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Effect of organics, biofertilizers and crop residue application on soil microbial activity in rice – wheat and rice-wheat mungbean cropping systems in the Indo-Gangetic plains

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Abstract: The aim of this study was to investigate the response of soil microbial parameters to nutrient management practices involving organic amendments, farmyard manure (FYM), vermicompost (VC), crop residues (CR) and biofertilizers (BF) in rice–wheat and rice–wheat–mung bean cropping system of the Indo-Gangetic Plains, India. Soil microbial biomass C (C_{mic}), basal respiration, ergosterol, glomalin, soil enzymes (glucosidases, phosphatases and dehydrogenases), FDA activity, organic carbon (C_{org}), C_{mic}-to-C_{org} ratio and metabolic quotient (qCO_2) were estimated in soil samples collected at 0–15 cm depth. The highest C_{org} (0.64%) and C_{mic} (103.8 μg g^{-1}) soil levels occurred in the treatment receiving a combination of VC, CR and BF. Soil respiration, C_{org} and C_{mic}-to-C_{org} ratio were significantly enhanced by the input of CR to plots receiving FYM and VC. The qCO_2 was the highest in plots receiving a combination of FYM, CR and BF followed by control (no nutrient input) and least in plots receiving a combination of VC, crop residue and biofertilizer. These results indicate that the organic practices involving VC, CR and BF improved soil microbial characteristics and C_{org} in rice–wheat systems.

ABOUT THE AUTHORS

The activity of our research group at the Division of Microbiology, Indian Agricultural research institute (New Delhi), is mainly related to the improvement of rice–wheat-based cropping systems in the Indo-Gangetic plains by integrated resource management (IRM) practice to minimize cost of production, reduce environmental damage in developing countries and improving food security in the developing countries. The research reported in the paper pertains to observe the effect of use of mixed organic fertilizers on soil enzyme activity, and hence soil fertility during rice–wheat cultivation. High input of resources like chemical fertilizers pollutes the environment and harms soil fauna. The present research demonstrates that integrated use of various organic fertilizers improves soil enzymatic activity and overall microbial activity of soil and thus fertility of soil.

PUBLIC INTEREST STATEMENT

Public concerns against resource-intensive agriculture include excessive use of chemical fertilizers, which reportedly leach into soil and water and pollute them. The present study demonstrates that mixed use of various organic fertilizers can reduce the dependence on chemical fertilizers besides improving soil enzyme fertility. It was observed in our study that important soil enzyme activities like glucosidase, alkaline and acid phosphatase, which circulate carbon (C) and phosphorous (P), were increased with mixed use of organic fertilizers. An increase in soil microbial activity was also observed. These observations confirmed increase in soil microbial activity and fertility. To check whether it affects the overall metabolic activity of soil fauna, soil respiration and soil microbial biomass carbon (SMBC), ergosterol content, soil glomalin content and FDA hydrolysis activity were calculated. It was observed that the overall enzyme activities of soil increased when organic fertilizers were used as compared to control suggesting improvement of soil fertility.

Subjects: Agriculture; Bioscience; Conservation - Environment Studies; Environmental Studies & Management

Keywords: crop residue management; Indo-Gangetic Plains; metabolic quotient; rice–wheat cropping system

1. Introduction

Rice–wheat cropping systems (RWCS) are the main source of food and income for millions of people in India, but crop productivity is either stagnating (wheat) or declining (rice) despite the use of higher yielding cultivars (Padre-Tirol & Ladha, 2006). This raises major concerns over the long-term sustainability of current farming practices and poses a threat to future food security against a background of climate change. Key factors responsible for deterioration in soil fertility and crop productivity include decline in soil organic matter (SOM) due to reduced inputs of bioresources and lack of an adequate rotation (Shibu, Van Keulen Leffelaar, & Aggarwal, 2010), negative macro and micro-nutrient balances, leading to depletion of soil fertility and nutrient deficiencies (Timsina & Connor, 2001), declining water availability and poorer quality water (Farooq, Kobayashi, Wahid, Ito, & Basra, 2009), and deterioration in soil structure of continuously puddled soils in rice paddies (Saharawat et al., 2010). The decline in the soil fertility, mainly due to the inadequate organic carbon (C_{org}) levels in soil, seems to be the most significant factor for decreased sustainability of the system.

The practice of adopting a cereal–cereal cropping system on the same piece of land over years has led to soil fertility deterioration, and questions are being raised about its sustainability (Prasad, 2005). However, introduction of summer legumes, such as mung bean, in the RWCS after the harvest of wheat and before the transplanting of rice can increase the productivity of these crops, besides improving the carbon and nitrogen status of soil (Prasad, 2011). After picking matured pods of mung bean, the plant biomass (3–4 t ha^{-1} dry matter) can be used for in situ green manuring.

Organic nutrient sources and crop residues (CR) are the primary source of C inputs (Lal, 2004), and the ways in which these are managed have a significant effect on soil's physical, chemical and biological properties (Kumar & Goh, 2000). The incorporation of CR alters the soil's physicochemical environment (Prasad & Power, 1991) which in turn influences the microbial population/activity in the soil and subsequent nutrient transformations. In general, soil enzymes are good markers of soil fertility since they are involved in the cycling of the most important nutrients. Keeping the above facts in view, the objective of the present study was to investigate the effects of different organic manure, crop residue and biofertilizer applications on soil biological functionality as described by enzyme activities, microbial biomass carbon (C_{mic}) and microbial signature molecules such as ergosterol and glomalin content in RWCS and rice–wheat–mung bean cropping (RWMCS) systems.

2. Materials and methods

2.1. Study site and experimental design

The field experiment was conducted in the main block 14-C of the research farm of the Indian Agricultural Research Institute, New Delhi, India, during 2003–2012. This is situated at 28.4° N and 77.1° E at an elevation of 228.6 m above mean sea level (Arabian Sea). New Delhi has a semi-arid and sub-tropical climate with hot and dry summers and cold winters. It falls under the Trans-Gangetic Plains' agro-climate zone. Summer months (May and June) are the hottest with the maximum temperature ranging between 41 and 48°C, while January is coldest with the minimum temperature ranging between 3 and 7°C. The temperature rises gradually through the months of February and March and reaches a maximum during June, then falls slightly with the advent of south-west monsoon rain. The mean precipitation of Delhi is 650 mm which is mostly received during July–September with occasional rain during winter. The soil of the experimental field is a sandy clay loam (typical Ustochrept) in texture, having 52.06% sand, 22.54% silt and 25.40% clay (pH 8.18, organic matter 1.25%). The physiochemical properties of the experimental field are given in Table 1.

Table 1. Physicochemical characteristics of soil of the experimental field

Property value	
Mechanical composition	
Sand (%)	52.06
Silt (%)	22.54
Clay (%)	25.40
Textural class Sandy clay loam	
Chemical composition and physical properties	
pH (1:2.5 soil:water ratio)	8.16
Electrical conductivity (dS m21 258C)	0.79
Cation exchange capacity (C.mol kg21 soil)	14.73
Organic C (g kg21 soil)	5.20
Total Kjeldahl N (mg kg21 soil)	580
0.5 M NaHCO3 extractable P (mg kg21 soil)	8.42
Neutral 1 N NH4OAC extractable K (mg kg21 soil)	187
Bulk density (Mg m23)	1.50
Field capacity at 1/3 atmospheric tension (%)	24.57

The experiment was laid out in a strip-plot design with three replications. No chemical pesticide/disease/weed control agent was supplied in the field, and hence study was carried out totally in organic farming conditions. Treatments consisted of 14 combinations of 2 cropping systems, namely rice–wheat and rice–wheat–mung bean, and 7 combinations of organic manures, CR, referring to incorporation of crop residue from the previous crop and biofertilizers (BF):- (T_1) farmyard manure (FYM) equivalent to 60 kg N ha^{-1}, (T_2) vermicompost (VC) equivalent to 60 kg N ha^{-1}, (T_3) FYM + CR, (T_4) VC + CR, (T_5) FYM + CR + BF and (T_6) VC + CR + BF, as well as (T_0), a non-amended control.

These treatments were applied to all the crops, i.e. rice, wheat and mung bean, during the period 2003–2012. The cropping history of the experimental field and treatment details are summarized in Table 2. The BF applied to the wheat, rice and mung bean crops consisted of azotobacter + cellulolytic culture + phosphate-solubilizing bacteria (PSB), blue green algae + cellulolytic culture + phosphate-solubilizing bacteria and rhizobium + phosphate-solubilizing bacteria, respectively. For the present study, soil samples were collected at the wheat harvest of 2011–2012, i.e. after completion of six cycles of the rice–wheat or RWMCS system.

Table 2. Cropping history of the experimental field from 2001–2012

Year	Kharif	Rabi	Summer	Remarks
2001–2002	Rice	Wheat	—	Conventional farming
2002–2003	Rice	Wheat		
2003–2004	Rice (Organic)	Wheat (Organic)	—	Transitional period
2004–2005	Rice (Organic)	Wheat (Organic)	—	
2005–2006	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	
2006–2007	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	Organic farming
2007–2008	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	
2008–2009	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	
2009–2010	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	
2010–2011	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	
2011–2012	Rice (Organic)	Wheat (Organic)	Mung bean (organic)	

2.2. Microbiological analysis

Soil was sampled manually from all the plots at 0–15 cm using a tube auger. Fifteen sub-samples per plot were taken and carefully mixed. Soil biological analyses were carried out on moist samples in triplicate and the results were expressed on a dry weight basis. The microbial biomass content of the soil was determined using the fumigation–extraction method of Vance, Brookes, and Jenkinson (1987). The levels of four enzymatic activities in soil were measured: dehydrogenase (EC 1.1.1.) (Casida, Klein, & Santoro, 1964), alkaline phosphomonoesterase (EC 3.1.3.1), acid phosphomonoesterase (EC 3.1. 3.2) (Tabatabai, 1994; Tabatabai & Bremner, 1969) and β-glucosidase (EC 3.2.1.21) (Eivazi & Tabatabai, 1988). The estimation of total glomalin (T-GRSP) was done by the procedure of Wright and Upadhyaya (1998) and the protein content was expressed as µg per g dry weight of soil. Soil microbial activity expressed as fluorescein diacetate (FDA) hydrolysis was determined following the method of Green, Stott, and Diack (2006). The soil respiration (SR) was measured by the alkali entrapment method (Stotzky, 1965) and the metabolic quotient was computed as respiratory activity in relation to microbial biomass (Anderson & Domsch, 1993). The C_{mic}-to-C_{org} ratio and the metabolic quotient (qCO_2) were calculated by dividing the C of CO_2 released from sample in 1 h by the C_{mic} content (Šantrucková & Straškraba, 1991). Soils were also analysed for the fungal biomarker, ergosterol. Ergosterol is a membrane-bound molecule commonly used as a fungal biomarker (Bååth & Anderson, 2003). Ergosterol was extracted from the samples by the microwave-assisted extraction method and determined by HPLC analysis (Young, 1995). The qCO_2, i.e. the respiration to biomass ratio, was calculated from qCO_2 = Basal respiration × 1000/C_{mic} (Insam & Haselwandter, 1989).

2.3. Statistical analysis

A two-factor analysis of variance (ANOVA) was performed to determine the effects of nutrient management/organic amendments, cropping systems and their interactions on soil biological and biochemical properties. Data analysis for all soil parameters was performed using the SAS software. For statistical analysis of data, least significant difference (LSD at p = 0.05) was used to determine whether means differed significantly.

3. Results and discussion

3.1. Phosphatases

Alkaline phosphomonoesterase (ALP) activity was higher in control than in the treatments (except in VC + CR + BF treatment in RWCS) with organic amendments, apparently leading to enhanced mineralization of native organic P fraction in soil (Table 3). Plots receiving a combination of VC + CR + BF in RWCS showed maximum ALP activity (though not significantly greater than control). But in case of all other organic amendment treatments, ALP activity is less than control in both RWCS and RWMCS. The addition of CR affected the ALP significantly. The ALP activity was significantly higher in VC + CR than in VC in RWCS, but significantly lower in RWMCS.

The acid phosphatase (ACP) activity was significantly high in plots receiving FYM + CR + BF and VC + CR + BF in RWCS and VC, CR and VC + CR + BF in RWMCS (Table 3) than in the control plots. The plots receiving VC showed a significantly lower acid phosphatase activity than without CR in RWMCS, but the addition of BF (VC + CR + BF) gave higher ACP activity than VC + CR alone in case of RWMCS. FYM alone or a combination of FYM and CR was at par with the control. Interestingly, ACP activity was stimulated by the application of BF compared with the control in both RWCS and RWMCS except FYM and FYM + CR treatments of RWCS.

3.2. β-glucosidases

In RWMCS, application of all combinations of organic nutrient sources significantly improved the enzyme activity compared with the control, whereas in RWCS, plots treated with FYM or VC were comparable to control plots (Table 3). Plots receiving VC alone or in combination with CR showed the highest stimulation of β-glucosidase activity in the RWMCS. The magnitude of increase in β-glucosidase activity over the control ranged from 43.8 to 55.5% in RWCS. While in RWMCS, the values ranged from 21.5 to 77.4%.

Table 3. Treatment details

Cropping systems											
Treatment	Rice		Wheat		Treatment	Rice		Wheat		Mung bean	
	Organic nutrients		Organic nutrients			Organic nutrients		Organic nutrients		Organic nutrients	
No.	Manures & compost	Biofertilizer	Manures & compost	Biofertilizer	No.	Manures & compost	Biofertilizer	Manures & compost	Biofertilizer	Manures & compost	Biofertilizer
1	FYM[1]	—	FYM	—	2	FYM	—	FYM	—	—	—
3	VC[2]	—	VC	—	4	VC	—	VC	—	—	—
5	FYM + CR[3]	—	FYM + CR	—	6	FYM + CR	—	FYM + CR	—	CR	—
7	VC + CR	—	VC + CR	—	8	VC + CR	—	VC + CR	—	CR	—
9	FYM + CR	BGA + Cellulolytic culture + PSB	FYM + CR	Azotobacter + Cellulolytic culture + PSB	10	FYM + CR	BGA + Cellulolytic culture + PSB	FYM + CR	Azotobacter + Cellulolytic culture + PSB	CR	Rhizobium + PSB
11	VC + CR	BGA + Cellulolytic culture + PSB	VC + CR	Azotobacter + Cellulolytic culture + PSB	12	VC + CR	BGA + Cellulolytic culture + PSB	VC + CR	Azotobacter + Cellulolytic culture + PSB	CR	Rhizobium + PSB
13	— (control)	— (control)	— (control)	— (control)	14	— (control)	— (control)	— (control)	— (control)	— (control)	— (control)

[1]FYM: Farmyard manure (equivalent to 60 kg N ha^{-1}).
[2]VC: Vermicompost (equivalent to 60 kg N ha^{-1}).
[3]CR: Crop residue (incorporation of crop residue of previous crop in succeeding crop.

The observed low activity of the β-glucosidase in FYM-treated plots corresponded with low-soil acid phosphatase in RWCS, dehydrogenase activity in both RWCS and RWMCS and glomalin content in RWMCS (Tables 3–5).

Table 4. Interactive effect of cropping system and nutrient management practices on glucosidase, alkaline phosphatase and acid phosphatase activities in soil

Cropping system/ Nutrient management	Glucosidase (µg pNPG per g^{-1} soil h^{-1})		Alkaline phosphatase (µg pNPP g^{-1} soil h^{-1})		Acid phosphatase (µg pNPP g^{-1} soil h^{-1})	
	Rice–Wheat	Rice–Wheat–Mung bean	Rice–Wheat	Rice–Wheat–Mung bean	Rice–Wheat	Rice–Wheat–Mung bean
Control	13.7	16.7	530	523	120	97
FYM	14.6	20.3	359	296	111	108
Vermicompost (VC)	14.9	27.3	297	411	147	136
FYM + Crop residue (CR)	19.7	24.0	200	386	112	127
VC + CR	20.6	27.8	374	322	139	104
FYM + CR + Biofertilizer (B)	21.0	22.1	253	383	158	123
VC + CR + B	21.3	23.8	536	289	156	165
Mean	18.0	23.2	364	373	135	123
LSD (p = 0.05)	Cropping system (CS): NS Nutrient management (NM): 5.0 CS × NM: 3.2		Cropping system (CS): NS Nutrient management (NM): 69 CS × NM: 50		Cropping system (CS): NS Nutrient management (NM):14.8 CS × NM: 29.3	

Note: Values are mean of the data (n = 3) and are statistically significant at $p < 0.05$. Data analysed by Two-way ANOVA at LSD < 0.05.

Table 5. Interactive effect of cropping system and nutrient management practices on FDA hydrolysis, dehydrogenase activity and microbial biomass in soil

Cropping system/ Nutrient management	FDA hydrolysis (µg fluorescein g^{-1} dry soil h^{-1})		Dehydrogenase activity (µg TPFg^{-1} soil 24 h^{-1})		Microbial biomass (µg MBC g^{-1}soil)	
	Rice–Wheat	Rice–Wheat–Mung bean	Rice–Wheat	Rice–Wheat–Mung bean	Rice–Wheat	Rice–Wheat–Mung bean
Control	319	280	1,285	954	51.6	62.8
FYM	295	291	1,065	881	66.9	87.9
Vermicompost (VC)	291	257	968	1,370	80.5	63.0
FYM + Crop residue (CR)	290	293	1,169	1,157	103.3	67.9
VC + CR	333	331	1,253	905	54.9	102.5
FYM + CR + Biofertil-izer (B)	302	347	1,152	1,022	55.4	69.4
VC + CR + B	335	251	1,234	810	105.5	102.0
Mean	309	393	1,161	1,014	74.0	79.3
LSD (p = 0.05)	Cropping system (CS): NS Nutrient management (NM): 13.6 CS × NM: 47		Cropping system (CS): 89 Nutrient management (NM): 133 CS × NM: 109		Cropping system (CS): NS Nutrient management (NM): 7.2 CS × NM: 9.3	

Note: Values are mean of the data (n = 3) and are statistically significant at p < 0.05. Data analysed by Two-way ANOVA at LSD < 0.05.

3.3. FDA hydrolysis

A significant increase in FDA activity was observed in the VC + CR + BF (5%) in RWCS and FYM + CR + BF (23.9%) in RWMCS over their respective controls, while FDA activity was reduced compared with the control in all organic treatments except with VC + CR and VC + CR + BF in RWCS. On the other hand, FDA activity was increased in RWMCS except with VC and VC + CR + BF (Table 4).

3.4. Dehydrogenase activity

Only soils receiving VC (43.6%) and FYM + CR (21.3%) in RWMCS were found to have significantly higher dehydrogenase activity among the fertilizer treatments over the control, while with RWCS, all dehydrogenase activity levels were lower than the control except VC + CR and VC + CR + BF. Nevertheless, this activity was not consistently correlated with other parameters such as CO_2 production or microbial biomass.

3.5. Microbial biomass C

Overall, the MBC values ranged from 51.6 to 105.5 µg g^{-1} soil in RWCS and 62.8 to 102.5 µg g^{-1} soil in RWMCS (Table 4). The results indicated statistically significant (p < 0.05) differences in the level of soil MBC between various combinations of organic fertilizers, their interaction with the cropping systems but not between the two cropping systems. The MBC values were significantly higher in the plots receiving organics (FYM, CR, C, BF and their combinations) than in the control except with VC + CR or FYM + CR + BF in RWCS and VC, FYM + VC or FYM + CR + BF in RWMCS, reflecting possibly qualitative and quantitative differences in the microbial communities, i.e. 6.4 to 104.5% increase as compared to control in RWCS and up to 63.2% increase in RWMCS, with different organic combinations. In RWCS, MBC with FYM was significantly lower than with VC, while in RWMCS, MBC with FYM was significantly higher than with VC. Application of CR significantly enhanced the soil MBC in conjunction with FYM in RWCS and with VC in RWMCS. The magnitude of increase recorded over control by the application of FYM alone and FYM + CR was 6.4 and 99%, respectively, in RWCS and 39.9 and 8.1%, respectively, in RWMCS. However, the increase of MBC by VC alone and VC + CR + BF was 56 and 104.5%, respectively, over control in RWCS and in RWMCS, increase was 63.2 and 62.4% over control with VC + CR and VC + CR + BF, respectively. A combination of VC + CR + BF was the best treatment as it enhanced microbial biomass significantly over the control in both RWCS and RWMCS, though FYM + CR in RWCS and VC + CR in RWMCS showed almost similar increase as observed in case of VC + CR + BF.

3.6. Basal respiration

Microbial biomass alone does not provide information on microbial activity. Therefore, measurements of microbial biomass turnover, such as SR, which is considered to reflect the availability of carbon for microbial maintenance, are required for that assessment. SR, a measure of the total activity of the soil microbial community, was significantly affected by nutrient management and its interaction with the cropping system. Input of organic nutrient sources significantly improved the SR activity over the control (Table 5). A comparison of the two cropping systems revealed a significant difference in soil CO_2 emission following the input of VC, as in RWCS, significant increase (31.6%) was observed, but in RWMCS, it was slightly lower than the respective control. These differences can be explained on the basis of differences in the C:N ratio of the rhizospheric soil. Leguminous crop fixes atmospheric nitrogen and improves the soil N status, thereby lowering the C:N ratio. In our experiment, respiratory activity was significantly increased with all treatments in RWCS. In RWMCS also, all organic treatments showed significant increase in SR except VC. The addition of CR stimulated the soil CO_2 emission in RWMCS. The SR increased significantly by the residue incorporation and the effect was more apparent where the FYM/VC either singly or in combination with CR was applied, though VC alone did not make any improvement in SR in RWMCS. A corresponding increase in the soil MBC content was also recorded. Carbon mineralization is known to be affected by the complexity of chemical constituents (lignocelluloses content) of organic amendments.

3.7. Metabolic quotient (qCO_2)

The elevated qCO_2 values detected with various organic treatments in RWCS except VC + CR and FYM + CR + BF and FYM + CR and FYM + CR + BF in RWMCS suggest less efficient microbial utilization of C compared to control. The treatment VC + CR in RWCS recorded the highest (5.9 μg CO_2_C μg^{-1} biomass C h^{-1}) (Table 6) followed by FYM + CR + BF (5.3 μg CO_2_C μg^{-1} biomass C h^{-1}) (Table 6), while in RWMCS, the highest value of qCO_2 is recorded in treatment FYM + CR followed by FYM + CR + BF.

Table 6. Interactive effect of cropping system and nutrient management practices on SR, ergosterol and glomalin content activities in soil

Cropping system/ Nutrient management	SR (mg CO_2 (100 g)$^{-1}$soil/week)		Ergosterol (μg g^{-1}soil)		Glomalin content (μg/kg)	
	Rice–Wheat	Rice–Wheat–Mung bean	Rice–Wheat	Rice–Wheat–Mung bean	Rice–Wheat	Rice–Wheat–Mung bean
Control	43.3	44.3	15.96	1.10	43.3	94.3
FYM	50.0	50.3	13.48	7.12	72.0	62.3
Vermicompost (VC)	57.0	43.2	2.97	2.35	61.0	103.3
FYM + Crop residue (CR)	57.1	59.0	10.29	1.04	92.3	102.0
VC + CR	54.3	56.3	7.50	3.14	85.3	103.0
FYM + CR + Biofertilizer (B)	49.6	50.8	1.30	8.46	91.3	64.0
VC + CR + B	53.0	54.2	3.53	6.63	76.7	102.7
Mean	52.0	51.2	7.83	4.26	74.6	90.2
LSD (p = 0.05)	Cropping system (CS): NS Nutrient management (NM): 4.3 CS × NM: 6.0		Cropping system (CS): 0.05 Nutrient management (NM): 0.38 CS × NM: 0.72		Cropping system (CS): NS Nutrient management (NM): 15.8 CS × NM: 30.7	

Note: Values are mean of the data (n = 3) and are statistically significant at $p < 0.05$. Data analysed by Two-way ANOVA at LSD < 0.05.

3.8. Total glomalin content

Glomalin content in the soil samples showed a significant effect of nutrient management and its interaction with the cropping systems. In RWCS, the highest value of glomalin content was observed in treatment FYM + CR (92.3 µg kg^{-1}) followed by FYM + CR + BF (91.3 µg kg^{-1}). FYM + CR and FYM + CR + BF applications had the maximum and significant ($p < 0.05$) impact in enhancing glomalin content (110.8–113.2%) over the control treatment in RWCS (Table 5). In contrast, in RWMCS, plots receiving FYM alone and FYM + CR + BF caused a significant reduction in this soil protein. Rest all other nutrient management practices in RWMCS recorded statistically identical values to the control. A comparison between the two cropping systems revealed that the quantity of glomalin under RWMCS was significantly higher in control, VC-, FYM + CR- and VC + CR-treated plots over the corresponding treatments in RWCS. These differences can be attributed to the differences in the soil organic carbon status in the two cropping systems. Overall, the nature of organic amendment was found to influence glomalin levels; for instance, application of FYM alone failed to improve soil glomalin content in RWMCS over the control, whereas VC application exerted a positive effect on soil glomalin content in both RWCS and RWMCS (Table 5).

3.9. Ergosterol

The RWCS and RWMCS, nutrient management and their interactions significantly influenced soil ergosterol content. Ergosterol is the main endogenous sterol of fungi, actinomycetes and some microalgae. Its concentration is an important indicator of fungal growth on organic compounds and mineralization activity. In the present study, the application of manure in combination with the CR in RWMCS favours fungal growth as the fungi are dominant decomposers in the soil. However, when bacterial biofertilizer is added along with the FYM + CR and VC + CR in RWCS, a lowered fungal/bacterial ratio may result in the observed decline in the soil ergosterol content.

3.10. Soil organic carbon

The soil organic carbon content, an indicator of soil fertility, was positively and significantly influenced by the cropping system and organic nutrient sources. In the present study, the treatment VC + CR + BF emerges as the best option in improving the soil organic carbon status for our experimental crops: rice, wheat and mung bean. Results indicated that at the end of nine years of crop rotation, application of FYM or VC either alone or in combination with CR increased the SOC (0.56–0.68%) compared to the control plot, where no organics were applied (Table 7). The application of FYM + CR + BF caused 34.04, 35.41 and 32.69% increase over their respective controls in rice, wheat

Table 7. Effect of treatments on organic carbon of soil (2011–2012)

Treatment	Soil organic carbon (%)	Soil organic carbon (%) After harvest of wheat (RWCS)	Soil organic carbon (%) After harvest of mung bean (RWMCS)
Cropping system			
Rice–wheat	0.56	0.58	0.60
Rice–wheat–mung bean	0.60	0.63	0.68
LSD (p = 0.05)	0.02	0.03	0.04
Nutrient sources			
Control	0.47	0.48	0.52
FYM	0.56	0.58	0.61
VC	0.57	0.62	0.65
FYM + CR	0.58	0.61	0.65
VC + CR	0.59	0.65	0.68
FYM + CR + B	0.63	0.65	0.69
VC + CR + B	0.64	0.67	0.71
LSD (p = 0.05)	0.03	0.04	0.06

and mung bean crops and VC + CR + BF caused 36.17, 39.58 and 36.54% increase over their controls in rice, wheat and mung bean crops, significantly higher over all other organic sources. A combination of CR with FYM or VC was the next best alternative source of organics. It is very difficult to increase the organic matter content in irrigated soils under sub-tropical climatic conditions due to their very high rates of C mineralization. The present studies suggest that FYM or VC, in combination with CR and BF, could be used as an effective mechanism to sequester SOC and improve soil nutrient status. Further, nature of crop also influences the soil organic carbon content. Although the increase in the amount of soil organic C is important, the increase in the amount of C associated with microbial biomass is more important.

4. Discussion

Among the soil enzyme activities studied, alkaline phosphatase (ALP) activity was the only enzyme activity not stimulated by addition of organic nutrient sources as the values of this activity in soil treated with organics were significantly lower than those found for the control in both RWCS and RWMCS. Phosphatases are a group of enzymes that catalyse the hydrolysis of organic compounds to phosphate. The demand for P by plants and soil micro-organisms can be responsible for the stimulation of the synthesis of this enzyme (Garcia, Hernandez, Roldan, & Albaladejo, 1997). According to Rao and Tarafdar (1992), increase in phosphatase activity indicates changes in the quantity and quality of soil phosphoryl substrates. The observed significant reduction in ALP activity in most organically amended plots may be attributed to the inhibition of phosphatase by an excess of inorganic P (Nannipieri, Grego, & Ceccanti, 1990). The acid phosphatases (ACPs) are reported to be contributed solely by the plant roots (Tarafdar, 1989) and conditions that favour plant root growth may also enhance the secretion of the enzyme. **However, phosphatase activity was found strongly correlated with extractable P (Nottingham et al., 2015), suggesting that increased microbial synthesis of phosphatases was a direct response to low available phosphate (Turner & Wright, 2014).** Additionally, the microbial degradation of CR and metabolic activity of the added BF possibly contribute to the organic acids which perhaps provide optimum pH for the observed high ACP activity.

The hydrolysis products of β-glucosidases are believed to be important energy sources for soil micro-organisms (Tabatabai, 1994). β-glucosidases are key enzymes in the carbon cycle and play a crucial role in hydrolytic processes that take place during organic matter breakdown. Overall, it appears that glucosidase enzyme activity increases with the use of organic nutrients which subsequently results in high available C in the soil and improves the microbial population in soil. Similar results have been reported by Zhang et al. (2010).

The FDA activity is widely accepted as an accurate and simple measurement of total microbial activity in soils, and includes the ubiquitous free and membrane-bound digestion enzymes, such as lipase, protease and esterase enzymes (Green et al., 2006). These differences may be due to the higher levels of organic matter, coupled with the presence of metabolically active micro-organisms (Taylor, Wilson, Mills, & Burns, 2002). A comparison of the two cropping systems revealed a lack of significant differences at all the tested nutrient management levels except VC + CR + BF treatment. However, it is important to interpret the FDA data cautiously because the measured enzyme activities depend on the contribution of both extracellular and intracellular enzyme activities. The enzymes that adhere to the colloids of the organic compost can be another factor to increase the rate of FDA hydrolysis in the organic cultivation (Nannipieri et al., 2003).

Dehydrogenase is involved in the oxidation of SOM and occurs in viable cells and not in stabilized soil complexes. Therefore, the present results are in disagreement with observations where soil amended with organics also exhibits the greatest dehydrogenase activity (Liang, Si, Nikolic, Peng, & Chen, 2005). The observed dissimilar enzymatic activity response to fertilizer treatments (Table 4) may be the result of the resiliency of the respective enzymes to external inputs.

The carbon of the microbial biomass (MBC) is one of the most important variables that reflects differences between organic and conventional areas (Monokrousos, Papatheodorou, & Stamou, 2008).

Microbial biomass is one of the most labile of the pools comprising organic matter. An increase in MBC is likely to better represent changes in the nutrient-supplying capacity of organic matter than an increase in total organic matter (Gunapala & Scow, 1998). The present results are supported by observations of previous workers (Albiach, Canet, Pomares, & Ingelmo, 2000), where they found that organic residues enhanced microbial population, soil microbial biomass and their activity. It has been reported that organic sources like FYM, green manure, CR and BF decompose slowly, resulting in organic carbon accumulation in soil (Sharma, Bali, & Gupta, 2001). Experiments conducted in Punjab, India, in the RWCS showed that the incorporation of CR increased SOC compared to their removal from field (Singh, Singh, Meelu, & Khind, 2000). In contrast, MBC did not result in significant increase over control with FYM + CR + BF in both RWCS and RWMCS. This is contrary to the previous reports where FYM in combination with CR and BF significantly improved the microbial biomass (Banerjee, Aggarwal, Pathak, Singh, & Chaudhary, 2006). The possible reason could be the antagonism among the microflora present in the FYM and the added BF. The present results highlight the importance of the input of CR along with VC in order to increase the microbial biomass carbon in soil. An increase in MBC is linked to changes in the nutrient-supplying capacity of organic matter (Gunapala & Scow, 1998).

The increase in SR activity following the addition of CR to FYM/VC over the FYM/VC alone may be attributed to the enhanced availability of C as an energy source for micro-organisms native to the soil as well as those present in FYM/VC, leading to enhanced mineralization and consequent release of CO_2, though in RWCS, SR decreased slightly in VC + CR than VC alone. CR supplies C as an energy source for micro-organisms and increases the microbial activity (Rousk & Baath 2007; Smith, Papendick, Bezdicek, & Lynch, 1993). Addition of the biofertilizer to the plots receiving FYM + CR caused a significant decline in the SR activity both in RWCS and RWMCS. The observed differential influence of the added bioinoculant may be due to the differences in the C:N of the FYM/VC and also by the interactions among inoculated microbes with native microflora of the FYM/VC. The C/N ratio of VC is much lower (16:1) than that of FYM (30:1). There may be efficient incorporation of C in the microbial biomass and less loss of the CO_2, causing C immobilization in microbial cells. Karmegam and Rajasekar (2012) have reported that microbial population in VC differs qualitatively and quantitatively from that of the compost, and VC is an efficient medium to support the growth of bioinoculants. Interestingly, the highest value of soil microbial biomass carbon was recorded following VC + CR + BF in both RWCS and RWMCS, indicating efficient incorporation of C in the microbial cell mass.

The metabolic quotient (qCO_2) evaluates the efficiency of soil microbial biomass in using the organic C compounds (Anderson & Domsch, 1989). The greater qCO_2 values in these treatments could reflect an increase in the ratio of active:dormant components of the microbial biomass. A low metabolic quotient (qCO_2) in plots receiving FYM may indicate either the presence of microbial populations, which are more efficient in incorporating C compounds, or availability of relatively less labile organic residues.

Application of CR in combination with FYM, VC and/or biofertilizer resulted in high qCO_2 values. This shows that those soils which receive inputs of easily degradable C account for the high qCO_2 values mainly due to more available C present in crop residue. A high microbial quotient generally implies a ready supply of fresh organic residues (Anderson & Domsch, 1989). Additionally, several factors such as low pH, qualitative changes within microbial population (e.g. increase in the proportion of fungi) and prevalence of zymogenous over autochthonous microbiota may explain the differences in metabolic quotient. In the present study, FYM-receiving plots showed the highest level of fungal population as measured by the ergosterol content. Microbiota of the r-strategy ecotype would thrive under such conditions (Insam, 1990). They respire more C per unit of degradable C than K-strategists, which are adapted to more complex C utilization patterns. The low qCO_2 may be due to the occurrence of K-strategists micro-organisms.

This could be possibly due to qualitative and quantitative changes in microbial community structure and function in response to the above ground plant (Patra et al., 2006). The increase of glomalin levels is usually related to greater AMF (arbuscular mycorrhizal fungi) activity in systems with organic substances (Oehl et al., 2004). Overall, the nature of organic amendment was found to influence glomalin levels. Greater availability of mineral nutrients in VC and their rich microbial populations account for the beneficial effects on the mycorrhizal fungi (Arancon, Edwards, Bierman, Welch, & Metzger, 2004). The greater pore volume in VC-amended soils possibly increased the availability of both water and nutrients to micro-organisms including mycorrhizal fungi in soils (Scott, Cole, Elliott, & Huffman, 1996). Addition of organic nutrient sources is known to significantly stimulate mycorrhizal development (Castillo, Rubio, Contreras, & Borie, 2004). However, input of BF in conjunction with FYM + CR led to a significant reduction in soil glomalin content in RWMCS, which was not observed in case of VC + CR. This observation may be attributed to the differences in the native microflora of the VC and FYM and their interaction with the added microbial biofertilizer.

The correlation coefficients between different soil biological properties under RWCS and RWMCS are furnished in Table 8 and 9. It was observed that FDA activity has a significant positive correlation with alkaline phosphatase in RWCS but not in RWMCS. In RWMCS, alkaline phosphatase and DHA showed significant/strong negative correlation with microbial biomass carbon. The correlations between microbial biomass and enzyme activity are influenced by many factors (Stark, Condron, Stewart, Di, & O'Callaghan, 2007).

Table 8. Correlation coefficients between different soil biological properties under rice–wheat cropping system

	GLC	AP	AcP	FDA	DHA	MBC	SR	ERG	GLO
GLC	1.000								
AP	−0.192	1.000							
AcP	0.530	0.108	1.000						
FDA	0.391	0.760*	0.375	1.000					
DHA	0.375	0.539	−0.027	0.766*	1.000				
MBC	0.318	−0.091	0.024	−0.115	−0.156	1.000			
SR	0.397	−0.565	0.163	−0.259	−0.458	0.631	1.000		
ERG	−0.608	0.281	−0.901**	−0.047	0.301	−0.245	−0.507	1.000	
GLO	0.833*	−0.621	0.201	−0.084	0.010	0.288	0.553	−0.471	1.000

*$p < 0.05$.

**$p < 0.01$.

Note: GLC, glucosidase; AP, alkaline phosphatase; AcP, acid phosphatase; FDA, fluorescein diacetate; DHA, dehydrogenase activity; MBC, microbial biomass; SR, soil respiration; ERG, ergosterol; and GLO, glomalin content.

Table 9. Correlation coefficients between different soil biological properties under RWMCS system

	GLC	AP	AcP	FDA	DHA	MBC	SR	ERG	GLO
GLC	1.000								
AP	−0.464	1.000							
AcP	0.383	−0.421	1.000						
FDA	0.040	−0.059	−0.524	1.000					
DHA	0.382	0.419	0.085	−0.168	1.000				
MBC	0.332	−0.817*	0.225	0.047	−0.723*	1.000			
SR	0.339	−0.585	0.182	0.338	−0.348	0.538	1.000		
ERG	−0.091	−0.605	0.287	0.288	−0.468	0.399	0.084	1.000	
GLO	0.485	0.158	0.311	−0.480	0.261	0.092	0.147	−0.724*	1.000

*$p < 0.05$.

Note: GLC, glucosidase; AP, alkaline phosphatase; AcP, acid phosphatase; FDA, fluorescein diacetate; DHA, dehydrogenase activity; MBC, microbial biomass; SR, soil respiration; ERG, ergosterol; and GLO, glomalin content.

A strong negative correlation between soil ergosterol and glomalin content in RWMCS may be explained by the input of organic nutrient sources in the soil which perhaps stimulate the fungal populations, thereby improving the available plant nutrients. These conditions are known to exert a negative effect on the growth and multiplication of the arbuscular mycorrhizal fungi, the source of glomalin protein in soils.

These results indicate that under identical nutrient management conditions, cropping system determines the soil microbial indices. This is supported by the observations that the above ground plant influences the composition and biomass of microbial communities (Jones, Hodge, & Kuzyakov, 2004) because rhizodeposits or organic compounds released by plant roots can be highly specific for a given plant species or even a particular cultivar (Prieto, Bertiller, Carrera, & Olivera, 2011). It indirectly supports the idea that plants also liberate enzymes to the soil through root exudates or after the death and rupture of the cells (Buée, Martin, van Overbeek, & Jurkevitch, 2009). The observed strong positive correlation between FDA activity and alkaline phosphatase as well as with dehydrogenase and in rice–wheat cropping system could be attributed to the fact that these enzymes reflect the hydrolytic and oxidoreductive abilities of the soil microflora. A strong positive correlation between soil glomalin and glucosidase is expected because soil glomalin contains 37% carbon and 3–5% nitrogen, and contributes to the storage of soil carbon (3%), and the glucosidase enzyme catalyses the conversion of the complex carbonaceous polymers into simpler carbon compounds, thereby improving C availability.

The above discussion establishes that organic amendments improve soil microbial activities. Soil microbial activities are directly related to soil biological properties and hence soil fertility. Thus, application of organics, biofertilizer and CR improves soil microbial activity in rice–wheat and rice–wheat–mung bean cropping systems in the Indo-Gangetic plains.

5. Conclusions

The overall microbial activity had been significantly enhanced in soils treated with VC or compost in combination with CR. In conclusion, compost or VC application in combination with CR was found to be beneficial in terms of improving the soil biological parameters in RWCS and RWMCS. The finding from this study possesses specific implications in agricultural, ecological and soil ecosystem restoration perspectives pertaining to maintenance of soil fertility. It is suggested that inclusion of leguminous crop (wheat–mung bean–rice cropping system) is better than wheat–rice cropping system for maintaining soil productivity under semi-arid Indo-Gangetic plains.

Supplementary material

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