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Soil biological pools in traditional long-term rainfed rice ecosystems in hill agriculture

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ABSTRACT

The soils of stable hill rice ecosystems *viz.* mono-cropped lowland and upland terraces in the present study were characterized in terms of biological pools of carbon (C), nitrogen (N) and phosphorus (P) and compared ($P<0.01$, paired t-test) and studied the pair-wise correlation for *kharif* and *rabi* season. Seasonal fluctuation in soil pH and different fractions of C, N and P *viz.* soil organic C (SOC), microbial biomass C, N, P (MB -C, -N, -P), dissolved organic C (DOC), basal respiration (BAS), substrate induced respiration (SIR), extractable organic N (EON), potentially mineralizable N (pMN), dehydrogenase (DHA) and phosphatase activity (PHA) were observed for different rice ecosystems. C and N components in soils of stable lowland and upland terrace rice ecosystems seem to be self-sustained, but the major limiting factor was availability of P. Moisture content were observed to be a critical variable that control the size and dynamics of biological pools of C, N and P and the interrelationships among the parameters in different rice ecosystems.

1. Introduction

Long term stable traditional rice ecosystems in Northeast India are the rainfed lowland in between the valleys and the upland terraces curve out in sloppy hill tops. These two rice ecosystem types are the rice production bowl of Northeast. The lowland rice ecosystems are waterlogged during a major part of the crop growing period and are predominantly anaerobic and harbour a unique microbial ecology. These ecosystems are different from upland terrace rice soils in several physico-chemical and biological properties (Adhya and Rao 2005). Both these type of rice ecosystems are purely mono-cropping area, sustains with huge weed biomass and rice crop residue as the source of nutrients with no external nutrient inputs. Soils of these rice ecosystems are acidic (<6 pH) in reaction with low nutrient use efficiency and high nutrient depletion rate due to high rainfall (≥760 mm to 1000+ mm average) and excess runoff.

The long term productivity and the sustainability of these rice ecosystems depends on the in-situ decomposition, microbial activity, mineralization and immobilization activity of the microorganism present in the system. The repeated intensified rice mono-cropping for several more years might begin to show a declining trend in productivity of rice as well as the soil deterioration over the years (Cassman and Pingali 1995). Soil organic matter (SOM) is the key element in determining the soil quality, productivity and sustenance of any ecosystem type and the labile fractions of SOM-microbial biomass carbon (MBC), dissolve organic carbon (DOC) and water soluble carbon *etc.*) are the indicators for any changes in soil health due to land use and management practices and their capacity to supply nutrients for both plants and microorganism. It is observed that >95% of total ecosystem carbon (C), nitrogen (N) and phosphorus (P) are contained in SOM, and their recycling and depletion rate is a function of many factors including soil moisture, temperature, aeration, decomposition rate, topography *etc.*

The long term rainfed rice ecosystems selected for the study is the traditional rice growing areas in Meghalaya. Rice crop in these regions occupy an area of 11,364 ha out of 26,921 ha net sown area and the site selected represents one mega-environment -based on the GGE-biplot analysis of multi-locational data of rice varietal trials (Tripathy *et al.*, 2007). Characterizing the soil biological pools in these traditional rice growing areas will be helpful to know the present soil health status and its probability of sustenance in the future.

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2. Materials and Methods

2.1 Description of the soil sampling sites

Two types of hill rice ecosystems at elevation range of >800 m above mean sea level were selected from the Kyrdemkulai village (Ri-Bhoi district) of Meghalaya plateau in North East India. These hill rice ecosystems were classified into lowland rice ecosystem and upland rice ecosystem. Lowland is the long term mono cropping rice ecosystem of >40 years old, whereas upland is the terrace rice cropping system of >15 years old. Both the ecosystems were never treated with any agro-chemicals and only input is weed biomass and crop residues incorporated in the field at ploughing time. Composite soil samples (0-15 cm) from 5 random locations were collected from each ecosystem during *kharif* and *rabi* season. Three same type of the rice ecosystems were taken as the replication. One part of each soil sample was immediately stored at 4°C until analysis for microbiological parameters and other part was air dried in laboratory and sieved through 2 mm sieve for chemical analysis.

2.2 Methods of soil analysis

Gravimetric soil moisture content (MC) was determined by oven drying at 105°C to constant weight. The relative proportion of sand, silt and clay (soil texture) in soil sample was determined by hydrometer method (Buoyoucous 1962). Air dry soil samples were analyzed for pH (1:2.5 soil/water suspension) using glass electrode pH meter. Soil organic carbon (SOC) was determined by wet oxidation method as described by Walkley (1947). Available nitrogen (Avl-N) was determined by alkaline permanganate oxidation method described by Subbiah and Asija (1956). Available P (Avl-P) in soil was determined by stannous chloride blue colour method (Bray and Kurtz 1945). The intensity of blue colour using Dickman Bray's reagent was measured at 660 nm. Soil total phosphorus (TP) was determined by digesting the soil sample with di-acid mixture and estimating in ammonium paramolybdate vanadate reagent and soil total nitrogen (TN) was determined by digesting air dry soil sample with concentrated H_2SO_4 in presence of digestion mixture followed by Kjeldahl distillation method described by Page *et al.* (1982). Dissolved organic carbon (DOC) and extractable organic nitrogen (EON) were extracted from the freshly collected soil samples. The DOC fraction was extracted by using 1*M* KCl solution at a ratio of 5:1 (v/w) followed by filtration through 0.45 µm Whatman nylon membrane (McDowell *et al.*, 2006) and quantified by wet oxidation method. The EON fraction was extracted by using 0.5 *M* K_2SO_4 at a ratio of 4:1 (v/w) (Ros *et al.*, 2009). A portion of the filtered supernatant was used for determination of total extractable N content by Kjeldahl method and the other portion was used for determination of inorganic extractable N (NH_4^+-N and NO_3^--N) by MgO-Devarda alloy method (Page *et al.*,1982). The difference between extractable total N and extractable inorganic N was considered as EON content.

Microbial biomass C, N and P (MBC, MBN and MBP) were determined by the chloroform-fumigation extraction method (Brookes and Joergensen 2006). For determination of MBC and MBN in the soil samples, 0.5 *M* K_2SO_4 (1:4 w/v) was used as an extractant and MBC was determined in the supernatant by wet oxidation method and MBN by Kjeldahl method. Analysis of MBP was done by following the Bray's I method (0.03 *N* NH_4F+0.025 *N* HCl). The difference in C, N and P content between fumigated and non-fumigated sub-samples was determined and then, calculated using conversion factors, K_{EC} = 0.38, K_{EN} = 0.45 and K_{EP} = 0.40 for MBC, MBN and MBP, respectively. Potentially mineralizable N (pMN) was determined by static anaerobic incubation method in freshly collected soil samples (Canali and Benedetti 2006). Soils were incubated at 40°C for 7 days and extracted using 4 *M* KCl solution. Mineralized N during 7 days incubation was calculated by subtracting the NH_4^+-N of non-incubated from that of incubated sample. Soil basal respiration (BAS) was measured at field capacity moisture content by using the standard base (NaOH solution) trap method (Pell *et al.*, 2006). Substrate induced respiration (SIR) was determined in the soil sample in incubation vessel by addition of glucose (Anderson and Domsch 1978). Soil and glucose mixed thoroughly with spatula and 1 *M* NaOH in scintillation vial was placed inside the incubation vessel and then, incubated at 22°C for 21 days. The excess of NaOH was titrated against 0.05 *M* HCl at 7, 14 and 21 days and the set up without soil sample was also maintained as check and CO_2 evolution was calculated per hour basis.

Dehydrogenase activity (DHA) was determined in air dried soil samples as per the method described by Casida *et al.* (1964) in which concentration of triphenyl formazan (TPF) in supernatant was determined against a standard graph prepared using known concentrations of TPF in 485 nm wavelength and the activity of DHA was expressed as µg TPF g^{-1} soil h^{-1}. Acid phosphomonoestarase activity (PHA) was determined in fresh soil samples as per the procedure described by Tabatabai and Bremner (1969) in which the intensity of yellow colour was measured in the filtrate at 400 nm using microtiter plate reader (Multiskan, Thermo Scientific, USA). The concentration of *p*-nitrophenol in the filtrate was determined against a standard curve prepared by using *p*-nitrophenol standard solution and the activity of PHA was expressed as µg PNP g^{-1} soil h^{-1}.

2.3. Statistical analysis

All univariate analyses were performed using SPSS v21.0 (SPSS Inc., Chicago, IL, USA). Within a site, each parameter analyzed for different seasons was normally distributed as determined using Kolmogorov-Smirnov test and any significance difference between seasons was performed by paired t-test ($P<0.01$). The pair-wise correlation matrix was also developed irrespective of site to find out the relationship between various parameters.

3. Results and Discussion

The waterlogged lowland rice ecosystems supported a diverse aquatic weed community with huge production of above and below-ground biomass. This weed biomass under high moisture content and the fluctuation of temperature with the season has differential effect on the decomposition and release of nutrients in rice ecosystems. Unlike lowlands, in upland terraces, the pronounced moisture conditions were observed for the *kharif* (61.4% MC) and *rabi* (22% MC) season (Table 1). The soil textural class of lowland and upland terraces was silt loam and loam respectively. Soil pH in *rabi* season was found to be significantly higher (paired t-test, $P<0.01$) as compared to that in *kharif* season in all rice fields. This seasonal fluctuation in soil pH and lower pH during the *kharif* might be the presence of organic acids in soil solution due to the anaerobic decomposition of weed biomass and organic matter decay under high temperature and excessive moisture in *kharif season*. Such activities considerably reduced with the decrease in soil temperature in *rabi* season and hence, the increment in soil pH was noticed. Another reason for the fluctuation in soil pH is the movement of salts into and out of the soil zones as the soil moisture moves up and down through the soil profile (Brady and Weil 2007). The significantly higher SOC and TN content observed in lowland rice ecosystem and higher in *rabi* season as compared to that in *kharif* ($P<0.01$). C:N ratio was also observed to be higher in lowland soils than the upland and significant higher soil C:N ratio in *kharif* season was observed in upland soils; whereas seasonal change in C:N ratios in soils of lowland was non-significant. Avl-N was higher (average 675 kg N ha^{-1}) in soils of lowland than upland rice, but then significant changes was not observed, however seasonal variation of Avl-N was pronounced in soils of upland rice. Availability of P was significantly higher in *rabi* season in comparison to that of *kharif* in rice soils. Irrespective of ecosystems and seasons, the total phosphorus (TP) content ranged from 1363 to 1436 kg ha^{-1} (Table 1) with non-significant seasonal variation ($P>0.01$). The non-fluctuation of TP in between the seasons is because the turnover rate of P content in soil is very slow to be identified within the short period of time, which is not in case of Avl-P (Venkatesh *et al.*, 2001).

3.1 Labile pools in rice ecosystems

The labile fractions *viz.* DOC, BAS, EON, pMN (Table 1) and enzyme activities DHA and PHA (Table 2) were studied for both the rice ecosystem types. These labile fractions are mostly generated by the microbial activities during the decomposition process from plants, litter and humus and the quantity of its content is highly influence by soil moisture, temperature and availability of biomass in the system (Kalbitz *et al.*, 2000). DOC and BAS content were higher in lowland than the upland soils and the content were higher in *kharif* season, which is similar to the study of Laik *et al.* (2009). EON and pMN also follow the similar trend like other labile fractions (Table 1). The studies on EON and pMN indicated the stability of the ecosystem and the less disturb ecosystems support larger amount of labile-N fractions (Haynes 2005; Schmidt *et al.*, 2007; Ros *et al.*, 2009) *viz.* EON, Avl-N, MBN and higher rate of pMN. These fractions are also highly dependent on the inputs from the rhizosphere, change in land use and biomass content. pMN, the rate of mineralization of active fraction of organic N through microbial action, gives the idea on N supplying capacity of the soil for crop growth (Doran and Parkin 1994). The higher pMN in lowland soils indicated the higher supplying capacity of lowland rice soils comparing to the upland terraces. DHA and PHA were also observed highest in lowland soils (Table 2). Higher phosphatase activity in soil coincided with higher contents of TP and microbial biomass in rice soils (Chhonkar and Tarafdar 1984). DHA on the other hand, is an important indicator parameter to study the biological activity of agricultural soils including flooded soils (Nannipieri *et al.*, 2002; Wlodarczyk *et al.*, 2002). Decreased redox potential in lowlands showed higher DHA activity (Makol *et al.*, 2008).

Glucose induced soil respiration (SIR) for the 1st, 2nd and 3rd week of incubation was significantly higher in *kharif* as compared to that in *rabi*, irrespective of rice fields (Table 2). Though glucose induced respiration was determined at constant temperature of 22°C at laboratory, it might be possible that inactive members of the soil microbial community due to low temperature in *rabi* season need a lag period to be activated. It was reported that on substrate availability soil respiration increases due to activation of certain groups of microbes resulting in microbial CO_2 production which is proportional to the mass of organisms (Hoper 2006; Bailey *et al.*, 2008).

3.2 Microbial biomass C, N and P to and their contribution to C, N and P in soils

MBC, MBN and MBP content was higher in lowland rice soils and content was significantly higher (P<0.01) in *kharif* season. Contributions of microbial biomass C, N, and P to the soil organic C and total N and P were found to be higher in lowland rice fields followed by upland soils respectively (Table 2). Diverse substrate availability and moisture content in soils of lowlands round the year supported higher microbial biomass in comparison to other rice ecosystems and this microbial biomass dynamics in rice soils is largely dependent on the organic substances released by roots (Lu *et al*., 2002). The C:N:P ratios of microbial biomass in soils of rice fields varied in the range from as low as 35.3:4.9:1 to a maximum of 54.0:6.5:1 with an indication of narrow ratio in lowland rice soils and wider ratio in upland soils. This finding clearly showed that MBC, MBN and MBP act as a major sink in replenishing pools of C, N and P in lowland rice ecosystems. Soil microbial biomass has an average C:N:P ratio of ~50:6:1 (Smith and Paul 1990). So, the mineralization potential of C, N and P is higher in lowland rice soils as compared to that in upland rice soils.

3.3 Relationship among various fractions of soil C, N and P

Season-wise all fractions of C, N, and P were analyzed for pair-wise correlations among themselves, irrespective of rice fields in Fig. 1 and Fig. 2 for *kharif* and *rabi* season, respectively. The correlations among the fractions of C, N and P in different seasons differ according to the availability of soil moisture and change in temperature condition. Strong either positive or negative correlations observed for almost all the fraction of C, N and P with the MC in both *kharif* and *rabi* seasons. MBC, the living and the most active part of soil organic carbon (SOC) (Friedel *et al*., 1996) showed strong positive correlation with SOC both in *kharif* and *rabi* seasons (Ingram *et al*., 2005). Several researchers earlier showed that there is a significant linear relationship between MBC and SOC in temperate environments and this relationships exist in tropical environments as well, where the SOC levels are very low (Leita *et al*., 1999; Banerjee *et al*., 2006). DOC is considered as the mobile phase plays a major role in the transport and supply of C and N to microbial populations (Cookson *et al*., 2005). The production and composition of DOC is largely dependent on its equilibrium with SOC (Gregorich *et al*., 2000). In our study too, irrespective of seasons, SOC maintained a strong positive correlation with DOC (P<0.01). Avl-P had positive correlation with soil pH in both the seasons (r=0.73 in *kharif* and r=0.79 in *rabi*). EON during *rabi* season had a either positive or negative correlation with almost all the fraction (MC, pH, MBC, SOC, DOC, BAS, MBN, Avl-N, pMN, Avl-P, MBP and PHA) which is not observed during *kharif* season, which might be because of the excess moisture during the *kharif*. PHA also indicated strong correlation with almost all the fractions except with TN and TP under study. pMN maintained a direct correlation with soil pH both in *kharif* and *rabi* season, which is similar to the reports of Paul *et al*. (2001), where he showed that soil pH affects the microbial activity as well as the decomposition in the soil.

4. Conclusion

The rice ecosystems *viz*. the lowland and the upland terraces prevailed in hilly regions varied greatly in terms of biological pools of C, N and P in soils, and these pools were strongly influenced by soil moisture content according to the season. The lowland rice ecosystem was waterlogged throughout the year with diverse aquatic weed biomass, which under high moisture and fluctuating temperature condition between the seasons had a differential effect on decomposition and release of nutrients. Overall, it can be concluded that the rice ecosystems are self-sustaining in C and N components and availability of P is the limiting factor.

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6. Declaration of Interest

The authors declared that there is no conflict of interest relevant to this research article.

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Table 1 Carbon, phosphorus and nitrogen fractions in soils of rice ecosystems as influenced by seasons

Site	Season	Textural class	MC (%)	pH	SOC (%)	TN	TP	Soil C:N
						kg ha^{-1}		
Lowland	*Kharif*	Silt loam	waterlogged	4.92±0.06^{a}	2.94±0.07^{a}	5905±90^{a}	1436±40^{a}	11.4:1^{a}
	Rabi		waterlogged	5.09±0.02^{b}	3.20±0.04^{b}	6071±146^{b}	1398±37^{a}	11.8:1^{a}
Upland	*Kharif*	Loam	61.4±1.2^{a}	5.20±0.04^{a}	2.13±0.04^{a}	5263±76^{b}	1385±30^{a}	9.3:1^{b}
	Rabi		22.0±0.4^{b}	5.30±0.02^{b}	2.12±0.06^{a}	5720±116^{a}	1363±34^{a}	8.2:1^{a}

Site	Season	kg ha^{-1}		μg CO_2 g^{-1} (dw) soil h^{-1}	μg g^{-1} (dw) soil		
		Avl-N	Avl-P	BAS	DOC	EON	pMN
Lowland	*Kharif*	647±34.0^{a}	6.2±0.3^{b}	0.43±0.04^{a}	1718±17.0^{b}	283±14.5^{b}	69.4±2.0^{a}
	Rabi	675±28.5^{a}	10.5±0.7^{a}	0.44±0.02^{a}	1557±43.2^{a}	454±51.5^{a}	68.4±2.7^{a}

	Kharif	552±23.0[a]	12.8±0.5[b]	0.41±0.07[b]	1280±22.2[b]	278±10.4[a]	62.8±3.1[a]
Upland	*Rabi*	384±16.1[b]	19.7±0.7[a]	0.25±0.01[a]	1033±14.5[a]	188±10.6[b]	52.6±3.2[b]

[MC- moisture content, SOC- soil organic carbon, TN- total nitrogen, TP– total phosphorus, Avl-N- available nitrogen, Avl- P– available phosphorus, BAS- basal respiration, DOC- dissolved organic carbon, EON- extractable organic nitrogen and pMN- potentially mineralizable nitrogen]. Within a site for each parameter, values that differ significantly (P<0.01) are followed by different letters, as determined by paired t-test

Table 2 Microbial activity indicators in soils of different rice ecosystems

Site	Season	MBC	MBN	MBP	MBC (x100) –to- SOC$^{\alpha}$	MBN (x100) –to- TN$^{\beta}$	MBP (x100) – to - TP$^{\gamma}$	C:N:P of microbial biomass
		µg g^{-1} (dw) soil						
Low land	*Kharif*	1405±21.4[b]	197.0±14.8[a]	39.8±3.6[a]	4.8	7.5	6.2	35.3:4.9:1
	Rabi	1377±26.0[a]	206.6±19.4[a]	36.5±3.5[a]	4.3	7.6	5.8	37.7:5.7:1
Upland	*Kharif*	992±7.0[b]	148.9±16.3[a]	26.1±2.4[a]	4.7	6.3	4.2	38.0:5.7:1
	Rabi	534±37.3[a]	64.0±5.4[b]	9.9±0.4[b]	2.5	2.5	1.6	54.0:6.5:1

Site	Season	SIR-1week	SIR-2week	SIR-3week	DHA	PHA
		mg C kg^{-1} (DW) soil			µg (TPF) g^{-1} (DW) h^{-1}	µg (p-nitrophenol) g^{-1}(DW) h^{-1}
Lowland	*Kharif*	107.88±3.34[a]	139.27±2.95[a]	131.21±2.66[a]	7.75±0.25[a]	10.46±0.20[b]
	Rabi	28.75±1.48[b]	97.61±1.87[b]	124.10±1.47[a]	7.72±0.13[a]	11.84±0.20[a]
Upland	*Kharif*	111.79±1.91[a]	148.39±3.14[a]	131.49±4.89[a]	4.16±0.19[b]	8.07±0.12[b]
	Rabi	20.11±0.47[b]	124.16±0.55[b]	114.53±0.23[b]	7.98±0.16[a]	8.54±0.07[a]

[MBC - microbial biomass carbon, MBN- microbial biomass nitrogen, MBP- microbial biomass phosphorus, SIR - substrate induced respiration, DHA – dehydrogenase activity, PHA – phosphatase activity]. Within a site for each parameter, values that differ significantly (P<0.01) are followed by different letters, as determined by paired t-test

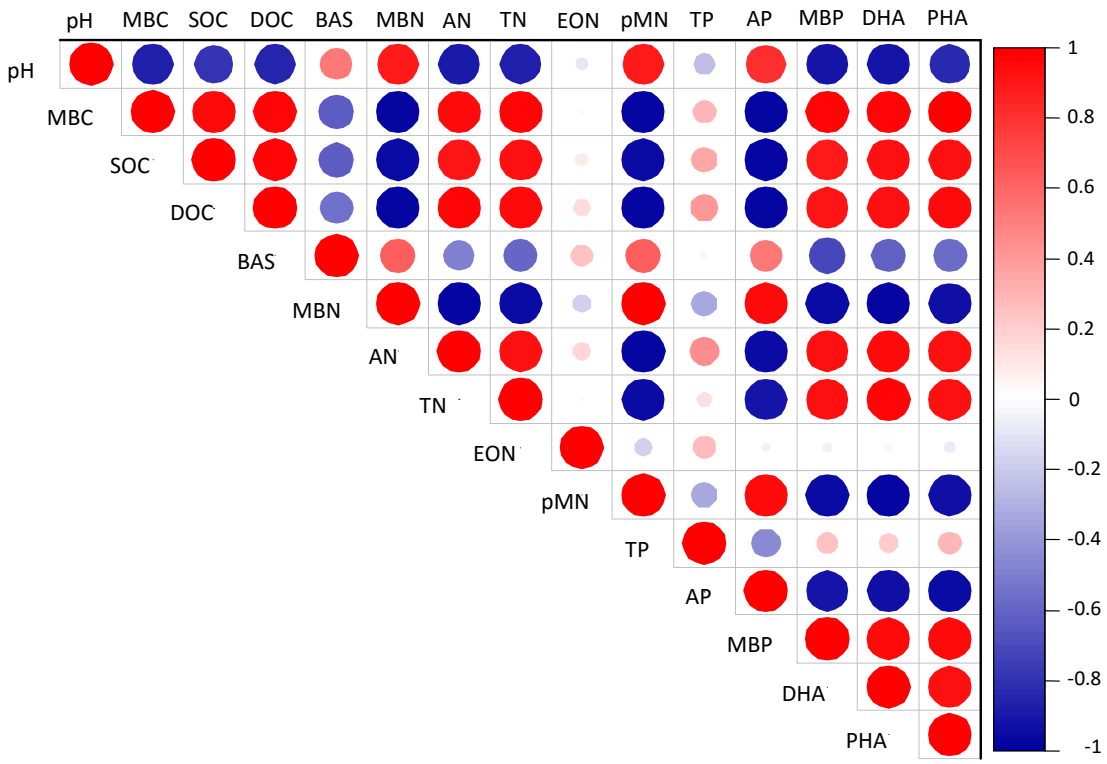

Figure 1. Pair-wise relationship between fractions of carbon, nitrogen and phosphorus in rice ecosystems during *kharif* season [SOC - soil organic carbon, DOC - dissolved organic carbon, MBC - microbial biomass carbon, BAS - basal respiration, TN – total nitrogen, AN – available nitrogen, MBN – microbial biomass nitrogen, EON – extractable organic nitrogen, pMN – potentially mineralizable nitrogen, TP – total phosphorous, AP – available phosphorous, MBP – microbial biomass phosphorous, DHA – dehydrogenase activity, PHA – phosphatase activity]

* significant at $r_{0.05}$= 0.63 and **significant at $r_{0.01}$= 0.80

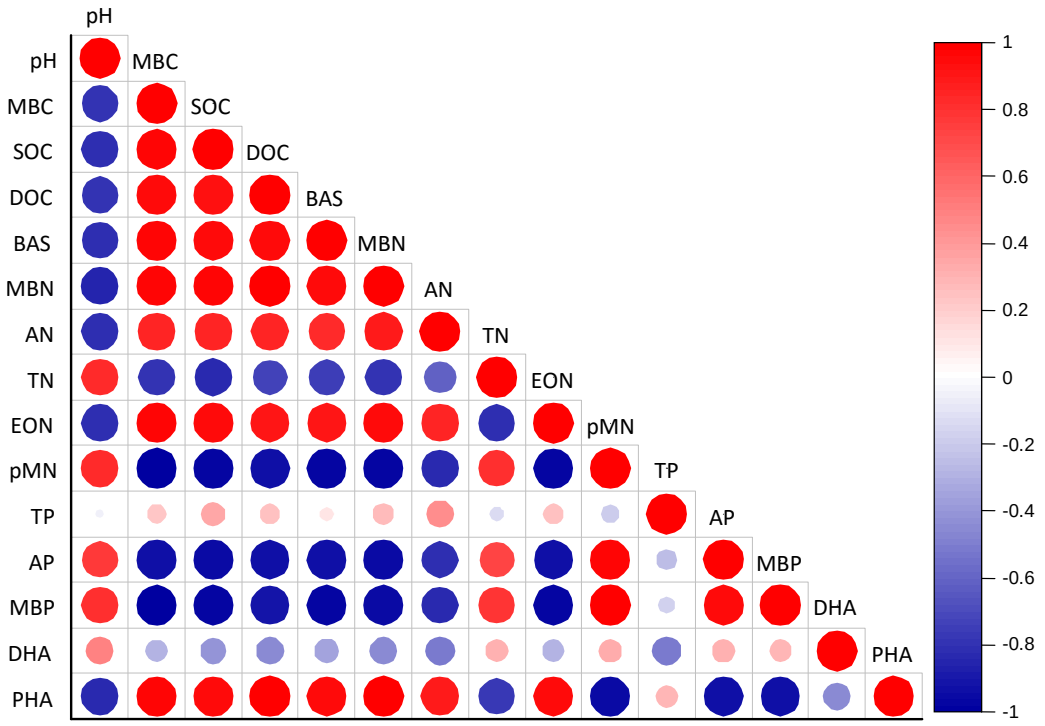

Figure 2. Pair-wise relationship between fractions of carbon, nitrogen and phosphorus in rice ecosystems during *rabi* season [SOC - soil organic carbon, DOC - dissolved organic carbon, MBC - microbial biomass carbon, BAS - basal respiration, TN – total nitrogen, AN – available nitrogen, MBN – microbial biomass nitrogen, EON – extractable organic nitrogen, pMN – potentially mineralizable nitrogen, TP – total phosphorous, AP – available phosphorous, MBP – microbial biomasss phosphorous, DHA – dehydrogenase activity, PHA – phosphatase activity]

* significant at $r_{0.05}$= 0.63 and **significant at $r_{0.01}$= 0.80