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Physicochemical and Biochemical Reclamation of Soil through Secondary Succession

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ABSTRACT

Conversion of forest to agricultural fields has become a common practice in India. Very often these fields have been abandoned due to lack of sustainable production. In course of time these fallow lands undergo natural secondary succession. The present study was carried out to find out the restoration of soil physicochemical and biochemical properties in a chronosequence of 2 yr, 4 yr, 6 yr, 11 yr, and 15 yr fallow lands. Soil enzyme activities play key roles in the biochemical functioning of soils, including soil organic matter formation and degradation, nutrient cycling, and reflect the change in soil management and land use. There was gradual improvement in the physical condition and nutrient status along with increase in soil amylase, cellulase, dehydrogenase, phophatase, and urease activity in the present study with the progress of fallow age which indicates the importance of natural secondary succession in soil restoration. However the PCA analysis indicated that natural vegetational succession could reclaim the soil quality and promote ecosystem restoration but it required a long time under the present local climatic condition.

Keywords: Sustainable Production; Secondary Succession; Soil Restoration; Land Use

1. Introduction

India has 329 million ha of total land area of which 43% is under cropping and 23% is under forest [1]. From the beginning human has exploited the natural resources for agricultural activities. As a result most of the forests have been converted into agricultural lands. Over recent years there have been intensive agricultural practice basically aimed to enhance the productivity to meet the food demands of huge population throughout the world. In spite of increased population, the productive potential has been decreased in many areas of tropical countries. Intensive cultivation leads to reduced soil fertility and increased soil erosion in many areas of tropics [2]. Specific detrimental effects on biological and biochemical characters of soil quality have also been noted due to changes in microclimate at the soil surface by tillage and on the rate and quality of organic matter input to the soil. Therefore, most of the agro-ecosystems are often abandoned due to unsustainable agricultural production. Maintenance of sustainable agricultural production has become a matter of great concern for the scientists all over the world. In many instances, the disturbed areas undergo natural recovery of vegetation within 5 to 20 years depending on population pressure and land availability [3].

Soil enzymes (microbial exoenzyme) are recognized as sensitive indicators of soil health and quality [4] due to the rapid response to changes in soil management. In particular, enzyme activities are especially significant in soil quality assessments because of their major contribution to degrading organic matter [5]. In fact they have been related to soil physico-chemical characters, microbial community structure and disturbance [6]. It has been reported that any change in soil management and land use is reflected in the soil enzyme activities, and that they can anticipate changes in soil quality before they are detected by other soil analyses [7]. The studies of soils from different regions indicated that enzyme activities are sensitive to soil changes due to tillage [8], cropping systems [9], and land use [10]. Most of the studies on soil enzyme activities in different land use systems have focused on temperate regions. Few literatures are available regarding the enzyme activities due to land use changes in tropics [11,12].

The present study of enzyme activity in soil along a chronosequence of vegetation regrowth in the fallow

lands can give insight into the role of soil enzymes in restoring soil fertility during secondary succession. Currently, no information is available on microbial biomass and enzyme activity that affected by conversion of forest into agricultural land, and then into fallow land in India except the work done by Maithani *et al.* [3] on microbial biomass in 7, 13, and 16 year regrowth of a disturbed subtropical humid forest in Meghalaya, India, and by Ralte *et al.* [13] on microbial biomass and activity in relation to shifting cultivation and horticultural practices in subtropical evergreen forest ecosystem of North-East India. Thus the present investigation was aimed to assess some key enzyme activities along with some of the selected physico-chemical characters involved in soil restoration in a chronosequence of abandoned rice fields in Western Odisha, India.

2. Materials and Methods

2.1. Study Sites and Climate

The present study was confined to the revenue district Sambalpur, located in the Western part of Odisha in India. Prior to 1950s the study sites were dense forest forming a part of Barapahar forest range. After the establishment of multipurpose hydroelectric Hirakud Dam project and subsequent industrialization, urbanization and increase in population pressure, the district forest coverage has been reduced to 30% [14]. Most of the forest lands were cleared and used for production of agricultural crops (rice, maize, beans and sugarcane). The climate is tropical monsoonal. The annual average rainfall during the study period (August, 2010-July 2011) was 1597 mm out of which about 80% fell during rainy season (July-October). The mean air temperature varied from 5˚C (during December) to 44˚C (during May). The soils of this district belong to mixed red and black soil, Red sandy soil, mixed red and yellow lateritic soil [15]. The climatological data during the study period are shown in **Figure 1**. In many places of the study area the rice fields were derived by clearing natural forest which had been subjected to abandonment by farmers due to lack satisfactory agricultural production. All the sampling sites were located on the same topographic situation and with the area of nearly 900 sq meters except 11 yr fallow land which covered 12000 sq meters. The age of the fallow period was ascertained by asking the land owners. By interviewing the elderly persons of the locality it was known that the peoples of these localities were practicing rain fed paddy cultivation since 1975. The selected study sites were paddy fields abandoned since 1995 (15 yr·F), 1999 (11 yr·F), 2004 (6 yr·F), 2006 (4 yr·F), and since 2008 (2 yr·F).

The 4-year-old field was adjacent to the 6-year-old field, while the 11-year-old field was adjacent to the 15-year-old field. All these sites were located at the bottom of hill, Chandili Dunguri, 1/2 Km towards south west from Sambalpur University campus at Burla (20˚43′N-20˚11′N and 82˚39′E-85˚13′E longitude, latitude at 263 m above mean sea level) in Sambalpur district of Odisha, India.

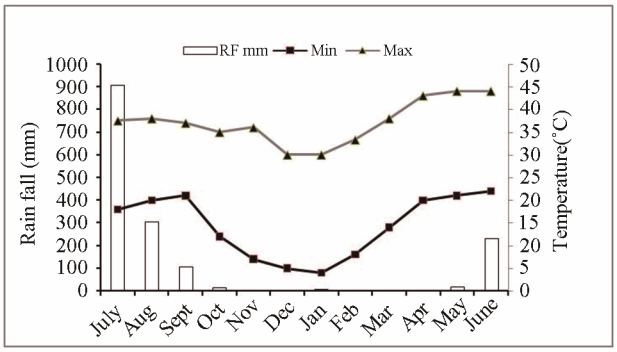

Figure 1. **Climate pattern of study sites.**

2.2. Soil Physicochemical Analysis

The soils were sampled from the experimental plots bimonthly during August 2010- June 2011. The soil samples were taken by using a cylindrical soil sampler having a diameter of 20 cm. and five random samples were taken from 0 - 10 cm, 10 - 20 cm, and 20 - 30 cm depths. The soil samples were packed in plastic zipper bag and brought into the laboratory and stored at 4˚C before analysis. The enzyme analyses were made within four weeks after sampling because storage beyond two weeks can cause decline in enzyme activities [16]. The soil samples were air dried, gently crushed and sieved through 2 mm sieve and then used for different physicochemical analysis.

The laboratory analyses were conducted for bulk density and water holding capacity following the method prescribed in TSBF hand book [17], soil pH by pH meter using 1:5 soil water suspension, the organic carbon content by Walkely and Black's titration method [18], total nitrogen (TN) by Kjeldhal method [19], the total phosphorus by Bray and Kurtz [20] .Soil microbial biomass carbon was calculated by chloroform fumigation-extraction method [21].

2.3. Soil Enzyme Activity

Soil amylase & cellulase activity was determined following Mishra *et al.* [22]. 3 g of soil sample was incubated with 0.2 ml tolune, 6 ml substrate solution (starch for amylase and carboxymethyl cellulose for cellulase) and 6 ml Sorenson's buffer (pH = 5.9) for 24 hr at 34˚C. After incubation the reducing sugar test was performed by adding 2 ml of 3, 5 dinitrosalicylic acid to 1 ml of supernatant of incubated mixture in a test tube and allowed to stand for 5 minutes for colour development.

The enzyme activity was quantified by spectrophotometer at 540 nm and expressed in μg glucose g^{-1} dry soil hr^{-1}.

Soil dehydrogenase was measured according to Casida *et al.* [23]. 5 g of soil was incubated with 2 ml of 1% soluble 2, 3, 5-triphenyl tetrazolium chloride (TTC) in a sealed screw cap test tube at 32˚C for 24 hr. The triphenyl formazon (TPF) formed in the incubated sample was extracted by methanol. The extrantant triphenyl formazon (TPF) was determined by spectrophotometer at 485 nm, using methanol as control.

Acid phosphatase was estimated following the method of Tabatabai and Bremnar [24] using paranitrophenyl phosphate (p-NPP) as a substrate. A mixture of 1 g fresh soil (<2 mm), 4 ml of modified universal buffer (pH 6.5) and 1 ml of 100 mM p-NPP were incubated in a sealed 100 ml Erlenmeyer flask at 30˚C for 30 min. The sample was adjusted to 100 ml with deionised water. After incubation the para- nitrophenol (p-NP) was filtered through Whatman-42 and quantified by spectrophotometer at 400 nm by taking p-NP as control.

Urease activity was determined as described by Dash *et al.* [25]. 0.2 ml toluene was added to 5 g soil and 9 ml of Tris-HCl buffer (pH = 9, 0.2 M) followed by 1 ml of 0.2 M urea (substrate solution) to the sample. The samples were subsequently incubated at 37˚C for 2 hrs. After incubation the volume was made up to the mark (50 ml) by adding KCl-$AgSO_4$. The suspension was centrifuged. To 1 ml of supernatant, 1 ml of phenol and 1 ml of alkaline hypo chlorite solution were added. After 5 min, the released ammonium was measured spectrophotometrically at 625 nm.

2.4. Statistical Analysis

The data were statically analyzed using three way analysis of variance (ANOVA) with SPSS 10 statistical software. Pearson's correlation analysis was performed to determine the significant level of correlation. The PCA was done using SX software to discriminate different sites on the basis of the principal components Z_1 and Z_2.

3. Results

3.1. Soil Physico-Chemical Properties

Soil physico-chemical properties of the present study sites have been presented in **Table 1**. Soil moisture content increased gradually along the chronosequence of abandoned field (**Table 1**). ANOVA revealed a significant impact of fallow period on soil moisture content.

Table 1. Soil physico-chemical properties of different abandoned fields at different depths.

Parameters	Depth (cm)	2 yr	4 yr	6 yr	11 yr	15 yr
Moisture (%)	0 - 10	5.17 ± 2.39	6.55 ± 3.05	7.96 ± 3.43	9.44 ± 4.32	14 ± 4.91
	10 - 20	4.70 ± 1.64	5.9 ± 2.34	7.24 ± 2.63	8.76 ± 3.62	12.12 ± 3.95
	20 - 30	3.57 ± 1.18	4.76 ± 2.02	5.94 ± 2.56	7.84 ± 3.15	10.39 ± 3.52
Bulk density $g \cdot cm^{-3}$	0 - 10	1.53 ± 0.065	1.48 ± 0.061	1.42 ± 0.06	1.35 ± 0.067	1.25 ± 0.08
	10 - 20	1.57 ± 0.051	1.52 ± 0.054	1.48 ± 0.063	1.41 ± 0.079	1.33 ± 0.074
	20 - 30	1.60 ± 0.054	1.56 ± 0.049	1.52 ± 0.054	1.48 ± 0.048	1.39 ± 0.098
WHC (%)	0 - 10	24.84 ± 4.03	27.10 ± 3.92	30.16 ± 4.19	32.47 ± 4.50	36.72 ± 4.52
	10 - 20	21.34 ± 2.87	24.00 ± 3.00	27.09 ± 3.25	29.52 ± 3.75	34.61 ± 4.03
	20 - 30	19.72 ± 2.54	22.32 ± 2.16	23.68 ± 2.83	28.16 ± 3.23	33.17 ± 3.72
pH	0 - 10	5.56 ± 0.08	5.75 ± 0.12	6.00 ± 0.27	6.45 ± 0.11	6.50 ± 0.12
	10 - 20	5.35 ± 0.39	5.80 ± 0.07	5.50 ± 0.14	5.64 ± 0.04	5.74 ± 0.03
	20 - 30	5.40 ± 0.18	5.45 ± 0.15	6.07 ± 0.16	6.17 ± 0.12	6.25 ± 0.14
SOC $mg \cdot g^{-1}$	0 - 10	6.17 ± 1.55	6.34 ± 1.61	8.7 ± 2.11	11.49 ± 2.82	13.43 ± 2.9
	10 - 20	5.42 ± 1.23	5.02 ± 1.3	7.61 ± 1.68	9.78 ± 2.55	11.25 ± 2.89
	20 - 30	4.64 ± 0.96	4.24 ± 1.43	6.02 ± 1.81	8.18 ± 2.37	9.57 ± 2.62
TN $mg \cdot g^{-1}$	0 - 10	0.66 ± 0.17	0.74 ± 0.18	0.91 ± 0.17	1.09 ± 0.23	1.19 ± 0.22
	10 - 20	0.06 ± 0.13	0.64 ± 0.12	0.86 ± 0.13	0.98 ± 0.22	1.07 ± 0.23
	20 - 30	0.53 ± 0.1	0.57 ± 0.13	0.72 ± 0.17	0.89 ± 0.2	0.93 ± 0.18
MBC $\mu g \cdot g^{-1}$	0 - 10	105.5 ± 32.12	155.36 ± 65.47	274.15 ± 95.26	398.97 ± 114.55	513.78 ± 109.57
	10 - 20	81.04 ± 24.49	115.40 ± 42.45	220.35 ± 82.01	290.47 ± 85.44	364.24 ± 92.64
	20 - 30	58.93 ± 17.91	84.97 ± 31.11	158 ± 71	214.39 ± 71.75	280.03 ± 100.19

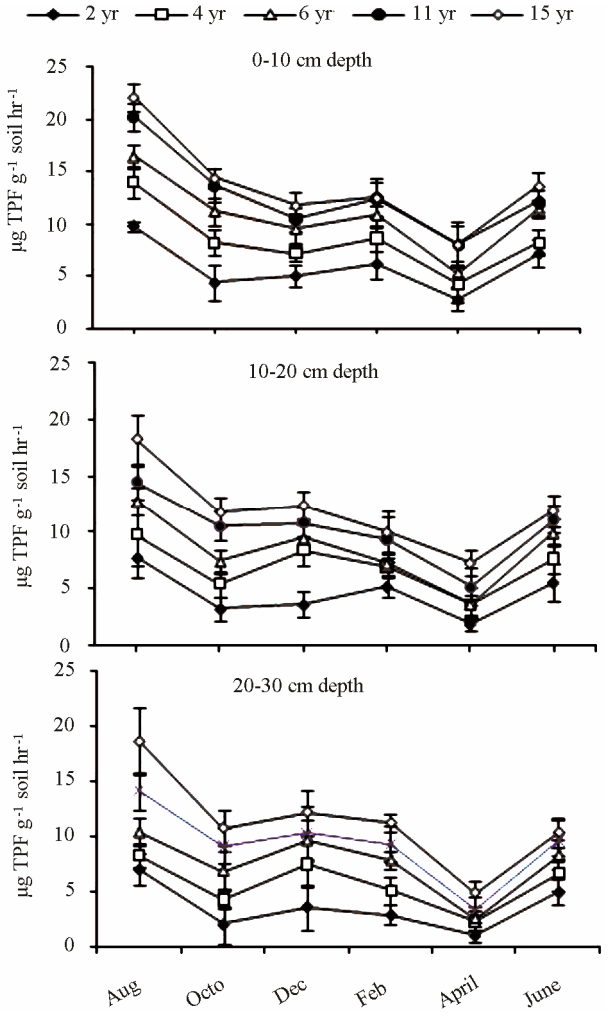

Figure 2. Amylase activity in soil of different abandoned fields during different months and at different depths.

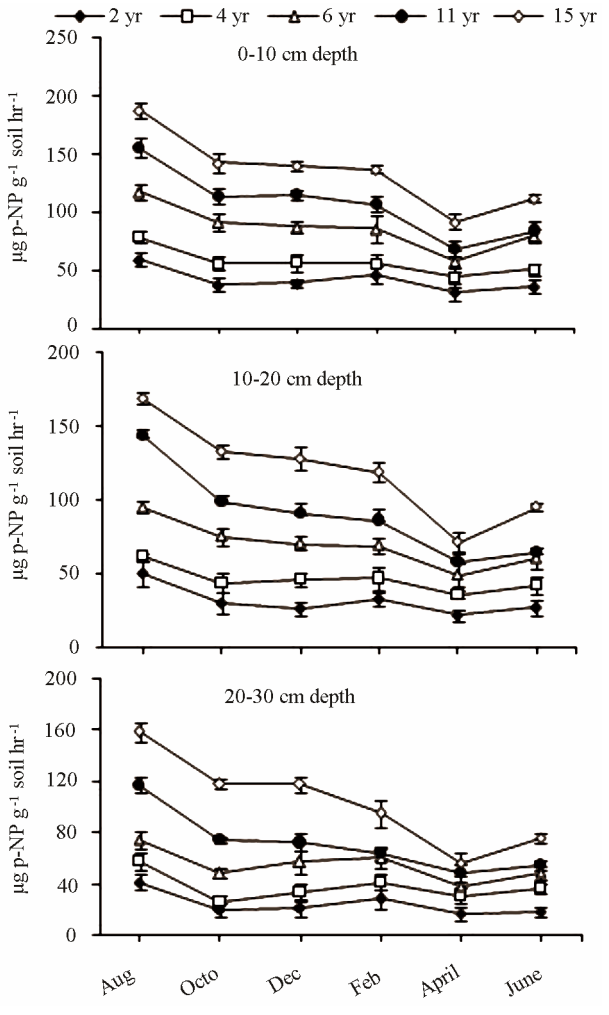

Figure 3. Cellulase activity in soil of different abandoned fields during different months and at different depths.

Decreasing level of soil moisture was found with increase in depth irrespective of fallow period. The bulk density ranged from 1.25 g·cm^{-3} in the surface soil layer (0 - 10 cm) of 15 yr·F land to 1.6 g·cm^{-3} in the subsurface layer (20 - 30 cm) of 2 yr·F land. Lowest water holding capacity (WHC) was recorded at 20 - 30 cm depth of 2 yr abandoned field (19.72%) and highest WHC was recorded in 0 - 10 cm depth of 15 yr abandoned field (36.7%). ANOVA revealed significant difference of WHC between different fallow lands and between depths at $p < 0.001$.

The soil pH was acidic in nature and varied from 5.35 to 6.28. The pH value increased over time since abandonment and highest value was seen in the upper soil layer (0 - 10 cm) of 15 yr·F land. The soil organic matter increased significantly with increasing year of abandonment being highest in 0 - 10 cm depth of 15 yr abandoned field (13.43 mg·g^{-1}) and lowest in 20 - 30 cm depth of 2 yr·F lands (6.17 mg·g^{-1}). Similar trend was observed for total nitrogen in the soil. The Soil microbial biomass carbon ranged from 104.50 to 513 µg·g^{-1} in top soil (0 - 10 cm), 81.04 to 364 µg·g^{-1} of soil in 20 - 30 cm depth and 58.93 to 280 µg·g^{-1} in 20 - 30 cm soil depth.

3.2. Soil Enzyme Activity

Figures 2-6 show amylase, cellulase, dehydrogenase, phosphatase and urease activities respectively in relation to sites, depth and season. Highest activities of all the enzymes were observed in top soil (0 - 10 cm), and were found to be decreasing with increasing depths. Seasonal variations in the enzyme activities in different sites indicated a peak during August (wet season) and lowest enzyme activities was observed in dry season *i.e.* during April. All the enzyme activities increased with the increase in years of abandonment. ANOVA revealed significant difference between sites, depths, and between different seasons at $p < 0.001$. Pearson's correlation analysis indicated significant correlation between en-

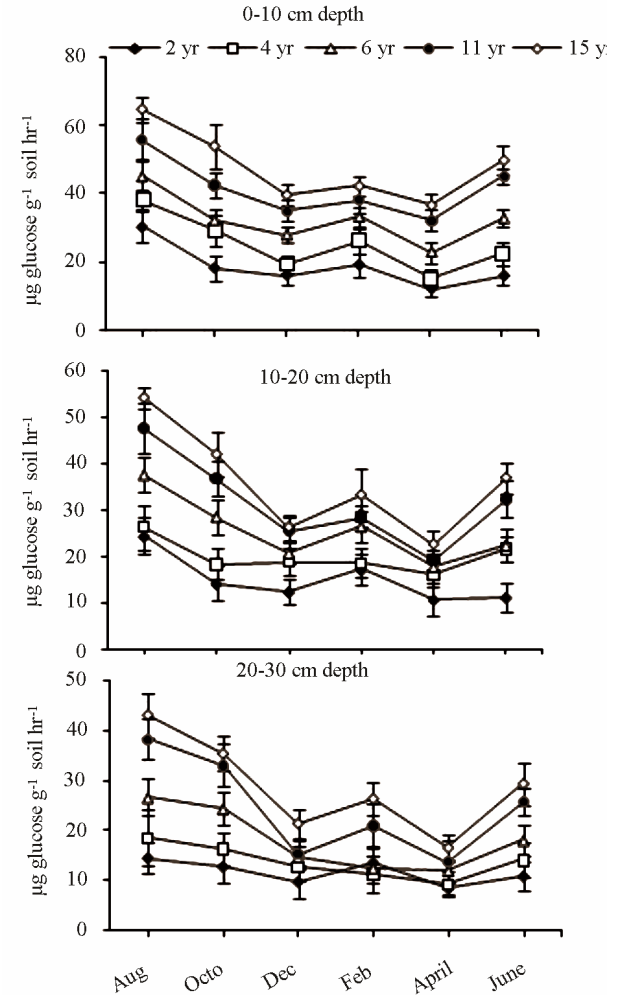

Figure 4. Dehydrogenase activity in soil of different abandoned fields during different months and at different depths.

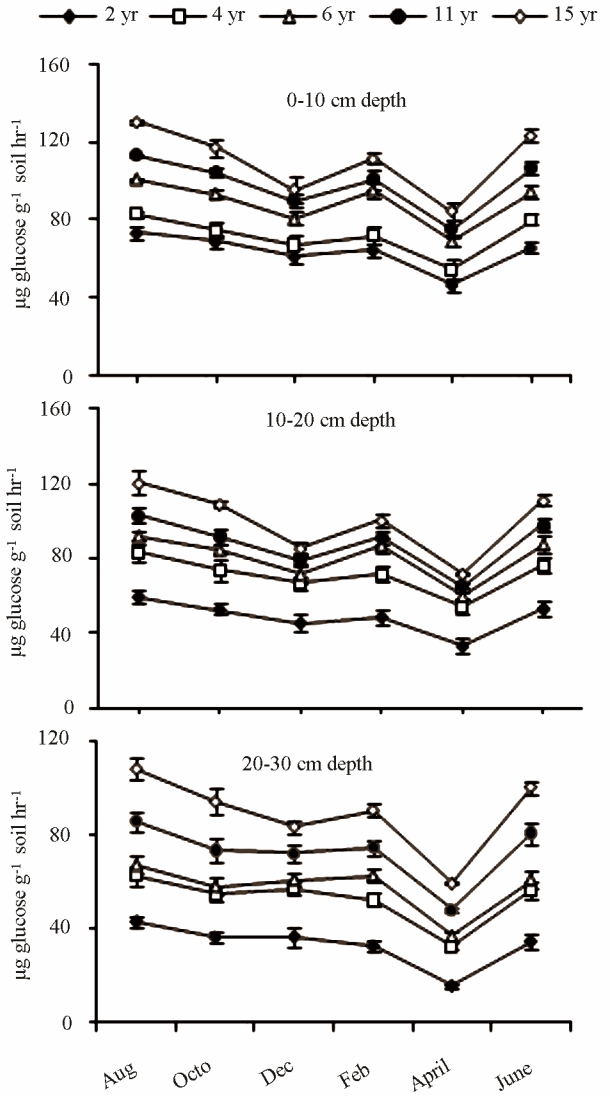

Figure 5. Phosphatase activity in soil of different abandoned fields during different months and at different depths.

zyme activities and soil physico-chemical parameters (**Table 2**).

4. Discussion

4.1. Soil Physico-Chemical Properties

In the present study, soil was slightly acidic ranging from 5.58 to 6.58. Leaching process tends to acidify soils and partially offset by plant growth [26]. Gradual establishment of plants with increasing year of abandonment is supposed to prevent much of the leaching in WHC and decrease the bulk density. As the fallow period gets extended, the inputs of organic residues from colonizing plants increased the carbon and nitrogen contents of soil. During the natural restoration in 3, 7, 10 year abandoned paddy fields an increase in pools of organic matter, total nitrogen, exchangeable K, Ca, Mg and pH was observed with increase of field age [27]. This supports the present findings. Microbial biomass reflects the degree of immobilization of C and N in soil. Gradual increase in microbial biomass carbon (MBC) was recorded in the present study with a maximum amount in the top soil of 15 yr·F land (513.78 $\mu g \cdot g^{-1}$).

This may be attributed to greater input of plant detritus that is ultimately incorporated into the soil and improves the nutrient pool thereby increases the MBC [28]. The MBC dynamic was significantly correlated with soil organic carbon content during the secondary succession as reported by Arunachalam and Pandey [29]. The present study supports the above findings. This result indicated that organic matter played a pivotal role in the building up and development of soil microbial biomass [30].

4.2. Soil Enzyme Activity

Soils enzymes are major indicators of microbial activity,

Table 2. Pearson's Correlation coefficients between soil properties.

	SM	WHC	BD	pH	OC	MBC	TN	AML	CEL	DEH	PHO	URE
SM	1											
WHC	0.45**	1										
BD	−0.34**	−0.71**	1									
pH	0.41**	0.54**	−0.51**	1								
OC	0.85**	0.56**	−0.51**	0.51**	1							
MBC	0.85**	0.6**	−0.54**	0.61**	0.90**	1						
TN	0.86**	0.55**	−0.47**	0.49**	0.97**	0.89**	1					
AML	0.86**	0.55**	−0.47**	0.51**	0.86**	0.88**	0.85**	1				
CEL	0.83**	0.59**	−0.6**	0.48**	0.81**	0.83**	0.81**	0.89**	1			
DEH	0.82**	0.54**	−0.44**	0.4**	0.81**	0.76**	0.81**	0.86**	0.86**	1		
PHO	0.85**	0.64**	−0.59**	0.53**	0.92**	0.88**	0.91**	0.87**	0.86**	0.87**	1	
URE	0.89**	0.57**	−0.52**	0.49**	0.85**	0.88**	0.84**	0.92**	0.93**	0.87**	0.89**	1

**Significant at $p < 0.01$; SMSoil moisture, WHC Water holding capacity, BDBulk density, OCOrganic carbon, TNTotal nitrogen, AMLAmylase activity, CELCellulase activity, DEHDehydrogenase activity, PHOPhosphatase activity, UREUrease activity.

and their activities always depend upon soil types, vegetation cover, microbial biomass, and microbial diversity during vegetation succession [31]. Dehydrogenase is an intracellular enzyme that exists only in viable microbial cells and it is considered as an index of microbial activity [32]. In the present study the enzyme activities showed peak value in rainy season (August) and lowest in summer (April). Some hydrolases have a tendency to increase in the rainy season [33]. During summer, due to moisture stress, the microbes are in dormant state and their activities become low, but the entry of rainy season caused spurt in flora and microbial population, and thus increased the enzyme activities. Prolonged precipitation along with enhanced plant growth and rhizoid position result in increased extracellular activities [34,35]. The activity of dehydrogenase, phosphatase and urease was found to decrease in summer [36]. Thus the present finding is in agreement with the above observations.

There was significant increase in extracellular enzymes like amylase, cellulase, phosphatase, urease, and intracellular enzyme *i.e.* dehydrogenase with increasing year of fallow period and significant decrease with increase in soil depth. Significantly positive correlation was found between all the enzyme activities with organic carbon, total nitrogen and microbial biomass carbon (**Table 2**). Increasing age of the land with respect to time caused gradual addition of organic matter to the soil through decomposition of shoot and root biomass of different vegetation developed on the surface layer. Higher organic matter content provides higher substrate which acts as energy source for microorganisms and supports high amount of microorganism, hence higher enzyme activities [30]. Later succession exhibited higher enzyme activity than the early period and upper soil layer showed higher enzyme activity than lower layer of soil. The decrease in these enzyme activities with depth might be attributed to the diminution of biological activity down the soil profile [37].

The data on physico-chemical parameters and enzyme activities of present study and the data of natural forest, natural grassland and crop field obtained in the same topography [38] were subjected to principal component analysis. **Figure 7** illustrates the discrimination of different sites on the basis of Z_1 and Z_2 whose cumulative percent variance was 85.9%. The PC separated the 2 yr, 4yr, 6 yr, 11 yr, 15 yr fallow far away from grassland and crop fields. However the 15 yr F is quite nearer to natural forest which indicates that the chronosequence of abandoned fields showed a general trend of reclamation in terms of some of the soil Physico-chemical and biochemical parameters but still required more time to achieve the soil status of natural forest, crop fields, and grasslands. Self regeneration of vegetation on a degraded land in tropics has relatively better reclamation potential

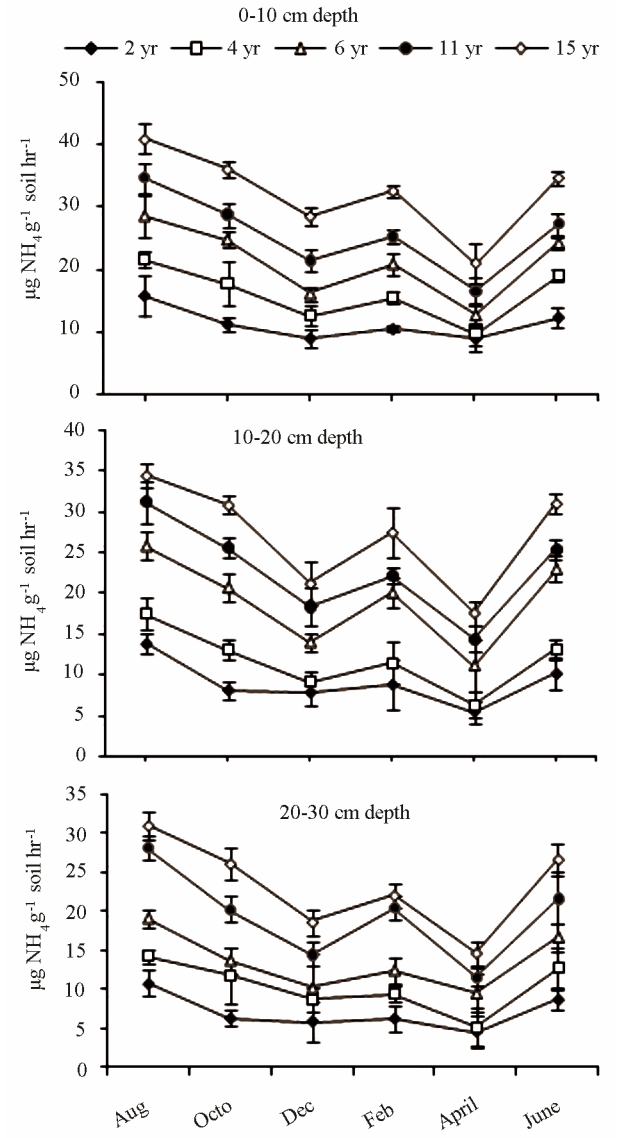

Figure 6. Urease activity in soil of different abandoned fields during different months and at different depths.

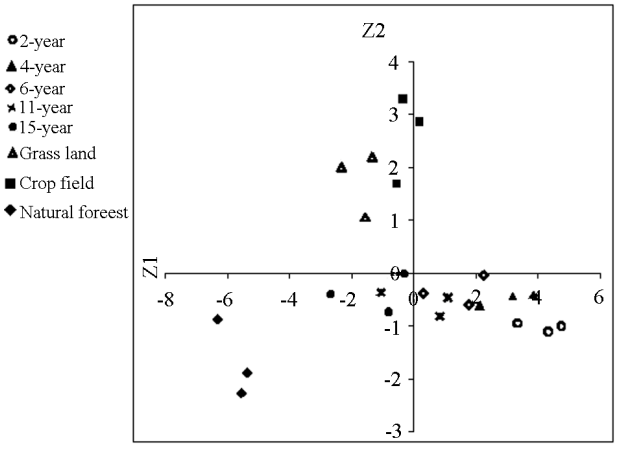

Figure 7. PCA analysis of different study sites.

than that of plantation species [39]. Thus the study justified that the degraded ecosystems derived from natural forest when subjected to abandonment showed the symptoms of reclamation during the course of natural secondary succession.

5. Acknowledgements

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