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Research article

Effect of different growth stages on rice crop on soil microbial and enzyme activities

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Abstract: The influence of growth stages of rice on soil microbial biomass carbon (MBC), soil microbial biomass nitrogen (MBN) and enzyme activities (amylase, dehydrogenase, alkaline and acid phosphatase) in a sub-tropical rice field has been screened. The above soil parameters were investigated at two soil depth (0-10 and 10-20 cm) and in five growth stages of rice crop, *i.e.* 30, 60, 90, 120 and 150 days after transplantation (DAT) of rice seedlings. Results shows that, contents of soil organic carbon, total nitrogen, MBC and MBN were highly influenced by the flowering stage (90 DAT) of the rice crop, at both 0-10 cm and 10-20 cm soil depths, but decline gradually when the crop reaches maturity (120 DAT) and late maturity stages (150 DAT). The soil organic carbon and total nitrogen were positively correlated with MBC and MBN. At both the sampling depths soil amylase activity showed a peak value at seedling and flowering stages (30 and 90 DAT) whereas dehydrogenase and phosphatase showed at flowering stage (90 DAT). Both dehydrogenase and phosphatase showed significant correlation with MBC and MBN.

Keywords: *Oryza sativa* - Soil microbial biomass - Soil enzyme activities.

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INTRODUCTION

Soil microbial biomass is the living component of soil organic matter. As organic matters are the preferred energy source for the microorganisms, ecosystems with high organic substances tend to have higher microbial biomass contents as well as its activities. Usually the highest microbial activity in soil takes place in the surface horizon compared to deeper horizons (Januszek 2011). Most of the enzymatic transformations in soil are accomplished by microbial biomass due to which a part of the organic materials are stabilized as humus and the remaining carbon and other nutrients are utilized by microorganisms for their own growth (Anderson & Domsch 1980). Seasonal changes in soil moisture, temperature and available residue have a strong influence on soil microbial biomass and its activity (Diaz-Ravina *et al.* 1995). Moreover, reduction in microbial biomass and enzyme activities due to excessive cultivation practices have been reported earlier (Gupta & Germida 1988). There is insufficient information on the dynamics of soil microbial biomass in sub-tropical rice field. On the other hand estimation of soil microbial biomass alone couldn't indicate microbial activity in soil, hence study of soil enzyme like dehydrogenase and phosphatase is essential to get a clear picture of soil microbial activities.

Soil enzymes are known to play an important role in the biochemical functioning of soils (Wang *et al.* 2011), including organic residue decomposition (Lei 2011), cycling of nutrients (Makoi & Ndakidemi 2008), and maintenance of soil structure (Dick *et al.* 1994, Balota *et al.* 2004). Their activity is controlled by many factors such as soil physico-chemical properties (Amador *et al.* 1997), soil microbial community (Kourtev *et al.* 2002), type of vegetation (Sinsabaugh *et al.* 2002) and ecological disturbances (Boerner *et al.* 2000). Soil enzymes may originate from plants, animals or microbes and can exist in bound or free form within the soil. Soil microorganisms produce quite a number of extra cellular enzymes to decompose the complex organic matter before it is utilized as a source of energy (Lalitha & Santhaguru 2012). Soil enzymes are specific with respect to the types of reactions they participate. For instance, amylase is a starch hydrolyzing enzyme which hydrolyzes α1-4D glucosidic linkage of amylase and amylopectin and consists of α-amylase and β-amylase. Although β-

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amylase is mainly synthesized by plants, studies indicate that α-amylase is synthesized by plants, animals and microorganisms (Pazur 1965). The microbial activity of soil, to a large extent, is dependent on the quantity of available carbon, and is reflected by dehydrogenase activity (Januszek 2011). Dehydrogenase is involved in biological oxidation of soil organic matter, and is responsible for dehydrogenation of organic matter by transferring hydrogen and electrons from substrates to acceptors (Maurya *et al.* 2011). Phosphatase, originating from microorganisms and root exudates, cleaves the phosphate from organic substrates and is involved in the P cycle in soil (Huang *et al.* 2011). Thus, microbial biomass and soil enzyme activities can potentially provide an integrated biological assessment of soil quality (Chhotaray *et al.* 2011). Evidence suggests that plant species have significant impacts not only on soil physicochemical properties but also more directly on the composition of soil microbial community and their activities (Ushio *et al.* 2008, Ushio *et al.* 2010). Moreover, rhizosphere zone of plants have profound effect on microbial population and activities (Viyas & Gupta 2014). Unlike other terrestrial ecosystems, relatively less effort has been made to elucidate possible influence of growth stages of rice crop on belowground processes in a sub-tropical rice ecosystem. Moreover, limited information is available on the depth wise changes in microbial biomass and soil enzyme activity of agro-ecosystems especially in the widely prevalent rice based cropping system of sub-tropical region.

So, the objectives of the present investigation were to determine the influence of different growth stages of rice on soil microbial biomass and enzyme activities from different soil depth in a subtropical rice field conditions, as well as to establish the relation between soil chemical parameters with microbial biomass and enzyme activities.

MATERIAL AND METHODS

Site description

The study was carried out in Jalukbari experimental field, Guwahati, Assam. The area is situated in south of the river Brahmaputra (26°12′ N, 91°50′ E) with an average altitude of 54 m (a.s.l). The soils of the experimental site are of old alluvial type and sandy loam in texture (Table 1). The climate of the area is monsoonal, characterized by long rainy season (May–September) and dry and cold winter (November–February). The average annual rainfall was about 1782 mm during the study period, with highest rainfall (315.5mm) in the month of September (Fig. 1). The hottest month of the year was August with a mean maximum temperature of 33.0°C and the coldest month was January with a mean minimum temperature of 9.9°C. Maximum relative humidity recorded during the study period was 85% in the month of July.

Table 1. Physical characteristics of soil**.**

Characteristics	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Textural class
Soil test values	5.49	61.00	21.10	14.98	Sandy loam

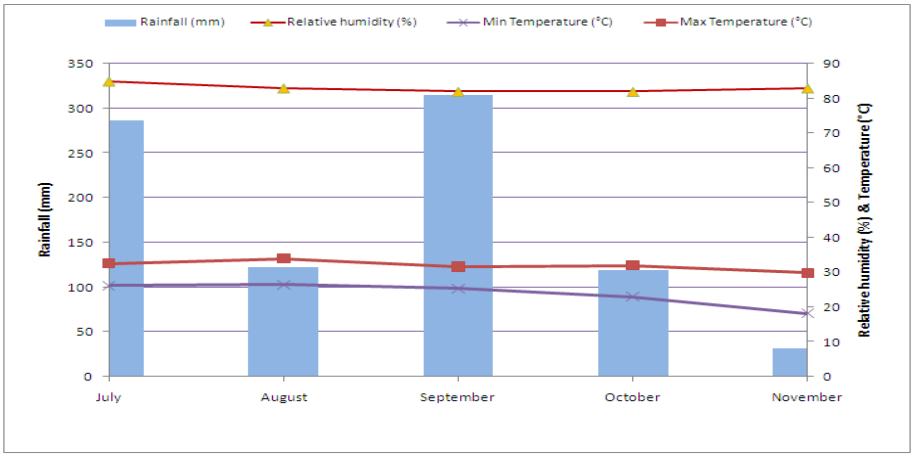

Figure 1. Mean monthly rainfall (mm), temperature (°C) and relative humidity (%) during the cropping season in the study area.

Soil sampling and physico-chemical analysis

Soil samples were collected from the experimental field at 30, 60, 90, 120, 150 days after transplanting (DAT) of rice seedlings during the cropping season. At each sampling date, five soil samples were randomly

collected from 0–10 and 0–20 cm depths using a soil corer. Collected samples from each depth were then put separately into sterilized plastic bags, tied to prevent moisture loss and transferred to laboratory as soon as possible for estimation of microbial biomass and enzyme activities or kept at 4°C until analysis for one week. The soil samples were mixed thoroughly to obtain a homogeneous sample, and sieved through a 2 mm sieve. Mechanical fractions were determined according to the standard procedure (Bouyoucos 1962). The pH of the soil samples was recorded on a digital pH meter in 1:5 (w/v) soil-water suspensions. Soil organic carbon was analysed using rapid titration method of Walkley & Black (1934), as described by Jackson (1973). Total nitrogen was estimated by Kjeldahl method (Jackson, 1973).

Estimation of microbial biomass and soil enzyme activities

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by fumigation-extraction method (Brookes *et al.* 1985, Vance *et al.* 1987). Enzyme activities like amylase is assayed by as per method of Kelly & Rodriquez (1975) and estimation of the reducing sugars released was estimated using the DNS method (Gascoigne & Gascoigne 1960). Dehydrogenase activity was assayed under standard conditions by the method of reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) (Casida 1977) and acid phosphatase and alkaline phosphatase were assayed as per the method of Tabatabai & Bremner (1969).

Statistical analysis

Statistical analysis was performed with SPSS program Version 17. Regression analyses and Pearson correlations were used to determine the interrelationships among the measured soil properties.

RESULTS

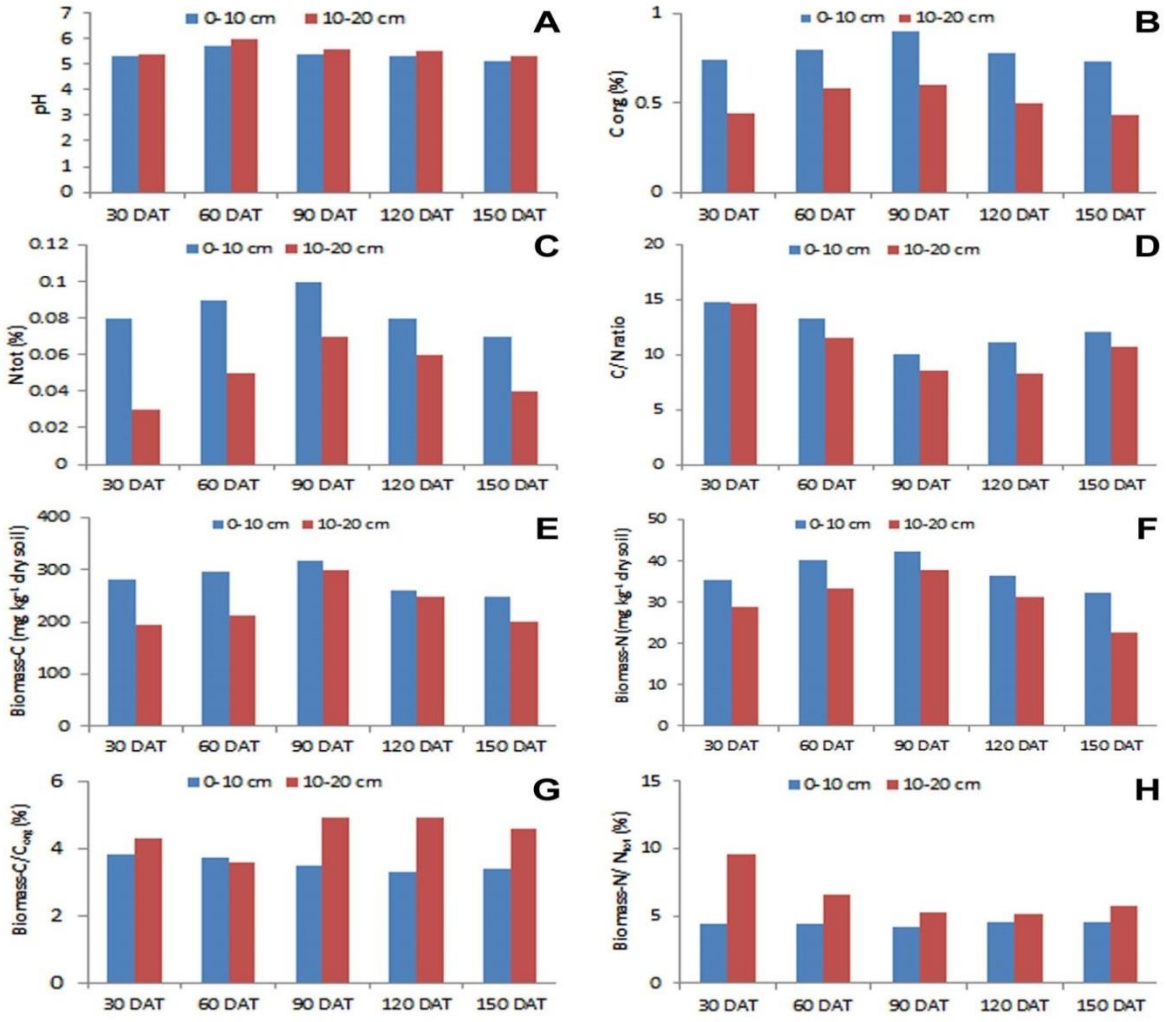

Figure 2. (A–D): Chemical characteristics of the soil samples collected from 0–10 and 10–20 cm depths at 30, 60, 90, 120 and 150 days after transplanting rice seedlings: **A,** pH; **B,** total organic carbon (C_{org}); **C,** total nitrogen (N_{tot}); **D,** C/N ratio; **(E–H):** Changes in the values of soil microbial biomass: **E,** Biomass-C; **F,** Bomass-N; **G,** Biomass-C/C_{org} (%); **H,** Biomass-N/ N_{tot} (%).

The basic chemical properties of the soils showed a range of soil conditions in the experimental site (Fig. 2A–D). Soil pH ranged from 5.1 to 5.7 and 5.3 to 6.0 in 0–10 and 10–20 cm, respectively. The concentration of soil organic carbon and total nitrogen in 0–10 cm layer was higher than that in 10–20 cm layer. At 90 DAT when the rice crops attain flowering stage, high organic carbon (0.90% and 0.60%) and total nitrogen (0.10% and 0.07%) levels were recorded in both the depth as compared to the preceding stages. Soil organic carbon showed high correlations with microbial biomass carbon, microbial biomass nitrogen, dehydrogenase and phosphatase activity (Table 2.). However, no such correlations were observed in case of total nitrogen. The C/N ratio was high at 30 DAT (14.8 and 14.6) *i.e.* when the rice crop was at seedling stage.

Table 2. Pearson's linear correlation coefficients (r) between soil chemical properties, microbial biomass, and soil enzymes.

Variables	A	B	C	D	E	F	G	H	I
pH (A)	1.000	0.650	0.307	0.384	0.698	0.451	0.688	0.420	0.540
Organic carbon (B)		1.000	0.863	0.939*	0.951*	0.348	0.934*	0.947*	0.981**
Total nitrogen (C)			1.000	0.920*	0.818	0.174	0.748	0.834	0.851
Microbial biomass carbon (D)				1.000	0.910	0.384	0.900	0.951*	0.939*
Microbial biomass nitrogen (E)					1.000	0.603	0.988**	0.840	0.881*
Amylase (F)						1.000	0.658	0.209	0.216
Dehydrogenase (G)							1.000	0.847	0.873
Acid Phosphatase (H)								1.000	0.987**
Alkaline phosphatase (I)									1.000

Note: ** & * Correlation is significant at the 0.01 & 0.05 level respectively (2-tailed), n = 5.

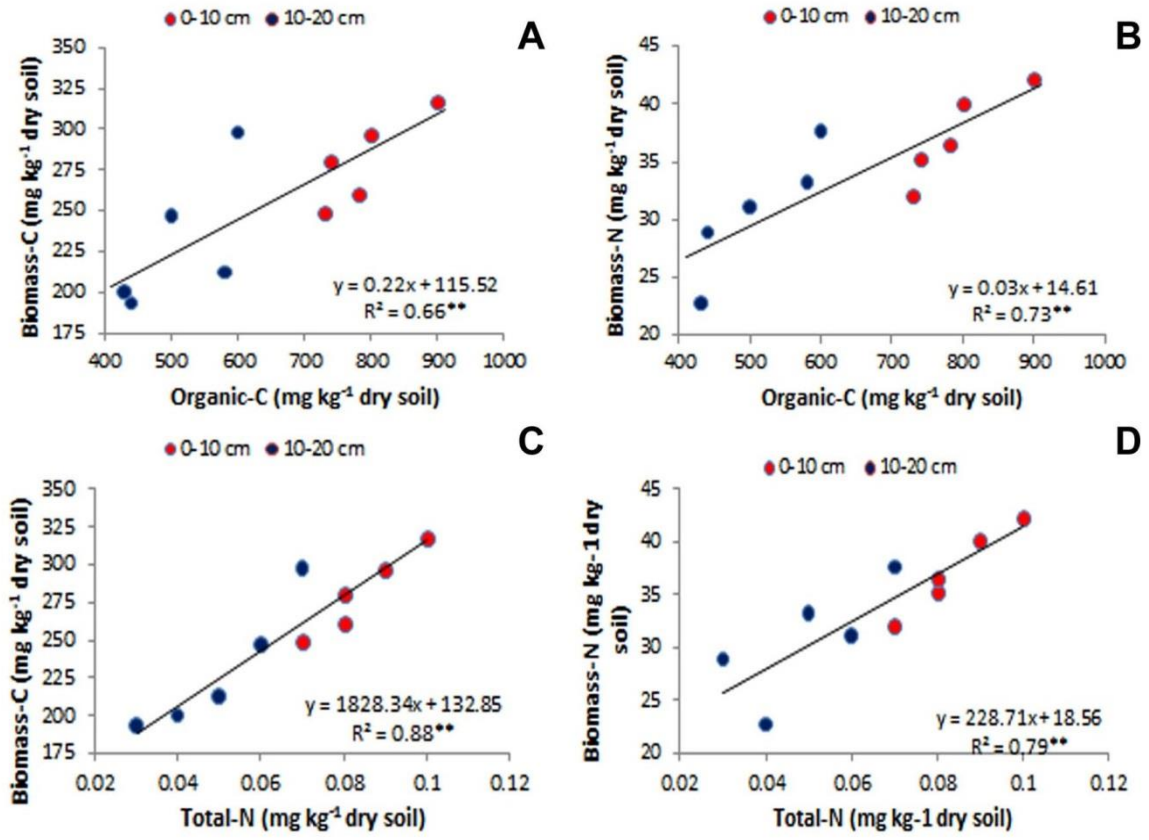

Figure 3. Relationships between: **A**, organic-C and biomass-C; **B,** organic-C and biomass-N; **C,** total-N and biomass-C; **D,** total-N and biomass-N.

Soil MBC and MBN contents were shown in figure 2E–H and the correlations with soil parameters are shown in table 2. MBC content varied from 249 to 317 mg kg^{-1}dry soil in 0–10 cm and 193 to 298 mg kg^{-1}dry soil in 10–20 cm depth, highest being at the flowering stage (90 DAT). Similarly, MBN varied from 32 to 42 mg kg^{-1}dry soil and 29 to 38 mg kg^{-1}dry soil in 0–10 and 10–20 cm soil depth respectively, with highest value at the flowering stage. Thus the soil organic carbon, total nitrogen MBC and MBN content were higher in surface soil layer (0–10cm) compared to sub-surface soil layer. According to earlier findings (Wang *et al.*, 2011) the

value of MBC and C_{org} decrease with soil depth. These results are in consistent with our present findings. Moreover, their concentrations were highest at 90 DAT (flowering stage) and lowest in 150 DAT (late maturity stage). This may be attributed to increase in the root exudates in the flowering stage leading to more intense microbial activity which gradually decline when the crop attained maturity to late maturity stages. High soil microbial biomass during flowering stage may also be attributed to sufficient moisture availability due to rainy summer period. The result is in consistence with earlier researcher (Singh *et al.*, 1989). There was a positive correlation of soil organic carbon and MBC (R^2 = 0.66, P< 0.05) or MBN (R^2 = 0.73, P< 0.05, and of total nitrogen and MBC (R^2 = 0.88, P< 0.05) or MBN (R^2 = 0.79, P< 0.05) in both the soil depth (Fig. 3). These relationships were in consistent with many studies, where soils with high organic matter content supported high levels of microbial biomass (Brookes *et al.* 1985, Wardle 1992, Bauhus & Khanna 1999, Friedel *et al.* 2006). Microbial C/N ratio ranged from 7.2 to 7.9 in 0–10 cm layer and 6.4 to 8.8 in 10–20 cm layer. Witt *et al.* (2000) reported microbial C/N ratio of 3.3 to 20.7 in Philippines rice soil. Inubushi *et al.* (1991) obtained unusually wide microbial C/N ratios of 9–12 in three waterlogged Japanese rice soils. Microbial quotient (MBC/ total organic-C and MBN/total N) has been reported as a useful indicator of changing soil processes and soil quality than microbial biomass or total organic matter alone (Anderson & Domsch 1989, Sparling 1992). In our study MBC/ organic-C varied from 3.3 to 3.8% and 3.6 to 4.9%, and MBN/total-N varied from 4.2 to 4.6 % and 5.2 to 9.6% in 0–10 and 10–20 cm depth respectively. Sparling (1997) suggested that if a soil is being used exploitively, microbial C pools will generally decline at a faster rate than total organic matter, and microbial quotient will decrease accordingly. Depending on the nutrient status and soil management, metabolic quotient values below 2.0% are regarded as critical (Anderson 2003). Soil pH is generally considered as one of the important regulator of microbial activity. In the present study total microbial biomass was lower under acidic soil conditions and with the increase in soil pH the microbial quotient was found to increase. The result is in agreement with the findings of Anderson (2003). Thus, seasonal variations, plant developmental stage, organic matter content and soil depth have been found to have a great influence on soil microbial biomass (Yang *et al.* 2010).

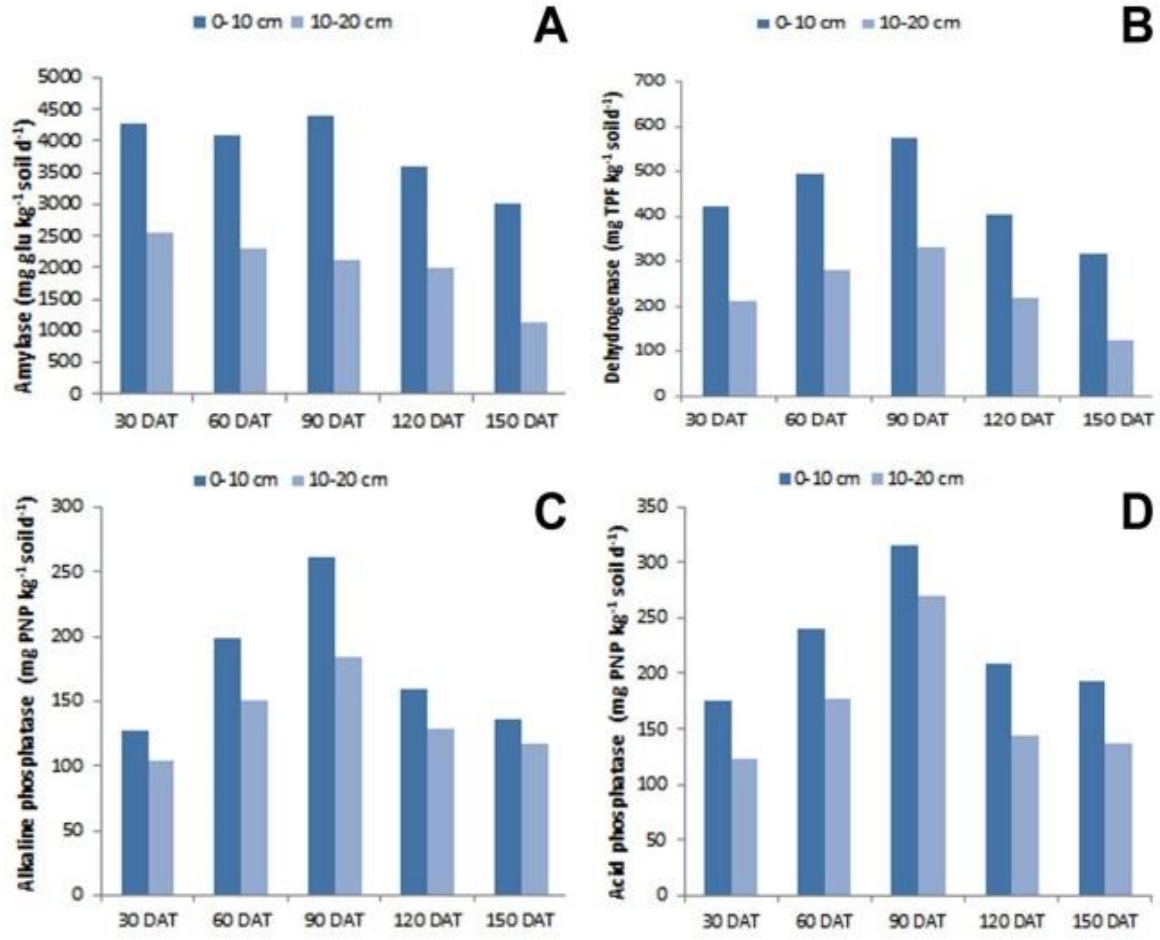

Figure 4. Changes in the enzyme activities of the soil samples collected from 0–10 and 10–20 cm depths at 30, 60, 90, 120 and 150 days after transplanting rice seedlings: **A,** amylase activity; **B,** dehydrogenase activity; **C,** alkaline phosphatase activity; **D,** acid phosphatase activity.

Figure 4 reveals soil enzyme activities at different stages of plant growth and in two different soil depths. The activity was more in 0–10 cm than 10–20 cm depth. The progressive decline in amylase activity with increasing soil depth in rice agroecosystem has been reported earlier (Chhotaray *et al.* 2011). The amylase activity increased with the increase in the rice growth and declined at late maturity stage. The activity was more in the soils collected at 30 DAT (4280.21 and 2543.67 mg glucose kg^{-1} soil d^{-1}) and 90 DAT (4390.25 and 2130.63 mg glucose kg^{-1} soil d^{-1}). At 150 DAT the level of activity declined (3024.43 and 1121.45 mg glucose kg^{-1} soil d^{-1}) in the two soil depth. The dehydrogenase activity of the soil ranged from 315.10 mg TPF kg^{-1} soil d^{-1} to 572.95 mg TPF kg^{-1} soil d^{-1} in 0-10 cm depth and from 124.25 mg TPF kg^{-1} soil d^{-1} to 332.56 mg TPF kg^{-1} soil d^{-1} in 10–20 cm depth. There was progressive decrease in the dehydrogenase activity from 90 DAT to 150 DAT (flowering to late maturity stage of the rice). The higher dehydrogenase activity at 90 DAT was likely due to high C input in the soil in the form of root mass that enhanced microbial activity. The relation between dehydrogenase activity and C inputs has been well established (Maurya *et al.* 2011). Phosphatase activity (alkaline and acid phosphatase) was maximum during flowering stage in both 0–10 cm (260.64 and 314.49 mg PNP kg^{-1} soil d^{-1}) and 10–20 cm (183.45 and 269.63 mg PNP kg^{-1} soil d^{-1}) depth. High phosphatase activity during flowering stages may be due to faster growth rate of plants and microbes owing to wet rainy period. As because, heavy rain in wet season often leads to nutrient loss and low available P detected in the wet season intensifies the phosphatase activity to meet the increasing P demand by plant and microbes growth (Huang *et al.* 2011). Both dehydrogenase and phosphatase showed significant correlation with MBC and MBN but no such correlation was showed by amylase. In our study no significant correlation between enzyme activities and soil pH were found. This may be due to the narrow range of pH values, 5.1–6.0 (Balota *et al.* 2004).

It is evident from the present study that soils collected at 90 DAT showed significantly higher enzymatic activities compared to those collected from other growth stages. At 90 DAT rice roots might have secreted more organic acid and carbohydrate, which stimulated higher soil enzymatic activities. The results corroborate with the findings of Zeng *et al.* (2005), Wang *et al.* (2011). Moreover, microbial biomass and enzyme activities were greater in the surface layer (0–10 cm). This is due greater nutrient availability in the surface layer which enhanced microbial activity (Ralte *et al.* 2005).

CONCLUSIONS

Increase in microbial biomass and enzyme activities indicates high rate of release of nutrients by rice crops which facilitate microbial activities. In addition, varying soil depth to certain extent is expected to influence increase in bioactivity. However, these findings are not sufficient to get a clear picture of the whole microbial activities in sub-tropical rice ecosystem, because present results were obtained from a short term study. Detail field level studies are necessary to expand the knowledge in this aspect.

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