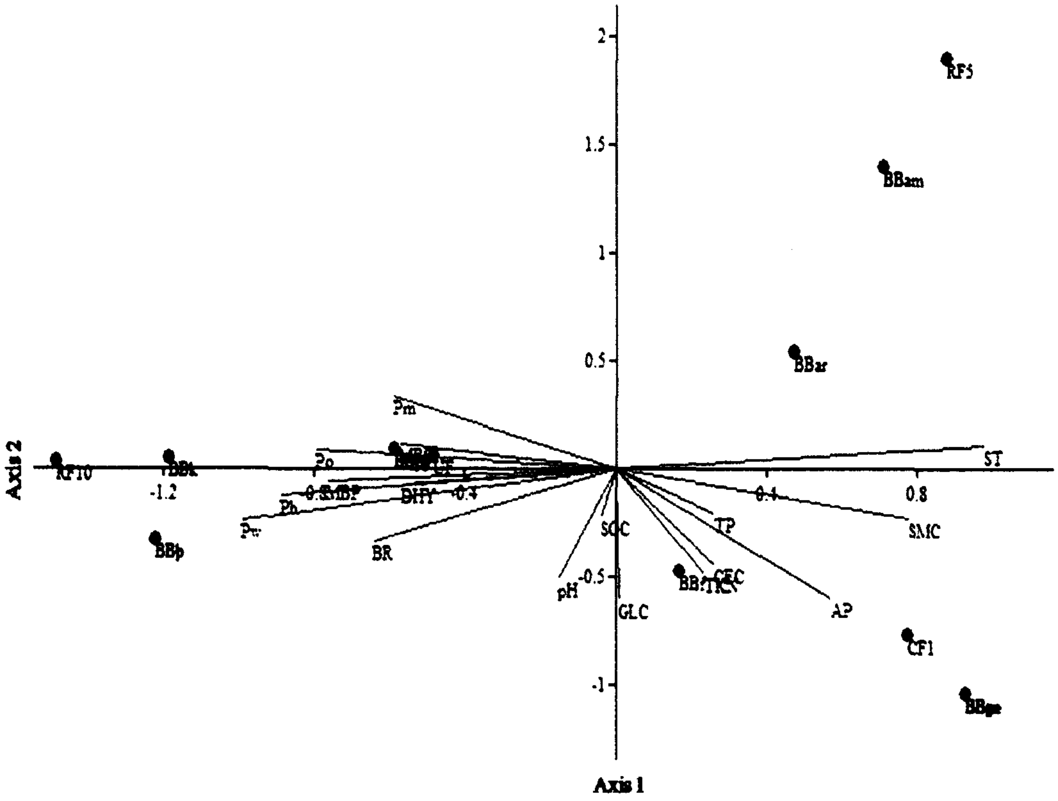

Fig. 7.3: Canonical correspondence analysis of PSB species (blue dots) and soil parameters of the undisturbed forest stands and the recovering stands in forest ecosystem.

(**PSB species: BBa** – *Bacillus amyloliquefaciens*; **BBam** – *Burkholderia ambifaria*; **BBar** – *B. arboris*; **BBb** – *B. bryophila*; **BBc** – *B. caledonica*; **BBce** – *B. cepacia*; **BBf** – *B. fungorum*; **BBg** – *B. glathei*; **BBm** – *B. mimosarum*; **BBp** – *B. phenazinium*; **BBpe** – *B. phenoliruptrix*; **BBpy** – *B. phytofirmans*; **BBs** – *B. sacchari*; **BBt** – *B. terrae*; **BBtr** – *B. tropica*; **BPk** – *Pseudomonas koreensis*).

(**Soil parameters: Po** – Porosity; **CEC** – cation exchange capacity; **SMC** – soil moisture capacity; **ST** – soil temperature; **pH** – soil pH; **SOC** – soil organic carbon; **TKN – total Kjeldahl nitrogen; TP – total hosphorus; Pb** – Bray's extractable phosphorus; **Pw** – water extractable phosphorus; **Pm** – phosphorus nineralization; **MBP** – microbial biomass phosphorus; **MBC** – microbial biomass carbon; **BR** – basal espiration; **DHY** – dehydrogenase activity; **AP** – acid phosphatase activity; **GLC** – β-glucosidase ctivity).

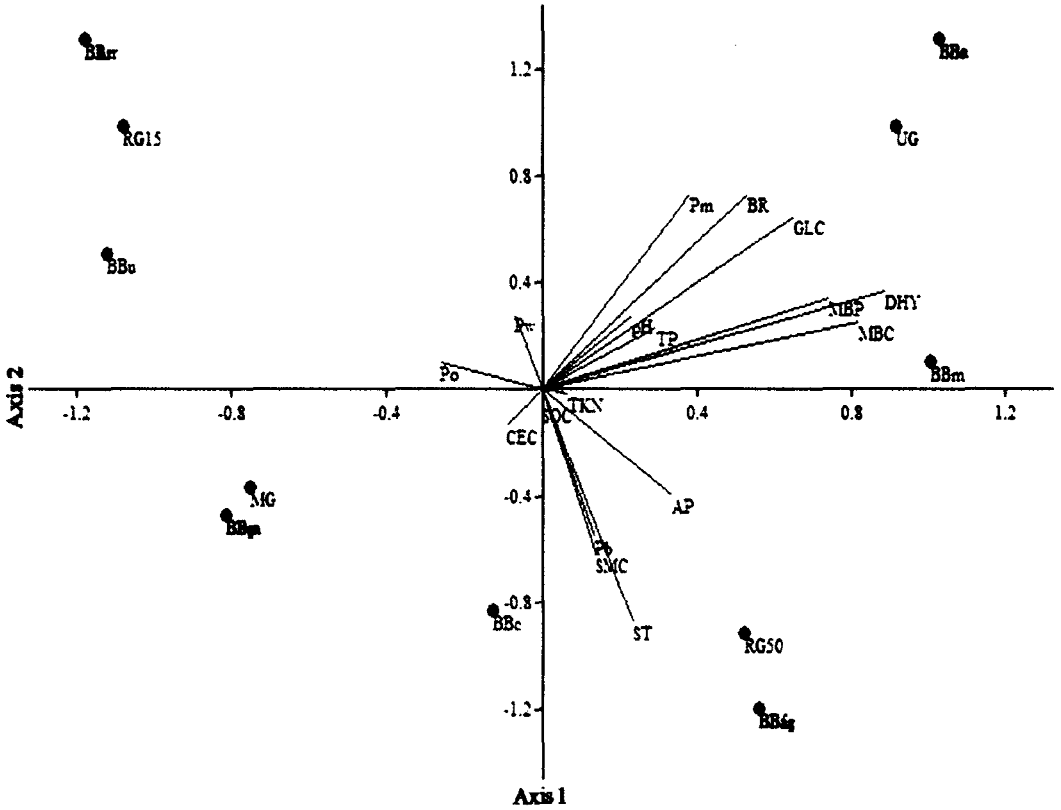

Fig. 7.4: Canonical correspondence analysis of PSB species (blue dots) and soil parameters of the undisturbed grassland sites and the recovering sites in grassland ecosystem.

(**PSB species: BAr** – *Agrobacterium rhizogenes*; **BBa** – *Bacillus amyloliquefaciens*; **BBag** – *Brevibacillus agri*; **BBar** – *Burkholderia arboris*; **BBc** – *B. cepacia*; **BBf** – *B. fungorum*; **BBg** – *B. glumae*; **BBm** – *B. mimosarum*; **BBn** – *B. nodosa*; **BBs** – *B. sabiae*; **BBsa** – *B. sacchari*; **BBsr** – *B. sartisoli*; **BBt** – *B. terrae*; **BBtr** – *B. tropica*; **BBu** – *B. unamae*; **BPm** – *Pseudomonas moraviensis*).

(**Soil parameters: Po** – Porosity; **CEC** – cation exchange capacity; **SMC** – soil moisture capacity; **ST** – soil temperature; **pH** – soil pH; **SOC** – soil organic carbon; **TKN** – **total Kjeldahl nitrogen**; **TP** – **total phosphorus**; **Pb** – Bray's extractable phosphorus; **Pw** – water extractable phosphorus; **Pm** – phosphorus mineralization; **MBP** – microbial biomass phosphorus; **MBC** – microbial biomass carbon; **BR** – basal respiration; **DHY** – dehydrogenase activity; **AP** – acid phosphatase activity; **GLC** – β–glucosidase activity).

Indicators for monitoring ecosystem recovery

The study clearly demonstrated that several structural and functional microbial parameters can act as potential indicators for monitoring the recovery of C and P pools in grassland and forest ecosystems. Most of these parameters showed sufficient level of sensitivity to recovering vegetation and soil properties (Table 7.1).

Table 7.1: Percentage recovery forest and grassland ecosystem in terms of soil properties and plant density.

	FOREST ECOSYSTEM				GRASSLAND ECOSYSTEM			
	UF	CF1	RF5	RF10	UG	MG	RG15	[illegible]
Plant density	100	34.3	92.0	149.1	100	7.0	78.1	81.6
Soil Temperature	100	112.0	108.8	93.0	100	101.0	103.1	104.1
Soil Moisture Content	100	154.9	125.3	103.0	100	137.7	69.5	102.9
Soil pH	100	102.0	98.8	100.1	100	81.1	105.6	108.3
Soil Organic Carbon	100	119.3	112.1	120.7	100	127.3	66.5	62.3
Soil Organic Matter	100	119.7	112.1	120.2	100	127.3	66.5	57.5
Total Kjeldahl Nitrogen	100	194.9	130.8	157.8	100	131.8	58.2	51.7
Total Phosphorus	100	174.2	142.2	142.0	100	96.4	60.5	53.7
Bray's Extractable Phosphorus	100	43.9	54.2	96.6	100	84.2	164.2	321.6
Water Extractable Phosphorus	100	97.4	97.5	101.6	100	98.3	101.8	96.7
C/N	100	57.5	79.9	71.6	100	98.9	114.7	131.0
C/P	100	70.4	77.9	89.5	100	127.9	100.7	107.0
Dehydrogenase activity	100	59.6	66.1	76.6	100	49.4	61.0	73.7
β–Glucosidase activity	100	115.7	103.1	111.5	100	45.6	74.6	69.0
Acid Phosphatase activity	100	111.5	99.6	98.2	100	104.8	89.3	96.1
Phosphorus mineralization	100	75.7	89.7	92.7	100	61.7	94.2	81.7
Microbial Biomass Carbon	100	48.9	66.6	75.7	100	42.8	67.2	87.0
Microbial Biomass Phosphorus	100	62.9	71.0	88.8	100	74.7	88.2	93.9
Basal Respiration	100	75.1	71.5	85.3	100	65.5	87.6	79.6
MBC/SOC	100	38.7	56.3	59.4	100	34.1	103.3	144.8
MBP/TP	100	34.3	48.5	60.7	100	78.5	133.0	162.0
Metabolic quotient	100	199.2	126.4	120.4	100	170.6	160.4	103.7

The parameters viz., plant density, soil moisture content, soil temperature, Bray's extractable phosphorus, water extractable phosphorus, C/N, C/P, dehydrogenase activity, acid phosphatase activity, phosphorus mineralization, microbial biomass carbon, microbial biomass phosphorus, MBC/SOC, MBP/TP and metabolic quotient in forest ecosystem, and plant density, pH, total Kjeldahl nitrogen, soil organic carbon, total phosphorus, Bray's extractable phosphorus, C/N, dehydrogenase activity, microbial biomass carbon, microbial biomass phosphorus, MBC/SOC, MBP/TP and metabolic quotient in grassland ecosystem exhibited a consistent trend (either increasing or decreasing) along the years of recovery. Among these, the parameters which showed greater percentage recovery were considered more sensitive indicators and were recommended for using them as indicators for monitoring the rate of ecosystem recovery. These indicators are: (i) plant density, Bray's extractable phosphorus, C/N, microbial biomass carbon, MBC/SOC, MBP/TP and metabolic quotient in both the ecosystems, (ii) soil moisture content and microbial biomass phosphorus only in forest ecosystem, and (iii) dehydrogenase activity in grassland ecosystem.

CHAPTER 8
SUMMARY

Soil microorganisms mediate many critical ecosystem processes and functions that determine nutrient availability to regenerating vegetation during the recovery of degraded ecosystems following disturbance events. Our understanding on how microbial communities are shaped due to changed environmental conditions during the recovery period following disturbance events, and how the structure and function of soil microbial communities influence rates of key ecosystem processes is limited. Considering the importance of P and C cycles in recovery of degraded ecosystems and relatively less knowledge on the role of microbial communities in regulating their dynamics particularly during post–disturbance recovery period, the present study was undertaken in Cherrapunjee plateau (Latitude 25° 16.49' N and Longitude 091° 43.59' E; altitudinal range 1480–1690 m a.s.l) of Meghalaya, Northeast India. The two natural ecosystems of Cherrapunjee viz., forests and grasslands are under constant anthropogenic pressure and highly degraded. Despite all the disturbances, the ecosystems of Cherrapunjee exhibit a high degree of resilience. This resiliency of Cherrapunjee landscape offered an interesting case study to understand the underlying processes and mechanisms, particularly the role of microbial communities that result in the ecosystem stability. The objectives of the present study were: (i) to study the changes in soil and vegetation characteristics during recovery process; (ii) to elucidate the role of microorganisms in C and P dynamics during recovery period; and (iii) to identify a few P solubilising microbial strains based on their relative efficiency during different years of recovery.

The present study was conducted in two natural ecosystems of Cherrapunjee viz., forest and grassland. The forest ecosystem was disturbed due to deforestation, and the grassland ecosystem was disturbed due to mining related activities such as dumping of coal and movement of trucks. The study followed a chronosequence approach to understand the recovery pattern of these ecosystems. Two replicate stands for each recovery age category and undisturbed forest/grassland in each ecosystem were selected at similar elevation within 2.7 km radius in Sohrarim and Laitryngew villages of Cherrapunjee plateau. The four categories of stands in forest ecosystem were: i) old–growth undisturbed forest stands (UF), and a chronosequence of three clearcut and regrowing stands viz., ii) freshly clear–cut forest representing the post–disturbance stands (CF1), iii) 5–year old recovering forest stands (RF5) and iv) 10–year old recovering forest stands (RF10). The four categories of stands in grassland ecosystem were: i) undisturbed grassland stands (UG), and a chronosequence of three mining affected and recovering stands viz., ii) mining affected grassland stands (MG), iii) 15–year old recovering grassland stands (RG15) and iv) 50–year old recovering grassland stands (RG50). All the stands were once the part of the undisturbed forest/grassland ecosystems having similar vegetation and soil characteristics.

The findings of the study may be summarized as follows:

Change in soil characteristics during the recovery of ecosystems

- In the forest ecosystem, the soil of CF1 stands, i.e. immediately after deforestation had the highest clay content (16.4%). As recovery progressed, clay content reduced significantly ($p<0.00$). There was a significant variation ($p<0.01$) in the proportion of sand and clay fractions in soils of CF1 stands with those of recovering RF5 and RF10, and the undisturbed forest stands. Soil of the CF1 stands was sandy loam, while soils of both the recovering stands and the undisturbed stand were loamy sand. In grassland ecosystem, only the sand proportion varied significantly ($p<0.00$) among different recovery ages. It ranged between 69% and 92% depending on the age of recovery. Therefore, soil textural class varied among the recovering ages. It was a sandy loam in UG and MG, sandy in RG15, and loamy sand in RG50.

- In forest ecosystem, bulk density (BD) significantly ($p<0.01$) decreased with recovery ages. It was highest in CF1 stands (0. 51 g cm^{-3}) i.e. immediately after deforestation followed by the recovering RF5 (0.41 g cm^{-3}), RF10 (0.30 g cm^{-3}) and the old growth undisturbed forest stands (0.26 g cm^{-3}). In grassland ecosystem, it increased due to disturbance and showed an overall reduction as recovery proceeded. The mining affected MG sites had significantly ($p<0.00$) greater BD than the recovering RG15 and RG50 sites. However, porosity followed a reverse trend as expected in both forest and grassland ecosystems.
- Water holding capacity (WHC) also followed the same trend as that of BD. With the increase in bulk density WHC also increased. It decreased significantly ($p<0.00$) as recovery followed. WHC ranged from 12–38% in grassland and 18–60% in forest.
- In forest ecosystem, cation exchange capacity (CEC) was significantly ($p<0.05$) greater in CF1 stands than the recovering i.e. RF5 and RF10 and UF stands. Overall, the value reduced significantly ($p<0.05$) as recovery progressed. Similarly in grassland ecosystem, CEC decreased with increasing recovery age. It was highest in MG and was significantly greater ($p<0.00$) than the recovering RG15 and RG50 and the undisturbed grasslands.
- In forest ecosystem, soil temperature (ST) reduced significantly ($p<0.01$) as recovery progressed. It varied significantly ($p<0.00$) among the recovery ages. However in grassland ecosystem, ST did not show any significant difference among MG, RG15, RG50 and the undisturbed grassland sites. It was significantly ($p<0.01$) higher in grassland ecosystem than forest ecosystem. ST varied significantly ($p<0.00$) among the seasons in forest ecosystem with highest during rainy season and lowest during winter season.
- Soil moisture content (SMC) was significantly ($p<0.01$) greater in CF1/MG stands than the recovering and undisturbed stands. The value reduced significantly ($p<0.01$)

as recovery progressed. SMC was significantly ($p<0.00$) greater in forest ecosystem than grassland ecosystem. It ranged from 22.4 – 34.6% in forest ecosystem and 15.9 – 31.5% in grassland ecosystem.

- The soils were acidic in nature in both the ecosystems. In forest ecosystem, pH had a relatively narrow range of 4.8–4.9 than the grassland ecosystem (4.0–5.3). In grassland ecosystem the disturbed MG sites had significantly ($p<0.01$) lower pH than the recovering RG15 and RG50 sites, and the undisturbed sites. Overall, the value increased significantly ($p<0.01$) as recovery progressed. pH also varied significantly ($p<0.00$) among the seasons in grassland ecosystem with highest value during winter and lowest during rainy.
- In forest ecosystem, soil organic carbon (SOC) was significantly ($p<0.01$) greater in CF1 stands (4.5%) i.e. immediately after deforestation than the undisturbed stands (3.8%). It reduced till 5th year of deforestation (RF5: 4.3%), after which it started to increase (RF10: 4.6%) indicating that the system recovered after 5th year of disturbance. In grassland ecosystem, due to coal dumping SOC was significantly ($p<0.00$) greater in MG sites (4.9%) than the recovering RG15 (2.6%) and RG50 (2.4%) sites, and the undisturbed grassland sites (3.9%). As coal dumping was stopped and the recovery started, the SOC values decreased sharply. It varied significantly ($p<0.00$) among recovery ages in both forest and grassland ecosystem. SOC was significantly ($p<0.00$) higher in grassland ecosystem than forest ecosystem. It ranged between 3.8 – 4.6% in forest and 2.4 – 5.0% in grassland ecosystem. SOC varied significantly ($p<0.00$) among the seasons and was highest during rainy season and lowest during summer season in both the ecosystems.
- Total phosphorus (TP) varied between 217.6 and 363.8 $\mu g\ g^{-1}$ in forest ecosystem, and 153.1 and 297.2 $\mu g\ g^{-1}$ in grassland ecosystem. In forest ecosystem, TP was significantly ($p<0.01$) greater in CF1 stands than the recovering RF5 and RF10 and

undisturbed forest stands. As recovery followed the TP content declined initially till 5th year of deforestation and increased thereafter. In grassland ecosystem, UG and MG sites had significantly ($p<0.00$) higher TP than RG15 and RG50. It was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. TP varied significantly ($p<0.00$) among the seasons, and was highest during autumn and lowest during summer/rainy seasons.

- Total Kjeldahl nitrogen (TKN) in forest soil ranged between 0.25 and 0.48%, and 0.13 and 0.32% in grassland ecosystem. In forest ecosystem, TKN was significantly ($p<0.00$) greater in CF1 stands (0.48%) i.e. immediately after deforestation than RF5 (0.33%), RF10 (0.39%) and UF (0.25%) stands. In grassland ecosystem, TKN showed a sharp decline as recovery progressed. The mining affected MG (0.32%) and UG (0.25%) sites had significantly ($p<0.00$) greater values of TKN than the recovering RG15 (0.14%) and RG50 (0.13%) sites. It was significantly ($p<0.00$) higher in forest ecosystem than the grassland ecosystem. It varied among the seasons significantly ($p<0.00$) and was highest during winter and lowest during summer.
- In forest ecosystem, C/N ratio was significantly ($p<0.01$) lower in CF1 stands than RF5, RF10 and UF stands. As recovery proceeded, it showed an overall increasing trend. C/P also followed a more or less same trend with an overall increase along the recovery ages. In grassland ecosystem, C/N ratio was significantly ($p<0.01$) lower in MG than RG15, RG50 and UG sites. It showed an increasing trend as recovery progressed. In contrast, C/P declined as recovery progressed. It was significantly ($p<0.01$) greater in MG than UG.
- PCA revealed higher impact of soil WHC, C/P and TP on the stands than the other physico–chemical properties.

Vegetation characteristics during recovery

- In forest ecosystem, due to disturbance a reduced number of tree species were recorded from the recovering stands. In the 1st year of deforestation, i.e. in CF1 stands, 27 tree species were recorded. In the recovering stands RF5 and RF10, 27 tree species and 34 tree species, respectively were recorded. The number of species under herbs and shrubs increased after disturbance. The number of herb species in CF1, RF5 and RF10 was 31, 22 and 13, respectively. The corresponding figures for shrub species were 23, 32 and 15. Only 12 tree species (15%) were common to all the stands. Three species viz., *Lithocarpus fenestratus*, *Photinia* sp. and *Toddalia asiatica* were exclusively found in CF1, 5 species viz., *Albizia odoratissima*, *Beilschmiedia assamica*, *Brassaiopsis speciosa*, *Ficus neriifolia* and *Litsea salicifolia* in RF5 and only one species viz., *Viburnum simonsii* was found in RF10 stands. Thirty two species were exclusive to UF stand only.
- In grassland ecosystem, from MG sites, nine herbaceous species belonging to 9 genera and 5 families were recorded. The recovering sites RG15 and RG50 had 20 and 21 plant species, respectively. Only 6 plant species (19%) were common to all the sites. One plant species viz., *Themeda intermedia* was found only in RG50 while 7 species viz., *Aeginetia indica*, *Habenaria goodyeroides*, *Malaxis acuminata*, *Satyrium nepalense*, *Spiranthes sinensis*, *Themeda villosa*, *Tripogon trifidus* were found only in UG. No species was exclusive to MG and RG15.
- In forest ecosystem, Shannon's diversity index and evenness index for tree species reduced due to disturbance. However, as recovery followed the diversity and evenness gradually improved to attain a more diverse and equitable community. In case of herbs and shrubs, Shannon's diversity index increased immediately after disturbance and subsequently reduced. Pielou's evenness index was greater for herbs than shrubs and trees in all the stands. In grassland ecosystem, Shannon's diversity

index was greater in the undisturbed site than the recovering sites. Disturbance reduced the species diversity, which improved as recovery followed. Pielou's evenness index exhibited quite high values in all the stands.

- The dominance–diversity curve followed a log normal distribution pattern i.e. low dominance or high equitability for trees in the undisturbed forest stands whereas in CF1, RF5 and RF10 the trees species showed low equitability or high dominance. The pattern of dominance–diversity curves for herbs and shrubs however, did not vary among the different recovery ages. In grassland ecosystem, the plant species exhibited a log normal distribution pattern in UG, RG15 and RG50 sites whereas MG sites had a steeper dominance–diversity curve depicting high dominance of a few species i.e. *Digitaria violascens* and *Arundinella khasiana.* The dominant species in all the vegetation components changed with recovery age in both the ecosystems.
- In forest ecosystem, tree density was lowest in CF1 (326 stems ha^{-1}) followed by RF5 (1052 stems ha^{-1}). The highest density value was in RF10 (1706 stems ha^{-1}) i.e. after 10 years of recovery. The tree density in the undisturbed forest stands was 1144 stems ha^{-1}. Tree basal area was lowest in CF1and increased as recovery followed. Densities of herb and shrub species increased due to disturbance. The density values reduced as recovery proceeded. In grassland ecosystem, disturbance reduced plant density. MG sites (27, 000 ha^{-1}) had significantly ($p<0.05$) lower density than the recovering RG15 (2,99,500 ha^{-1}) and RG50 (3,13,000 ha^{-1}) sites, and undisturbed grassland site (3, 83,500 ha^{-1}). With increase in recovery age the density values increased.
- Tree species composition during different recovery ages of the forest ecosystem were assessed by PCA. *Quercus glauca* had higher impact on the CF1 stands and the recovering stands RF5 than the other species. *Symplocos glomerata* and *Neolitsea cassia* were found important for the recovering stands RF10. In grassland sites,

Digitaria violascens, *Borreria articularis*, *Eulalia quadrinervis* and *Arundinella khasiana* were found important for mining affected MG sites. *Eragrostis nigra* and *Osbeckia capitata* had higher impact on recovering sites RG15 and RG50 respectively.

Microbial community structure and functions related to C–dynamics

- Disturbances impacted the total amount of soil organic matter in both the ecosystems, which had a direct effect on the biomass, population size and activities of microbial community. A decline in the biological properties associated with C cycle viz., enzyme activity, microbial biomass carbon (MBC), MBC/SOC ratio and basal respiration (BR) was observed following disturbance.
- MBC ranged between 324 μg g^{-1} and 663 μg g^{-1} in forest ecosystem and 206 μg g^{-1} and 482 μg g^{-1} in grassland ecosystem. It reduced significantly ($p<0.05$) in the disturbed sites in both the ecosystem and showed gradual increase with progress in recovery. MBC was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. It varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with highest during winter and lowest during rainy.
- MBC/SOC ratio ranged between 0.7% and 1.9% In forest ecosystem and 0.4% and 1.9% in grassland ecosystem. This ratio increased as recovery progressed and indicated accumulation of labile C in soil and favourable environment for microbial growth. As microbial populations are largely dependent on the soil labile C as their C source, low MBC/SOC ratio after disturbance was indicative of poor quality of organic matter.
- A decline in β–glucosidase activity following disturbance was observed. This indicated that the microbial populations allocated most of their energy towards maintenance of a minimum viable population size rather than enzyme production after disturbance. β–glucosidase ranged between 257.4 μg g^{-1} soil h^{-1} and 309 μg g^{-1}

soil h^{-1} in forest ecosystem, and 116.7 μg g^{-1} soil h^{-1} and 256.0 μg g^{-1} soil h^{-1} in grassland ecosystem.

- Dehydrogenase (DHY) activity also followed the similar trend. The values of dehydrogenase ranged between 21.8 μg g^{-1} soil h^{-1} and 36.6 μg g^{-1} soil h^{-1} in forest ecosystem and 14.8 μg g^{-1} soil h^{-1} and 29.9 μg g^{-1} soil h^{-1} in grassland ecosystem. DHY activity was significantly higher ($p<0.00$) in forest ecosystem than grassland ecosystem. DHY activity varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystems with its peak during rainy season and trough during winter.
- Basal respiration ranged between 2.7 μg g^{-1} soil h^{-1} and 3.8 μg g^{-1} soil h^{-1} in forest ecosystem, and 1.8 μg g^{-1} soil h^{-1} and 2.7 μg g^{-1} soil h^{-1} in grassland ecosystem. It declined significantly ($p<0.05$) after disturbance due to the decrease in microbial population in both the ecosystem and showed gradual increase with recovery progress. Basal respiration was significantly ($p<0.00$) higher in forest ecosystem than grassland ecosystem. It varied significantly ($p<0.00$) among the seasons in both forest and grassland ecosystem with highest during rainy season and lowest during winter.
- The microbial metabolic quotient (qCO_2), a bioindicator of ecosystem development as well as of environmental stress on microbial communities, had greater values in the post disturbance sites. This was due to increased availability of readily degradable C indicating that a relatively large percentage of the substrate was decomposed to meet the energy demand of the microflora. As recovery progressed, the prevailing stress on soil microbes gradually subsided resulting in decreased qCO_2. It ranged between 7.0 and 13.9 in forest ecosystem, and 6.7 and 11.4 in grassland ecosystem.
- Among the soil biological parameters studied, dehydrogenase activity, glucosidase activity and basal respiration showed significant correlation with soil moisture and temperature. SOC showed a significant positive relationship with dehydrogenase

activity. BR was significantly correlated with SOC and Bray's extractable P in forest ecosystem. MBC showed a significant positive correlation with total P and extractable P in forest ecosystem and with TKN in grassland ecosystem. Therefore, it may be concluded that the microbial populations were limited by P in forest ecosystem and by N in grassland ecosystem.

Microbial community structure and functions related to P–dynamics

- While total P increased immediately after the disturbance both in forest and grassland ecosystems, extractable P declined. The decline in extractable P after disturbance was partly attributed to weathering of soil due to removal of vegetation cover. Microbial biomass played a crucial role in P dynamics as it acted as a transient sink of labile P. The percentage contribution of microbial biomass P to total P (7–21%) was higher than the values reported by earlier workers (2.6–7.9%). This suggests that microbial communities in the present study sites have evolved a more complex system of substrate–use efficiency with the heterogeneous input of organic matter, enabling them to immobilise a greater portion of P in their biomass. This higher rate of immobilization of P in the microbial biomass observed in the present study might be a possible reason for the very low available P in these ecosystems.
- There was a decline in phosphatase activity during the initial years of recovery. After 5th year of recovery the activity increased showing net recovery. Acid phosphatase activity was highest during rainy season and lowest during dry season. Significant positive relationship of acid phosphatase activity with SOC and total N was obtained.
- The extractable P was higher during wet season than the dry seasons, which was attributed to the enhanced water filled pore space due to rainfall that restricted the diffusion of oxygen from the atmosphere to the soils creating anaerobic condition in soils. This anaerobic condition reduced the redox potential of soil promoting Fe reduction and the concomitant release of Fe–bound P. A negative correlation between

the inorganic P fractions (water extractable P and Bray's extractable P) and acid phosphatase activity was due to the inhibition of acid phosphatase activity by available P (negative feedback regulation).

- MBP was positively correlated with total P ($p<0.00$) in forest ecosystem and with SOC in grassland ecosystem. The significant positive correlation between microbial biomass P and total P indicates that it could be used as an indicator of ecosystem recovery.

Phosphorus solubilising bacterial diversity during ecosystem recovery, and their relative efficiency

- A combination of approaches viz., morphological, biochemical and 16S rRNA gene sequences were used for the identification of PSB from undisturbed and recovering stands of forest and grassland ecosystems. A total of 47 PSB isolates were obtained after screening which were identified. The phosphate solubilization potential of PSB species was evaluated through *in vitro* experiments as well as crop application.
- The decrease in the population size and diversity of PSB after disturbance was observed. *Burkholderia* was the dominant genus involved in P solubilization in both the ecosystems. In spite of growing in selective NBRIP medium, only a few isolates formed a halo around the colony. On the other hand, all the isolates growing on NBRIP medium reduced pH suggesting that pH reduction assay is a far superior tool to screen PSB.
- Crop application of selected PSB showed positive effects on the growth of maize plants. Inoculation with PSB made more soluble phosphates available to the growing plants and therefore, was the reason for improved growth of maize plants in pot experiment. The PSB species with inherent high solubilising efficiency in *in vitro* conditions failed to retain the similar efficiency when exposed to stress in terms of reduced nutrients presented by the pot experiment. In contrast, species with

intermediate level of efficiency in *in vitro* conditions excelled in the stressed conditions. The stress originating from disturbance events such as mining related activities, resulted in occurrence of the most efficient strains of PSB e.g. *Burkholderia tropica* in the disturbed grassland ecosystem indicating the positive response of microbes to high intensity disturbances.

- Recovering stands varying in recovery age and the undisturbed stands had different PSB species composition. New groups of PSBs colonised each stand and no species was common to all the recovering/undisturbed stands. This pattern of species composition during different recovery ages indicates that PSB as a community exhibits a microbial succession model that is very close to the "Relay floristic model" as exhibited by higher plants during the succession process.

Factors affecting the distribution of PSB during ecosystem recovery

- The disturbance events resulted in reduction in PSB populations from 16 x 10^4 CFU g^{-1} soil to 29 x 10^4 CFU g^{-1} soil in forest ecosystem and 13 x 10^4 CFU g^{-1} soil to 27 x 10^4 CFU g^{-1} soil in grassland ecosystem.
- CCA revealed a significant association of PSBs with some plant species and soil parameters. *Castanopsis tribuloides*, *Quercus glauca*, *Elaeocarpus lanceifolius* and *Neolitsea cassia* were important tree species in forest ecosystem influencing PSB distribution. The PSB species *Burkholderia arboris* showed association with two tree species viz., *Castanopsis tribuloides* and *Quercus glauca*, PSB species *Burkholderia fungorum* was influenced by *Elaeocarpus lanceifolius* and *Castanopsis purpurella*, and the PSBs *Burkholderia phytofirmans* and *Burkholderia cepacia* showed association with *Symplocos glomerata* and *Neolitsea cassia* tree species. In grassland ecosystem, *Arundinella khasiana*, *Cyanotis vaga*, *Eriocaulon cristatum* and *Themeda intermedia* were important plant species influencing PSB distribution. Only two

species of PSB viz., *Burkholderia unamae* and *Burkholderia mimosarum* showed association with the grass species *Arundinella khasiana* and *Cyanotis vaga.*

- CCA also revealed that soil temperature, soil moisture, acid phosphatase activity, water extractable phosphorus and phosphorus mineralization were important for the distribution of PSB species among the different recovering stands. Two PSB species viz., *Burkholderia phytofirmans* and *Burkholderia phenoliruptrix* showed strong association with porosity, microbial biomass carbon (MBC) and phosphorus (MBP). A third species viz., *Burkholderia fungorum* was associated with total nitrogen (TKN).

Conclusion

- Notwithstanding the limitations of chronosequence studies, the study revealed that microbes did play a major role in both the ecosystems during post–disturbance recovery process. As stress resulting from ecosystem degradation reduced with recovery age, the functional diversity and activity of the soil microbial communities increased. The microbes influenced the recovery process by impacting various ecosystem processes and functions through their activities such as enzyme activities, mineralization and immobilization.
- Several structural and functional microbial parameters were identified as potential indicators for monitoring the recovery of C and P pools in grassland and forest ecosystems. Most of these parameters showed sufficient level of sensitivity to recovering vegetation and soil properties. Among these, the parameters which showed a consistent trend and greater percentage recovery were considered more sensitive indicators and were recommended for using them as indicators for monitoring the rate of ecosystem recovery.

- These indicators are: (i) plant density, Bray's extractable phosphorus, C/N, microbial biomass carbon, MBC/SOC, MBP/TP and metabolic quotient in both the ecosystems, (ii) soil moisture content and microbial biomass phosphorus only in forest ecosystem, and (iii) dehydrogenase activity in grassland ecosystem.

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AISSE	1999	C.B.S.E	Eng, Hindi, Science, Maths, Social Studies	1st	77.80
AISSCE	2001	C.B.S.E	Maths, Physics, Chem, Bio, Eng	1st	77.60
B.Sc (Biochem.)	2004	N.E.H.U	Biochemistry, Zoology, Chem	1st	71.75
M.Sc	2006	University of Kalyani	Microbiology & Microbial technology	1st	64.80

Title of Ph.D. thesis: *Role of soil microorganisms in C and P dynamics during recovery of degraded terrestrial ecosystems of Cherrapunjee plateau*

Work Experience:

Post Held	Employer	Nature of work	Period with dates
Project Fellow	CAS in Botany North Eastern Hill University	Role of soil microorganisms in C and P dynamics during recovery of degraded terrestrial ecosystems of Cherrapunjee plateau	16th May 2007 - 31st March 2011
Junior Research fellow	UGC-Meritorious	Role of soil microorganisms in C and P dynamics during recovery of degraded terrestrial ecosystems of Cherrapunjee plateau	9th May 2011 – 9th June 2011
Junior Research fellow	DBT Biotech hub	Role of soil microorganisms in C and P dynamics during recovery of degraded terrestrial ecosystems of Cherrapunjee plateau	9th June 2011 -till date

Training programmes attended:

Summer training at Saha Institute of Nuclear Physics, Kolkata on "Isolation of halophilic bacterium from the skin surface of dry fish", for one month duration in M.Sc. 2nd semester.

In plant training at Mother Dairy Calcutta on "the application of microbiology and microbial technology in diary industry", for one month duration in M.Sc. 4th semester.

A short term training course in "Data analysis using excel" for one month in August, 2009 conducted by Computer centre, NEHU, Shillong.

Training at Sardar Patel University on "culture dependent and cultivation-independent molecular techniques viz. PCR amplification of 16S rRNA gene and metagenomics to analyze bacterial diversity" for 20 days in September, 2009 under DST's visiting fellowships to research students from NER for work in other regions.

A short term training programme on "Techniques in Molecular Biology" organized by the Department of Botany, NEHU, Shillong from 17th to 25th November, 2011 under DBT's State Level Biotech Hub.

Participated in the 18th DBT-sponsored training course on "Bioinformatis: A Practical Approach in Genomics and Proteomics" conducted by Bioinformatics Centre, NEHU during 26-29 September, 2011.

Symposium/ Workshops/ Training programmes/ Seminars Attended:

Attended National symposium on "Frontiers in Biocomplexity & Biodiversity of Plants" organised by Centre for Advanced Studies in Botany, NEHU, Shillong from March 14-15, 2008.

Attended training-workshop on "Capacity building of media persons on access and benefit sharing (ABS) from genetic resources and associated traditional knowledge",

Participated in 96th Indian Science congress held in NEHU from January 3-7, 2009.

DECLARATION

I declare that the above information is true and correct to the best of my knowledge and belief.

Place: *Shillong*

Date: 15·10·2013

Debashree Nath

Signature of the candidate