



Interpretations of Elemental and Microbial Phosphorus Indicators to Understand P Availability in Soils Under Rice–Wheat Cropping System

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Received: 4 June 2019 / Accepted: 1 November 2019 / Published online: 27 November 2019
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Abstract In order to address the scarcity of phosphorus nutrient reserves and the variations usually observed in the P nutrient storage in soils, a long-term experiment was targeted to assess the impact of integrated fertilizer schedule on soil phosphorus pools and ecophysiological ratios of phosphorus element. P-related soil enzymes like acid phosphatase activity and alkaline phosphatase activity were improved by 6.53–15.93% and 6.14–11.41%, respectively, over the recommended dose of fertilization. Addition of FYM recorded highest DHA (45.24 $\mu\text{g TPF released g}^{-1}$ dry soil h^{-1}) followed by wheat straw (41.25 $\mu\text{g TPF released g}^{-1}$ dry soil h^{-1}) and green manure (38.98 $\mu\text{g TPF released g}^{-1}$ dry soil h^{-1}). Integrated fertilizer schedule improved the microbial biomass phosphorus content by 8.97–29.72% as compared to 100% recommended dose through mineral fertilizer. In the integrated system, only 5–7% of the organic phosphorus was ascribed to microbial biomass, reflecting the accumulation of organic P forms. Higher ratio of DHA (0.20) and pyrophosphatase (0.23) to microbial C in integrated treatments confirmed that the enzyme activities were from extracellular enzymes released by microorganisms. The lower ratios of alkaline or acid phosphatase to pyrophosphatase indicate domination of phospho-monoesters in the P pools. These ratios are important to understand the P availability in soil systems especially under the integrated fertilization schedule.

Keywords Phosphorus · Long-term experiment · Ecophysiological ratios · INM · Fertilizer · Microbial biomass carbon

Introduction

Phosphorus (P) is one of the indispensable elements of any biotic systems, and its supply to living organisms depends upon the dynamics of P. Deficiency of phosphorus is a prevalent nutrient usually limited to crop production especially under soils of tropical regions [7]. But it is a concern for resource-poor farmers in countries like India where correcting this P deficiency with surplus P application is not a practicable choice. However, reduced supply of P confines plant growth in managed ecologies and hence

improved P management practices could essentially decrease the stress on the natural sources of phosphorus. Since the sources of P fertilizers are limited [3], there is a need to detect the sustainable P management practices.

Phosphorus occurs in a number of forms with various range of solubility *vis-a-vis* availability. It is present either in inorganic or organic forms in which organic forms are the prime forms [27]. Phytate-P is considered as the major organic P in soils, whereas phosphate esters exist as minor forms. There is a substantial amount of phytate-P due to its nature of fewer solubility and strong association with the solid phase [34]. This creates a major barrier for plants to take up P from the soils. The availability and assimilation of phosphorus by a crop is the result of a number of synchronized processes like mineralization and immobilization of P and the rate at which P diffuses in soils.

Organic P cycling is the key to retain the availability of soil P over the long run. Several enzymes participate in the

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mineralization of organic phosphorus. Extracellular phosphatases releases orthophosphate by acting on the organic P compounds [22] and hence play a central role in P cycling. Microbial biomass P reacts rapidly to any changes in soil environment and hence affects the availability of P in soils. The role of microbes influencing the P nutrition to plants is dealt with a great interest over the decades. This interest is strengthened specifically in tropical soils where P deficiency is common. Although soils contain a large amount of total P, it is hardly available due to its sparingly soluble nature besides the use efficiency of P fertilizers ranges from 15 to 20%.

Microbial biomass significantly recycles a large portion of soil P, which estimates around 100 mg P per kg soil per year [26]. The microbial biomass is highly influenced by the quality and quantity of organic inputs, crop management practices and soil pH. Long-term experiments provide a means of assessing the P cycle to ascertain the function of soils and to work out the drivers that affect these soil functions under integrated fertilizer management. Study on these experiments provides a supplementary data on the factors that increase the soil P availability, thereby enhancing the efficient acquisition of P by plants under the integrated fertilizer management [14, 29] especially under tropical and subtropical agro-ecosystems. Considering an experiment started on 1984–1985 with a number of integrated treatments, we hypothesized that long-term fertilization regimes and addition of organic residues could have considerable implications on the elemental and ecophysiological ratios of phosphorus. To test the hypothesis, soil samples were collected from the said experiment to evaluate the forms of P (total, inorganic and organic) and related enzymes to appraise the impact of integrated fertilizer application on soil P pools and enzyme activities with an idea to interpret the ecophysiological ratios of phosphorus element under long-term fertilizer management.

Materials and Methods

Site Description and Experimental Details

The experiment was initiated in 1984 at the Bihar Agricultural College Research Farm (25° 23' N, 87° 07' E, 37.19 m.s.l.), Bhagalpur, Bihar, India, under the network project research programme of the ICAR—Indian Institute of Farming System Research (formerly Project Directorate on Farming System Research) Modipuram with Rice–Wheat cropping system. The soil is sandy clay loam soil (sand—50%, silt—22% and clay—28%) having pH 7.40 and organic carbon 4.6 g kg⁻¹, and extractable soil nutrient status in no fertilizer soil sample were: 194 kg N ha⁻¹

10.12 kg P ha⁻¹ and 128.65 kg K ha⁻¹. The experiment was constituted with four replications and twelve treatments in randomized block design. The treatments were different regimes of nutrient management practices imposed as control (T₁) (no fertilizer no organic manure); 50% RDF of NPK through fertilizer to both rice and wheat (T₂); 50% RDF + 50% N through farm yard manure (FYM) to rice and 100% RDF to wheat (T₃); 75% RDF of NPK through fertilizer to both rice and wheat (T₄); 100% RDF of NPK through fertilizer to both rice and wheat (T₅); 50% RDF of NPK through fertilizer + 50% N through FYM in rice and 100% RDF of NPK through fertilizer in wheat (T₆); 75% RDF of NPK through fertilizer + 25% N through FYM in rice and 75% RDF of NPK through fertilizer in wheat (T₇); 50% RDF of NPK through fertilizer + 50% N through wheat straw in rice and 100% RDF of NPK through fertilizer in wheat (T₈); 75% RDF of NPK through fertilizer + 25% N through wheat straw in rice and 75% RDF of NPK through fertilizer in wheat (T₉); 50% RDF of NPK through fertilizer + 50% N through green manure in rice and 100% RD of NPK through fertilizer in wheat (T₁₀); 75% RDF of NPK through fertilizer + 25% N through green manure in rice and 75% RDF of NPK through fertilizer in wheat (T₁₁); Farmer's Practice (N₇₀P₃₀K₁₀) in rice and (N₈₀P₃₀K₁₅) in wheat (T₁₂). The recommended dose of fertilizers (N:P₂O₅:K₂O) for rice (cv. Sita) and wheat (cv. HD-2967) is 80:40:20 (N:P₂O₅:K₂O) kg ha⁻¹ and 120:60:40 kg ha⁻¹, respectively. The average phosphorus content of FYM, wheat straw and green manure used in this experiment was 0.30%, 0.22% and 0.12% P, respectively.

Soil Sampling

Composite surface (0–15 cm) soil samples were collected from each plot at the harvesting stage of rice crop. The 48 composite samples (12 treatments × 4 replicates) were kept in plastic bags and transported to the laboratory, where fresh soil samples were immediately sieved through a 2-mm sieve, homogenized, and preserved at 4 °C in a refrigerator for determination of soil enzymes. The moisture content was determined by gravimetric method. Portions of the sampled soils were air-dried and passed through a sieve (2-mm) for initial characterization.

Analysis of Soil Samples

Fresh soil samples were analysed for soil enzymes like acid phosphatase, alkaline phosphatase, dehydrogenase, pyrophosphatase, microbial biomass carbon and phosphorus. Air-dried samples were analysed for chemical parameters like total soil P, inorganic soil P and organic soil P.

Enzyme Activities

Dehydrogenase activity (DHA) in soil was determined by the spectrophotometric method as described by Klein et al. [15] using 3% triphenyl tetrazolium chloride (TTC) as substrate. Phosphomonoesterases activity (acid and alkaline) in soils was estimated following the most rapid, accurate and precise method of Tabatabai and Bremner [32]. *p*-Nitrophenyl phosphate was used as a substrate in modified universal buffer (MUB; pH 6.5 for acid phosphatase and 11 for alkaline phosphatase) solution. Inorganic pyrophosphatase activity was estimated by the method as described by Dick and Tabatabai [13] using tetra sodium pyrophosphate decahydrate substrate in MUB buffer at pH 8.0.

Microbial Biomass Carbon (MBC)

Two sets of 10 g fresh soil were taken in beakers; the first one was fumigated with chloroform for 24 h, while the second one was kept unfumigated. Both the fumigated and unfumigated soil samples were extracted using 0.5 M K₂SO₄. A blank was run simultaneously. Then, 10 ml K₂Cr₂O₇, 10 ml H₂SO₄ and 5 ml H₃PO₄ were added to 10 ml of the extracts. They were kept at 160 °C for 30 min in a microwave. The carbon which is available for oxidation is oxidized by potassium dichromate. The unconsumed K₂Cr₂O₇ was determined by titration with standard 0.1 N Ferrous ammonium sulphate (FAS) using Diphenyl amine indicator. The MBC was calculated using the formula given below and reported on oven dry soil basis [38].

$$\text{MBC (mg C kg}^{-1}\text{)} = \frac{C_F - C_{UF}}{K_{EC}}$$

where C_F is the carbon in fumigated soil, C_{UF} the carbon in unfumigated soil, K_{EC} the efficiency of extraction (0.45).

Microbial Biomass Phosphorus (MBP)

Two sets of 10 g fresh soil were taken in beakers; the first one was fumigated with chloroform for 24 h, while the second one was kept unfumigated. Both the fumigated and unfumigated soil samples were extracted using NaHCO₃. Phosphorus content of the aliquot was estimated by blue colour method using ascorbic acid as reducing agent, and the intensity of blue colour was measured at 720 nm wavelength by a spectrophotometer.

The MBP was calculated using the formula given below and reported on oven dry soil basis [5].

$$\text{MBP (mg P kg}^{-1}\text{)} = \frac{P_F - P_{UF}}{K_{EP}}$$

where P_F is the phosphorus in fumigated soil, P_{UF} the phosphorus in unfumigated soil, K_{EP} the efficiency of extraction (0.40).

Inorganic Phosphorus

The method followed for the estimation of inorganic phosphorus was Olsen's method [25]. Five gram soil was mixed with a teaspoonful of activated charcoal followed by the addition of 50 ml of Olsen's reagent (0.5 N NaHCO₃, pH 8.5) in plastic beakers and shaken for 30 min. It was filtered with Whatman 42 filter paper and stored. Then, 5 ml of the extract was taken into 25 ml volumetric flasks, and drop-wise *p*-nitrophenol indicator was added. If the yellow colour persisted, 7 N H₂SO₄ was added to make it colourless. It was followed by the addition of 4 ml of mixed reagent, and volume was made up with distilled water. The colour development was followed in the same manner for the standards. The blue colour intensity of the samples and the standards was read at 720 nm by a spectrophotometer.

Organic Phosphorus

Organic phosphorus in the samples was calculated by deducting the values of inorganic phosphorus from total phosphorus. To calculate the inorganic phosphorus, the soil was treated with HCl–NaOH mixture and finally digested with H₂SO₄ and HClO₄, and the absorbance reading was taken at 840–880 nm [24].

Total Phosphorus

The method followed for the estimation of total phosphorus was wet digestion. One gram air dry soil was digested in a tri acid mixture comprising of conc. HNO₃, HClO₄ and conc. H₂SO₄ in the ratio of 10:4:1 on a hot plate at 150 °C until the sample became colourless. Then, the volume was made to 50 ml with distilled water. Five millilitre of the extract was transferred to 25-ml volumetric flasks followed by the addition of 4 ml of mixed reagent. The blue colour intensity of the samples and standards was measured by a spectrophotometer at a wavelength of 720 nm.

After analysing the above parameters, the elemental and ecophysiological ratios were worked out to understand the relative contribution, shift between P pools and metabolic activity of microbial community [14]. Ratios such as organic P/total P, inorganic P/total P, microbial P/total P and microbial P/organic P denote the relative contribution. The ratio between inorganic P and organic P shows the relative shift, organic C/organic P relates to the changes in organic matter composition. The relevance of enzyme

activities in relation to microbial C is to present the metabolic efficiency of microbial community. The ratio between various enzymes characterizes the substrate composition and their utilization in the soil systems.

Results

Soil Enzymes and Microbial Biomass Carbon

The pattern of acid phosphatase activity in soil was significantly influenced by the integrated fertilizer management practices. The acid phosphatase activity was lowest in the control (T₁-100.37 µg *p*-nitrophenol released g⁻¹ dry soil h⁻¹) where no fertilizer/manure was used since last 33 years. Substitution of half the recommended N dose through wheat straw produced the highest acid phosphatase activity in soil (T₈-139.22 µg *p*-nitrophenol released g⁻¹ dry soil h⁻¹). Suboptimal level of nutrient application (T₁-T₃) slows down the acid phosphatase activity by 3.84–16.6% than 100% recommended dose of fertilizer (RDF) application, while organic intrusion (T₆-T₁₁)

improved the acid phosphatase activity by 6.53–15.93% (Table 1).

Substitution of nitrogen requirement through organics was significantly superior, improving the alkaline phosphatase activity over 100% recommended NPK through mineral fertilizer (Table 1). Improvement in the alkaline phosphatase activity was in the range of 6.14–11.41% (T₆-T₁₁) over T₅ (157.88 µg *p*-nitrophenol released g⁻¹ dry soil h⁻¹). The highest activity was in T₉ (178.22 µg *p*-nitrophenol released g⁻¹ dry soil h⁻¹) where 25% of nitrogen requirement was supplemented with wheat straw (Table 1). Inadequate nutrition (T₂-T₄ and T₁₂) lowers down the enzyme activity by 3.56–11.6% as compared to T₅.

Dehydrogenase activity essentially reflects total respiratory activity of the viable soil micro-flora in soil which is considered to be a good indicator of microbial activity. Conjoint application of organic amendments and mineral fertilizer significantly ($p < 0.05$) influenced the dehydrogenase activity of soil (Table 1). Substitution of half of the nitrogen through FYM (T₆) recorded highest DHA (45.24 µg TPF released g⁻¹ dry soil h⁻¹) followed by T₈ (41.25 µg TPF released g⁻¹ dry soil h⁻¹) and T₁₀

Table 1 Enzyme activities and microbial biomass as influenced by long-term nutrient management practices under rice–wheat cropping system

Treatment	Acid phosphatase (µg <i>p</i> -nitrophenol released g ⁻¹ dry soil h ⁻¹)	Alkaline phosphatase (µg <i>p</i> -nitrophenol released g ⁻¹ dry soil h ⁻¹)	Dehydrogenase (µg TPF released g ⁻¹ dry soil h ⁻¹)	Pyrophosphatase (µg PO ₄ ⁻ -P released g ⁻¹ dry soil h ⁻¹)	MBC (µg g ⁻¹ dry soil)
T ₁ -control	100.37	141.37	12.15	20.28	190.22
T ₂ -50% RDF-both R and W	108.55	148.88	18.22	24.88	201.22
T ₃ -50% RDF-R and 100% RDF-W	112.71	149.55	20.22	37.55	205.22
T ₄ -75% RDF-both R and W	118.21	152.44	26.25	41.25	210.22
T ₅ -100% RDF-both R and W	117.04	157.88	34.58	45.78	218.55
T ₆ -50% RDF + 50% N-FYM-R and 100% RDF-W	128.33	168.22	45.24	53.55	230.22
T ₇ -75% RDF + 25% N-FYM-R and 75% RDF-W	125.22	170.22	39.55	50.11	225.22
T ₈ -50% RDF + 50% N-WS-R and 100% RDF-W	139.22	175.48	41.25	49.11	242.25
T ₉ -75% RDF + 25% N-WS-R and 75% RDF-W	138.22	178.22	40.22	51.32	245.55
T ₁₀ -50% RDF + 50% N-GM-R and 100% RDF-W	135.88	173.22	38.98	52.11	232.55
T ₁₁ -75% RDF + 25% N-GM-R and 75% RDF-W	132.11	174.82	37.64	50.41	235.55
T ₁₂ -FP in R and W	110.55	146.22	26.22	38.25	202.55
LSD ($p < 0.05$)	7.21	8.01	4.53	4.03	10.77
SEm (±)	2.50	2.78	1.57	1.40	3.74

RDF recommended dose of fertilizers, R rice, W wheat, FYM farm yard manure, WS wheat straw, GM green manure, FP farmers' practice

(38.98 μg TPF released g^{-1} dry soil h^{-1}). Integrated nutrient management through FYM, wheat straw and green manure significantly improved the DHA by 11.3–23.6% over recommended dose of mineral fertilization (34.58 μg TPF released g^{-1} dry soil h^{-1}).

Data on pyrophosphatase activity of soil revealed the significance of various treatments. Result showed that the lowest value of pyrophosphatase activity in the control (T_1) (20.28 μg $\text{PO}_4^{-}\text{-P}$ released g^{-1} dry soil h^{-1}) and the highest in T_6 (53.55 μg $\text{PO}_4^{-}\text{-P}$ released g^{-1} dry soil h^{-1}). Application of wheat straw (T_8 and T_9) and green manure (T_{10} and T_{11}) also resulted in 6.78–14.51% higher pyrophosphatase activity over T_5 (45.78 μg $\text{PO}_4^{-}\text{-P}$ released g^{-1} dry soil h^{-1}) (Table 1).

Perusal of data revealed that integrated nutrient management practices significantly ($p < 0.05$) influenced the microbial biomass carbon in soil. Results represented in Table 1 showed that lowest value of microbial biomass carbon in the treatment where no nutrient was applied, i.e. control (T_1) (190.22 μg C g^{-1} dry soil). As the availability of nutrient increases, the microbial biomass carbon content in soil also improved. Thus, the microbial biomass carbon content increases from 201.22 to 218.55 μg C g^{-1} dry soil where treatment received 50–100% of recommended dose of fertilizer. But integrated nutrient management practices showed higher microbial biomass carbon even with reduced doses of mineral fertilizer. The highest microbial biomass carbon was found in the treatment T_9 (245.55 μg C g^{-1} dry soil). Application of FYM and green manure improved the microbial biomass carbon content in soil by 2.96–7.22% over the 100% NPK through mineral fertilizer (218.55 μg C g^{-1} dry soil).

Bio-available P

Microbial biomass phosphorus was significantly influenced by the different nutrient management practices (Table 2). Combined application of organics and mineral fertilizer showed higher microbial biomass phosphorus. The highest microbial biomass phosphorus was found in the treatment T_8 (77.52 μg P g^{-1} dry soil) where 50% of the nitrogen was substituted by wheat straw which was significantly superior over treatment receiving FYM (T_6) (69.68 μg P g^{-1} dry soil) and green manure (T_{10}) (61.82 μg P g^{-1} dry soil). Decline in the level of organic matter application (i.e. 25% N substitution) significantly reduced the microbial biomass phosphorus content so that the lower values were observed in T_7 (61.89 μg P g^{-1} dry soil), T_9 (69.62 μg P g^{-1} dry soil) and T_{11} (59.85 μg P g^{-1} dry soil). Integrated nutrient management practices improved the microbial biomass phosphorus content by 8.97–29.72% over 100% recommended dose through mineral fertilizer (T_5) (54.48 μg P g^{-1} dry soil).

Addition of mineral fertilizer significantly improved the Olsen phosphorus content in soil in the order of T_2 (3.87 μg P g^{-1} soil) < T_3 (6.98 μg P g^{-1} soil) < T_4 (7.53 μg P g^{-1} soil) < T_5 (8.54 μg P g^{-1} soil) (Table 2). The highest Olsen phosphorus content was obtained from the treatment T_6 (10.48 μg P g^{-1} dry soil). Integrated nutrient management practices improved the Olsen phosphorus content by 2.73–18.51% over T_5 . Farmer's practice of nutrient management resulted in ~ 40% lower value of total phosphorus content than 100% recommended dose of fertilizer treatment.

The organic phosphorus content was significantly ($p < 0.05$) influenced by the nutrient management practices. The organic phosphorus content was lowest in control (458.24 μg P g^{-1} soil) among the all treatments (Table 2). Addition of mineral fertilizer significantly improved the organic phosphorus content in soil as of order: T_2 (602.87 μg P g^{-1} soil) < T_3 (700.50 μg P g^{-1} soil) < T_4 (741.93 μg P g^{-1} soil) < T_5 (778.28 μg P g^{-1} soil); integrated nutrient management upholds the organic phosphorus content by 31.4–49.2% over T_5 . The highest organic phosphorus content was obtained from the treatment receiving 50% recommended dose of NPK through mineral fertilizer and 50% N through FYM in rice (T_6) (1161.25 μg P g^{-1} dry soil) which was significantly ($p < 0.05$) superior over the treatment receiving wheat straw (1080.28 μg P g^{-1} dry soil) and green manure (1061.11 μg P g^{-1} dry soil). Integrated nutrient management practices improved the total phosphorus content by 11.97–19.68% over recommended dose of fertilizers. The highest total phosphorus content was obtained from the treatment receiving 50% recommended dose of NPK through mineral fertilizer and 50% N through FYM in rice (T_6) (2513.55 μg P g^{-1} dry soil) which was followed by the treatment receiving 75% recommended dose of NPK through mineral fertilizer and 25% N through wheat straw in rice (T_9) (2210.48 μg P g^{-1} dry soil) and the treatment receiving 75% recommended dose of NPK through mineral fertilizer and 25% N through green manure in rice (T_{11}) (2301.78 μg P g^{-1} dry soil).

Grain yield and System Productivity

Data indicated that substitution of inorganic fertilizers through farm yard manure (FYM), wheat straw and green manuring proved significantly effective in raising the yield of rice as well as wheat through their residual effect (Table 3). Results revealed that substitution of 50% N through FYM along with 50% NPK applied through inorganic fertilizers (T_6) produced the highest rice grain yield (5556 kg ha^{-1}) and straw yield (7375 kg ha^{-1}). Rice yield with the application of wheat straw (T_8) and green manures (T_{10}) in combination with mineral fertilizers were at par

Table 2 Bio-available fractions of phosphorus as influenced by long term nutrient management practices under rice–wheat cropping system

Treatment	MBP ($\mu\text{g g}^{-1}$ dry soil)	Olsen P ($\mu\text{g P g}^{-1}$ soil)	Organic P ($\mu\text{g P g}^{-1}$ soil)	Total P ($\mu\text{g P g}^{-1}$ soil)
T ₁ -control	30.54	2.58	458.24	1526.83
T ₂ -50% RDF-both R and W	37.90	3.87	602.87	1722.52
T ₃ -50% RDF-R and 100% RDF-W	44.25	6.98	700.50	1843.55
T ₄ -75% RDF-both R and W	51.36	7.53	741.93	1902.39
T ₅ -100% RDF-both R and W	54.48	8.54	778.28	1945.67
T ₆ -50% RDF + 50% N-FYM-R and 100% RDF-W	69.68	10.48	1161.25	2513.55
T ₇ -75% RDF + 25% N-FYM-R and 75% RDF-W	61.89	10.11	1138.50	2422.52
T ₈ -50% RDF + 50% N-WS-R and 100% RDF-W	77.52	9.99	1080.28	2400.55
T ₉ -75% RDF + 25% N-WS-R and 75% RDF-W	69.62	8.78	1007.44	2210.48
T ₁₀ -50% RDF + 50% N-GM-R and 100% RDF-W	61.82	9.55	1061.11	2289.71
T ₁₁ -75% RDF + 25% N-GM-R and 75% RDF-W	59.85	9.48	1022.75	2301.78
T ₁₂ -FP in R and W	38.53	5.12	630.58	1751.26
LSD ($p < 0.05$)	5.87	1.04	58.53	104.29
SEm (\pm)	2.04	0.36	20.32	36.21

RDF recommended dose of fertilizers, R rice, W wheat, FYM farm yard manure, WS wheat straw, GM green manure, FP farmers' practice

Table 3 Grain and straw yield of rice and wheat under permanent plot experiment after 33rd cycle of rice–wheat system

Treatment	Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})		REY (kg ha^{-1})
	Rice	Wheat	Rice	Wheat	
T ₁ -control	942	771	1328	1141	1982
T ₂ -50% RDF-both R and W	2764	2024	3781	2895	5492
T ₃ -50% RDF-R and 100% RDF-W	2865	3302	3937	4392	7316
T ₄ -75% RDF-both R and W	3787	2804	5079	3869	7566
T ₅ -100% RDF-both R and W	4762	3712	6372	4900	9766
T ₆ -50% RDF + 50% N-FYM-R and 100% RDF-W	5556	4277	7375	5645	11,321
T ₇ -75% RDF + 25% N-FYM-R and 75% RDF-W	5280	3907	7006	5197	10,546
T ₈ -50% RDF + 50% N-WS-R and 100% RDF-W	5329	4007	7056	5290	10,731
T ₉ -75% RDF + 25% N-WS-R and 75% RDF-W	4999	3845	6692	5114	10,181
T ₁₀ -50% RDF + 50% N-GM-R and 100% RDF-W	5452	4210	7295	5599	11,127
T ₁₁ -75% RDF + 25% N-GM-R and 75% RDF-W	5167	3892	6971	5138	10,414
T ₁₂ -FP in R and W	3371	2736	4640	3749	7059
LSD ($p < 0.05$)	735	642	989	852	1177
SEm (\pm)	255.2	222.9	343.4	295.8	408.7

RDF recommended dose of fertilizers, R rice, W wheat, FYM farm yard manure, WS wheat straw, GM green manure, FP farmers' practice

with the yields obtained with integrated application of FYM and inorganic fertilizers (T₆). Substitution of 25% N through FYM, wheat straw and green manuring and adding 75% NPK as inorganic fertilizers also helped to raise yield level of rice but the differences were not significant.

In wheat, treatments receiving 50% N through FYM and green manuring + 50% NPK through inorganic fertilizers in rice and 100% recommended dose in wheat as inorganic fertilizers (T₆ & T₁₀) significantly out-yielded the treatment

getting the recommended dose of inorganic fertilizers both in rice and wheat (T₅). The highest yield of 4277 kg ha^{-1} was obtained with T₆ (FYM) as against only 3712 kg ha^{-1} with the application of inorganic fertilizers alone (T₅).

Rice equivalent yield (REY) of the system was utmost in the treatment receiving 50% recommended N through FYM + 50% NPK through inorganic fertilizers (T₆) (11,321 kg ha^{-1}) and was significantly superior over rest of the treatments. Rice equivalent yield under various

organic–inorganic combinations increased by 4.25–15.92% over the application of recommended dose of fertilizer alone (T₅).

Elemental and Ecophysiological Ratios of P

Elemental phosphorus ratios imply the apportion of bio-available phosphorus to total phosphorus in soil. Organic phosphorus accounts major proportions of total phosphorus (~ 30–46%) followed by microbial assimilates (MBP) (~ 2.0–3.2%) and least in Olsen P (~ 0.17–0.44%). Essentially two different substrate-induced apportion in bio-available phosphorus was found in different treatments (Table 4). Combined application of organic and inorganic sources of phosphorus significantly improved the organic phosphorus content in soil (T₆–T₁₁), whereas T₅ possess highest inorganic phosphorus content in soil. Microbial P to total P and microbial P to organic P was highest in T₈.

Ecophysiological ratios provide more insight about the relative abundance of bio-available phosphorus fractions. Wider microbial C to microbial P ratio indicates phosphorus insufficiency in soil. Integrated nutrient management practices significantly improved the phosphorus availability in soil. Suboptimal level of fertilization reduces the phosphorus accumulation in microbes. Ratio of phospho-monoesterase (acid and alkaline) activity to microbial

biomass carbon remains unaffected irrespective of the treatments, whereas dehydrogenase and pyrophosphatase activity to MBC was significantly influenced by the nutrient management practices (Table 5). Ratio of alkaline and acid phosphatase activity to pyrophosphatase activity provides an indication of the dissolution of native inorganic phosphorus. Long-term application of FYM (T₆) showed lowest ratio (alkaline phosphatase: PP-3.14 and acid phosphatase: PP-2.40) in contrast to the control (T₁) which possess the highest ratio (alkaline phosphatase: PP-6.97 and acid phosphatase: PP-4.95) (Table 5).

Discussion

The results obtained from the current investigation have revealed several aspects pertaining to the nutrient P accumulation, enzyme activities and microbial biomass under permanent plot experiment and their levels after 33rd cycle of rice–wheat system. The increased response under integrated treatments was due to the positive impact of organic inputs used over the years. Organic input supply nutrient and are able to maintain a balanced supply of nutrients to plants [36]. Besides there is solubilization of insoluble nutrients due to fulvic and humic acids released by the organic materials supplied in the integrated schedule. The

Table 4 Elemental P ratio of the treatments under permanent plot experiment after 33rd cycle of rice–wheat system

Treatment	Organic P/total P (%)	Avail P/total P (%)	Microbial P/total P (%)	Microbial P/organic P (%)	Avail P/organic P (%)
T ₁ -control	30.01	0.17	2.00	6.66	0.56
T ₂ -50% RDF-both R and W	35.00	0.22	2.20	6.29	0.64
T ₃ -50% RDF-R and 100% RDF-W	38.00	0.38	2.40	6.32	1.00
T ₄ -75% RDF-both R and W	39.00	0.40	2.70	6.92	1.01
T ₅ -100% RDF-both R and W	40.00	0.44	2.80	7.00	1.10
T ₆ -50% RDF + 50% N-FYM-R and 100% RDF-W	46.20	0.42	2.77	6.00	0.90
T ₇ -75% RDF + 25% N-FYM-R and 75% RDF-W	47.00	0.42	2.55	5.44	0.89
T ₈ -50% RDF + 50% N-WS-R and 100% RDF-W	45.00	0.42	3.23	7.18	0.92
T ₉ -75% RDF + 25% N-WS-R and 75% RDF-W	45.58	0.40	3.15	6.91	0.87
T ₁₀ -50% RDF + 50% N-GM-R and 100% RDF-W	46.34	0.42	2.70	5.83	0.90
T ₁₁ -75% RDF + 25% N-GM-R and 75% RDF-W	44.43	0.41	2.60	5.85	0.93
T ₁₂ -FP in R and W	36.01	0.29	2.20	6.11	0.81
LSD ($p < 0.05$)	2.22	0.04	0.19	0.18	0.06
SEm (\pm)	0.77	0.01	0.07	0.06	0.02

RDF recommended dose of fertilizers, R rice, W wheat, FYM farm yard manure, WS wheat straw, GM green manure, FP farmers' practice

Table 5 Ecophysiological P ratio of the treatments under permanent plot experiment after 33rd cycle of rice–wheat system

Treatments	Microbial C/microbial P	Alkaline phosphatase/microbial C	Acid phosphatase/microbial C	DHA/microbial C	PP/microbial C	Alkaline phosphatase/PP	Acid phosphatase/PP
T ₁ -control	6.23	0.74	0.53	0.06	0.11	6.97	4.95
T ₂ -50% RDF-both R and W	5.31	0.74	0.54	0.09	0.12	5.98	4.36
T ₃ -50% RDF-R and 100% RDF-W	4.64	0.73	0.55	0.10	0.18	3.98	3.00
T ₄ -75% RDF-both R and W	4.09	0.73	0.56	0.12	0.20	3.70	2.87
T ₅ -100% RDF-both R and W	4.01	0.72	0.54	0.16	0.21	3.45	2.56
T ₆ -50% RDF + 50% N-FYM-R and 100% RDF-W	3.30	0.73	0.56	0.20	0.23	3.14	2.40
T ₇ -75% RDF + 25% N-FYM-R and 75% RDF-W	3.64	0.76	0.56	0.18	0.22	3.40	2.50
T ₈ -50% RDF + 50% N-WS-R and 100% RDF-W	3.13	0.72	0.57	0.17	0.20	3.57	2.83
T ₉ -75% RDF + 25% N-WS-R and 75% RDF-W	3.53	0.73	0.56	0.16	0.21	3.47	2.69
T ₁₀ -50% RDF + 50% N-GM-R and 100% RDF-W	3.76	0.74	0.58	0.17	0.22	3.32	2.61
T ₁₁ -75% RDF + 25% N-GM-R and 75% RDF-W	3.94	0.74	0.56	0.16	0.21	3.47	2.62
T ₁₂ -FP in R and W	5.26	0.72	0.55	0.13	0.19	3.82	2.89
LSD ($p < 0.05$)	0.88	0.02	0.02	0.01	0.01	0.115	0.077
SEm (\pm)	0.306	0.007	0.007	0.003	0.003	0.040	0.027

RDF recommended dose of fertilizers, R rice, W wheat, FYM farm yard manure, WS wheat straw, GM green manure, FP farmers' practice

biological activities in soils were improved because of the readily available source of nutrients in integrated treatments which resulted in a better crop growth and yields. Increase in organic matter is attributed to decaying roots, litter and residues added over the years [2].

The phosphatase, a hydrolysing enzyme, acts on organic P to release PO_4^{3-} . The alkaline phosphatase activities were much higher than acid phosphatase activity because of the higher pH (alkaline reaction) of the tested soils. Phosphatase activity was strongly influenced by soil pH [28]. The higher activities of alkaline phosphatase in the integrated treatments were due to the diversity of bacteria capable of solubilizing phosphorus [33]. Total phosphorus was never established as a good predictor of phosphatase because it includes the primary mineral as well as the occluded recalcitrant forms, which cannot be acted upon by the phosphatase [21]. Phosphatase acts upon a very small portion of organic P to release the available P [16]. However, available P is not well correlated with phosphatase activity, signifying that available P not relates to the potential capacity of the systems but the actual available P. This available P is a result of the competing ability of plants and microbes to take up P from the soils, which is partly controlled by the activity of phosphatase.

Since dehydrogenase activity is an indicator of the viable cells, it might reveal the oxidative activity of soil microbes and considered as a good indicator of microbial activity. The data revealed that integrated application of fertilizer increased the dehydrogenase activity in the soil because of the increased ability of the microbes to efficiently utilize the native pool of organic carbon [4]. Further, there is an increase in the availability of substrates for dehydrogenase in the plots incorporated with organic inputs [19]. Pyrophosphatase activity in soils is dominant in the top layers if C sources are applied over the years, and analysis shows that activity of pyrophosphatase is highly correlated with organic C (data not presented). Application of organic materials showed a significant escalation in microbial biomass carbon which can be ascribed to the increased availability of mineralizable as well as readily hydrolysable carbon [17].

High P stocks found under integrated treatments were due to the organic inputs applied for almost 33 years. These changes enhanced the activity of microbes resulting in higher microbial biomass P in soils with integrated fertilizers. Higher activity of microbes does immobilize P and sustains long-term P availability after microbial biomass turnover [10].

The results of the present study also revealed a higher level of available P under integrated nutrient management treatments. This significant increase had occurred due to additional P added through organic inputs [35]. It was observed that lowering P in the fertilizer schedule lowers the available P content in the soil. Besides, decomposition of organic materials releases certain acid which enhances the solubilisation of native as well as added phosphorus. Besides, organic inputs mineralize to contribute a significant amount of phosphorus to available P pools [20], whereas lesser organic input leads to a decline in SOM vis-à-vis mineralization [18]. The addition of P fertilizers in plots with 100% RDF enhances P availability but cannot be considered sustainable as this P is either utilized by plant or locked somewhere in the soil system [30]. Integrated treatments stimulate the production of organic P through the process of immobilization [31], clearly signifying an increased MBP. Applications of organic fertilizer have a direct role in increasing plant biomass through providing readily available nitrogen. This increased biomass resulted in more crop residues which accumulates organic matter in the soils over the period of experimentation [36]. Organic matter provides a congenial environment for microorganisms and supports higher microbial biomass [40]. Higher microbial biomass helps in faster mineralization of organic P, resulting in higher availability of organic P under integrated treatments.

The ratios of various P pools to total P were calculated to specify their relative contribution, whereas ratio of inorganic to organic P indicates swing between those P pools [39] (Table 4). The ratio of microbial C to microbial P designates the P availability to the soil microorganisms, because mineralization of organic phosphorus was driven by microbial carbon demand [9]. Enzyme activity per unit of microbial C was considered to appraise the effect of organic inputs on metabolic activity of soil microbes. Ratios between enzymes activities were determined to assess the substrate compositions essential for their activity [6]. Any shift observed in these ratios would also reflect the persistence of tested enzymes in soils. The relationships among P pools reflect the partitioning of P, and these relative shifts can be better understood by the ratios of organic P to total P. This ratio is 40 (expressed in percentage) for treatments with recommended dose of fertilizers, whereas it ranges from 44.43 to 47 for integrated treatments reflecting an accumulation of P in organic forms in treatments T₆–T₁₁. Despite the accumulation of organic P in the integrated treatments, the reduced ratio of available P to organic P signifies the presence of easily metabolizable P. The microbial P to organic P ratio signifies that application of wheat straw improves the transfer the P to the microbial cells by efficiently utilizing the organic P compounds indicating the improved assimilation efficiency with straw

because of its higher carbon and N content (0.65% in this study) [41]. The same can also be observed in the microbial P to total P ratio with the application of wheat straw (3.23%) which implies the presence of efficient microbial communities utilizing the soil nutrients for their biosynthesis. Although there is an accumulation of organic P in the integrated treatments, only 5.8–7.2% attributes to the microbial biomass indicating the stability of organic P compounds. There are reports that inositol phosphates get stabilized by the soil colloids through adsorption reactions [12].

The microbial C to P ratio reflects the ability of microorganisms to utilize P for their growth [1]. Chauhan and co-workers [8] in 1981 reported that microbial C to P ratio ranged from 12:1 to 45:1 for readily available P and in limited P supply condition, respectively. In this study, microbial C to P varied from 3:1 to 6:1, demonstrating a sufficient P supply environment for the microbes. Significantly higher ratio of DHA to microbial C activity confirms the accumulation of extracellular enzymes in the soil environment [14]. Enzyme activity ratios reflect the relative concentrations substrates in the environment [6]. The lower ratios of alkaline or acid phosphatase to pyrophosphatase signify governance of phosphomonoesters which accounted around 61–73% of the organic P in soils [23]. It has been reported that soils with large proportions of monoesters provide a favourable environment for the mineralization of organic P [11]. Besides, the amount of pyrophosphate is low in the top organic layer, but pyrophosphatase activity traced in the current study was attributed to the stabilization of pyrophosphate in the SOM [37].

Conclusions

Integrated application of fertilizers significantly improved the dehydrogenase and phosphatase enzyme activities after 33 years of experimentation. Permanent plot experiment with integrated nutrient management led to significant increase in the microbial biomass carbon and the bio-available fraction of phosphorus in soils. Of the total P in the integrated systems, 38% constitutes the organic phosphorus, while 2.6 and 0.3% of the total P attributes to the microbial biomass P and Olsen P, respectively. Microbial C to P ratio in the treatments with integrated fertilizer scheduled demonstrates a sufficient P supply environment for the microbes. Respiratory enzyme like DHA-to-MBC ratio signifies the accumulation of DHA in soils, one of the important soil biological indicators. Since these ratios are highly dependent on the management practices, additional studies are needed to explore the nature of P assimilating microorganisms and the existing P compounds in soils.

Acknowledgements Authors are thankful to the Vice Chancellor, Bihar Agricultural University (BAU), Bhagalpur, Bihar, India, for providing necessary facilities. Special thanks go to the scientists associated with AICRP-IFS, Sabour, and ICAR—Indian Institute of Farming system Research, Modipuram.

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