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Long-term effects of organic manure and inorganic fertilization on biological soil quality indicators of soybean-wheat rotation in the Indian mid-Himalaya

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ABSTRACT

A field experiment was accompanied to scrutinize the effect of long-term (21-year) mineral and organic manure fertilizer treatments on carbon mineralization, glomalin related soil protein and some microbial characteristics of arable soils in Indian mid-Himalaya. The experiment was initiated in 1995 includes six treatments: Control, N 120, NPK, FYM, N + FYM and NPK + FYM. Long-term chemical fertilization has been reported to negative effects on soil biological properties, however, the impact of combined use of organic and inorganic fertilization on soil biological properties and its relationship remains poorly understood. Results showed that integrated use of organic and inorganic (NPK + FYM) for 21 years significantly increased in carbon mineralization, easily extractable glomalin related soil protein (EEGRSP) and total glomalin related soil protein (TGRSP). Soil microbial indices (microbial quotient: *q*MIC, microbial metabolic quotient: qCO_2 and metabolic potential: MP) were enhanced in the NPK + FYM treatment as compared to rest of the treatments. The activities of seven enzymes, viz. dehydrogenase (DHA), β-glucosidase (β-GA), invertase (IA), acid phosphatase (Acid-PA), alkaline phosphatase (Alkaline-PA), arylsulphatase (ASA) and urease activity (UA) were significantly influenced by the application of NPK + FYM as compared rest of treatments. Soil fertilization with NPK and FYM was significantly higher fluorescein diacetate assay (FDA) activity as compared to other treatments. Soil microbial properties had strong positive correlation with *q*MIC, microbial biomass carbon (MBC) and soil organic carbon (SOC), while qCO_2 had negative correlation under both soil layers. Results showed that the 21-year fertilization experiment clearly indicated that NPK + FYM embodied the best management practice (BMPs) for soil biological sustainability and for sustainable food production.

1. Introduction

The long-term fertilization is important to all agricultural production systems and contributed considerably to the remarkable escalations in sustainable food production systems (Li and Han, 2016; Ghosh et al., 2019). Best management practices (BMPs) of soil fertility and health, understanding of the mineralization process of carbon is required. C-mineralization is vital biogeochemical processes that fortify soil sustainability (Cai et al., 2019; Chen et al., 2020). Application of mineral fertilization with and without organic manure is very common farming practice {traditional management practices (TMPs)} for augmentation and mineralization of soil organic matter (SOM) as results of improvement in soil sustainability (Jiang et al., 2014; Pan et al., 2020). Soil microbes are a vital part of soil, playing a noteworthy role in essential processes such as SOM dynamics, nutrient transformation, decomposition of crop residues (Padhan et al., 2020) and also role regulate of the nutrient accessibility of soil system by immobilization of nutrients as microbial biomass and mineralization of nutrients (Li and Han, 2016; Wang et al., 2019). Hence, soil biological properties have been measured as most subtle indicators of fluctuations in the soil quality (Zornoza et al., 2015). The long-term fertilization had more insistent on soil characteristics, like microbial diversity, activity and biomass (Hartmann et al., 2015). Considering fact that, glomalin related soil protein (GRSP) may be changed by nutrient management (Singh et al., 2013); whether it can be used as a common parameter for evaluating soil quality (Bhattacharjya et al., 2017) still needs to be

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further examined. Soil biological properties respond quickly and are affected by the nutrient management and strong relationship was reported among microbial biomass, soil respiration and enzymatic activities (Frankenberger Jr. and Johanson, 1983; Li and Han, 2016). Arbuscular mycorrhizal fungi (AMF) also plays a role in dynamic soil processes mainly carbon cycling along with influencing binding protein and thrives through a spatial hyphal network that develops throughout the soil (Turgay et al., 2015; Parihar et al., 2020).

The microbial metabolic quotient (qCO_2), which is the amount of CO_2-C produced per unit MBC has been used as an eco-physiological measure of ecosystem succession or disturbance (Wardle and Ghani, 1995). Soil microbial biomass, *q*MIC, qCO_2, metabolic potential (MP) have been used as potential soil quality indicators (Kaschuk et al., 2010) because of their sensitivity to environmental changes, land use, BMPs and TMPs (Ghosh et al., 2019). Fertilization management significantly influenced biological properties of soil system (Frindte et al., 2019; Averill et al., 2019). Long-term fertilization stimulated the production of hydrolytic enzyme or extracellular enzyme activities related to C (β-glucosidase and invertase), N (Urease), P (phosphatase) and S (arylsulphatase) cycles (Li and Han, 2016). Consequently, observing change in various enzymatic activity offer a potential for better understanding of the nutrient cycling, availability, and soil quality. Some long-term effects of combined use NPK and FYM on soil biological properties have been studied in different cropping systems under varying climatic conditions in India (Saha et al., 2008; Bedi et al., 2009; Bhatt et al., 2016).

Nevertheless, very little evidence is available regarding changes in carbon mineralization, fluorescein diacetate hydrolysis (FDA), glomalin related soil protein (GRSP) and enzymatic activities under sub-temperate conditions, more specifically in irrigated soybean-wheat cropping system. Hence, the present investigation was undertaken with the objectives (i) to assess the changes in carbon mineralization, glomalin related soil protein and enzymatic activity as influenced after long-term fertilization under irrigated soybean-wheat rotation on silty clay loam soil of the Indian mid-Himalaya, and (ii) to find out the relationship between soil biochemical properties and soil enzymatic activity under influence of nutrient management.

2. Materials and methods

2.1. Site description

A long-term (21 years) field experiment was commenced at ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Experimental Farm, Hawalbagh (29°36′N; 79°40′E at 1250 m MSL), in the state of Uttarakhand, India, during the winter season of 1995. Experimental site has sub-temperate climate with annual precipitation around 1005 mm (most of which was confined to a three-month period from June–September). Mean air temperatures ranged from 9 to 25 °C experimental periods. Initial soil properties and weather conditions are given in Supplementary Tables 1 and 2.

2.2. Field experiment and design

The treatment details of this study were: control (no fertilizer), N (120 kg N ha^{-1}); NPK (120–26-33 kg ha^{-1}); FYM (10 Mg ha^{-1}); N + FYM (120 kg ha^{-1} + 10 Mg ha^{-1}); NPK + FYM (120–26-33 kg ha^{-1} + 10 Mg ha^{-1}) which were laid out in a randomized block design with four replications. The experiment included, soybean (June–October)-wheat (November–April) crop rotation, and the plot size of 15 m^2 was used for harvesting the crop to minimize the border effects on the crop yields. Chemical composition of FYM had 372 g moisture kg^{-1} and contained 7.2–7.6 g N kg^{-1}, 2.2–2.5 g P kg^{-1} and 5.2–5.6 g K kg^{-1} on dry weight basis.

2.3. Crop management

Initial irrigation was applied before sowing of wheat to facilitate proper soil moisture for crop establishment (October–November). Three days after irrigation, FYM (10 Mg ha^{-1} on a fresh weight basis) was applied and incorporated with a spade during field preparation. Inorganic fertilizers were applied before last tillage to wheat every year and soybean crop grown under residual nutrient management (full PK and half dose of N were applied at the land preparation and remaining N was top-dressed at tillering stage). Fields were manually tilled with a spade to ~20 cm soil depth and levelled.

Wheat (cv. VL-421 was grown from 1995 to 1996 to 2000–2001, VL-616 was grown 2002–2003 to 2004–2005 and VL-804 was grown from 2004 to 2005 to till date) was sown manually with 100 kg ha^{-1} in rows 20 cm apart at a depth of 5–6 cm. Wheat was harvested by manually cutting 5 cm above the ground using sickles and aboveground biomass was removed, but some stubble incorporated into the soil during land preparation of soybean.

Soybean (cv. Bragg was grown from 1996 to 2000, and VL Soya-2 was grown from 2001 to till date) was grown in row apart 40 cm using 80 kg ha^{-1} in the first fortnight of June each year. Before seeding, the land was ploughed manually. After seeding, seed covered manually. Soybean was harvested manually in the month of October.

2.4. Soil sampling and analysis

Composite surface (0–15 and 15–30 cm) soil samples (using core sampler of 15 cm length and 7.6 cm diameter from each plot were thoroughly mixed together) were collected from each plot after harvesting of soybean during 2016 and stored at 4 °C for subsequent carbon mineralization, glomalin related soil protein and soil enzymatic activity measurement. The methodology was followed for analysing of carbon mineralization given by Anderson (1982), microbial biomass carbon by Vance et al. (1987), microbial metabolic quotient, metabolic quotient and metabolic potential indices by Anderson and Domsch (1993). The methodology for biochemical analysis, like fluorescein diacetate assay (FDA) was outlined by Adam and Duncan (2001), glomalin related soil protein by Wright and Upadhyaya (1996). The full details methodology of enzymatic activities given in Supplementary Table 3.

2.5. Statistical analysis

Statistical analysis of the data was done by using analysis of variance (ANOVA), assessed by Duncan's multiple range tests (Duncan, 1955) with a probability, the treatment mean was compared at $P < 0.05$ by using SPSS 10.0 and SAS 9.3 software. All parameters were taken into consideration for Principal Component Analysis (PCA) was performed using SAS 9.3 software.

3. Result and discussion

3.1. Carbon mineralization (Cmin)

Cumulative C-mineralization significantly ($p < 0.05$) varied under different treatments from 224 to 318 mg CO_2-C 100 g^{-1} and 182 to 278 mg CO_2-C 100 g^{-1} soil in 0–15 and 15–30 cm soil depth after 120 days after incubation (DAI) (Fig. 1a, b). Higher cumulative C-mineralization recorded under surface soil as compared subsurface layer under different treatments. Initially cumulative values of evolved CO_2-C increased rapidly from 0 to 50 DAI, subsequently the increases were less for the rest of the incubation period. The application of NPK + FYM had greatest cumulative C-mineralization (318 and 278 mg CO_2-C 100 g^{-1} soil) throughout the incubation period, while the lowest mineralization was recorded in control as 224 and 182 mg CO_2-C 100 g^{-1} soil under 0–15 and 15–30 cm soil layers, respectively. The changes in

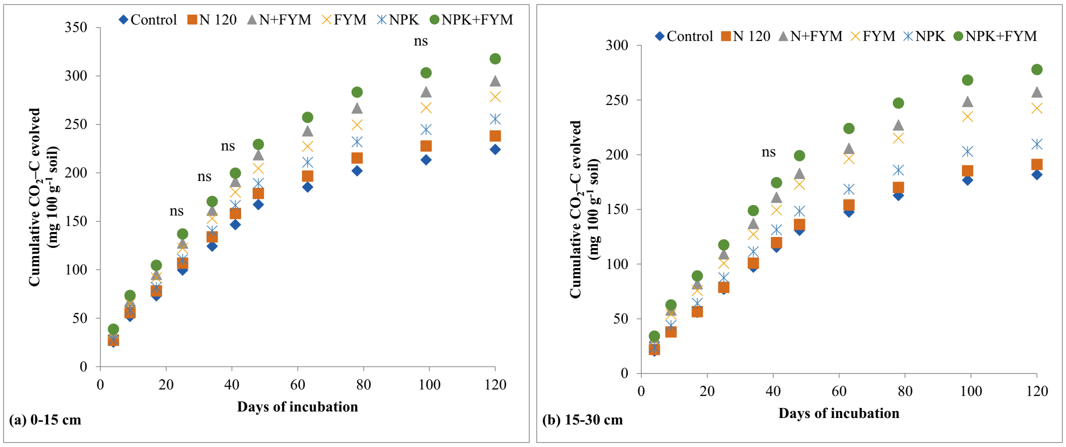

Fig. 1. Cumulative CO_2-C evolution in 0–15 (a) and 15–30 (b) as influenced by long term application of mineral fertilizer and organic manure under irrigated soybean-wheat rotation in Indian mid-Himalayan (ns denotes non-significant).

the rate of C-mineralization are symbolic of the variable amounts of labile organic C-accumulated in different fertilizer treatments. The imbalanced use of nitrogen fertilizer (N only) leads to lower C-mineralization as compared to balanced application (NPK). The impact of long-term use of FYM had higher C-mineralization than NPK and N + FYM treatments. However, there was no significant difference between N + FYM and FYM under both the soil layers. Carbon mineralization process in soil represents amount of organic matter present in soil and is reflected as sign of microbial activity. It can be qualified to the higher microbial population and biomass in soil, which stimulate biological activity due to annual addition of fresh C source as organic manure (Liu et al., 2018). Application of N (nitrogenous fertilizer) along with organic manure (N + FYM) increased soil acidity in the soil, which leads to lower microbial population and activity as compared to FYM treatments.

The significantly highest C-mineralization under NPK + FYM treatments can be associated to build-up of more root biomass and crop residue which leads to better crop growth and yield and improved soil health ultimately enhanced soil micro-biological activity (Ingle et al., 2014; Bhatt et al., 2016; Cai et al., 2019). Carbon mineralization had significant and positive correlation with SOC ($r = 0.77$ and 0.85; $p < 0.01$), MBC ($r = 0.78$ and 0.94; $p < 0.01$) and FDA (0.87 and 0.76; $p < 0.01$) in 0–15 and 15–30 cm soil layers, respectively (Suppl. Table 4).

3.2. Microbial indices

The use of microbial quotient (*q*MIC: is the ratio of MBC to SOC) significant for assess the microbial quality and dynamics (Li and Han, 2016). Results showed that *q*MIC values varied from 4.30 to 4.78% in 0–15 cm and 3.52 to 4.46% in 15–30 cm soil layer (Table 1). The treatment NPK + FYM had highest value of *q*MIC and the lowest under control in both the soil layers. Plot received NPK had highest value of *q*MIC as compared to control, N, FYM and N + FYM plots in 0–15 cm soil layer. The significantly highest value of *q*MIC was recorded under NPK + FYM plot might be due to organic manure induced higher microbial activity. Similar consequences have also been reported by some other researchers (Liu et al., 2018; Cai et al., 2019). The positive and significant correlation was reported between *q*MIC and MBC ($r = 0.67$ and $r = 0.85$; $p < 0.01$) in 0–15 and 15–30 cm soil layers, respectively (Suppl. Table 4).

Microbial metabolic quotient (qCO_2) significantly varied from 2.38 to 2.69 mg CO_2-C mg^{-1} MBC h^{-1} and 2.39 to 3.00 mg CO_2-C mg^{-1} MBC h^{-1} under 0–15 and 15–30 cm soil layers, respectively (Table 1). Plot received NPK had lower value of qCO_2 than that of N and control plots because application of balanced fertilizer had higher MBC, which was responsible for lower value of qCO_2. Higher quotient value under N treated plot than NPK, firstly it might be due to nitrogenous fertilizer causes soil acidic condition decreases microbial activities and growth of soil microbes (Li and Han, 2016), consequently reduces microbial biomass under such soils, secondly lower microbial respiration under imbalanced fertilization (N plot) than balanced fertilization (NPK plot) ultimately higher value of quotient was recorded (Anderson and Domsch, 2010). Plot with NPK + FYM showed lowest value of quotient as compared to rest of treatments. Result obtained from current study was accordance with Ding et al. (2012), Li and Han (2016) and Liu et al. (2018). The qCO_2 had a significant and negative correlation with MBC ($r = -0.54$ and $r = -0.83$; $p < 0.01$) in 0–15 and 15–30 cm soil

Table 1
Microbial quotient (qMIC), microbial metabolic quotient (qCO_2) and metabolic potential (MP) in different soil layers as influenced by long-term fertilization under irrigated soybean-wheat rotation.

Treatments	*q*MIC (%)		qCO_2 (mg CO_2-C mg^{-1} MBC h^{-1})		MP (μg TPF h^{-1} μg C^{-1})	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Control	4.30 ± 0.13b	3.52 ± 0.22b	2.69 ± 0.13bc	3.00 ± 0.10c	0.87 ± 0.07c	0.51 ± 0.04d
N	4.35 ± 0.15b	3.62 ± 0.21b	2.64 ± 0.13bc	2.77 ± 0.10bc	0.93 ± 0.09c	0.53 ± 0.08d
NPK	4.50 ± 0.17ab	3.72 ± 0.13b	2.60 ± 0.07bc	2.65 ± 0.08ab	1.06 ± 0.07bc	0.72 ± 0.10c
FYM	4.39 ± 0.16b	3.90 ± 0.16ab	2.63 ± 0.10c	2.64 ± 0.05ab	1.28 ± .007ab	0.83 ± 0.03bc
N + FYM	4.40 ± 0.18b	4.15 ± 0.21ab	2.52 ± 0.20a	2.58 ± 0.12ab	1.33 ± 0.11a	0.96 ± 0.06ab
NPK + FYM	4.78 ± 0.11a	4.46 ± 0.19a	2.38 ± 0.13a	2.39 ± 0.07a	1.45 ± 0.05a	1.12 ± 0.04a
Mean	4.45	3.89	2.60	2.67	1.15	0.78

Different letters in the same column indicate the significant difference at $p < 0.05$ according to Duncan Multiple Range Test for separation of means (values are mean ± SE).

Table 2
Microbial traits in both soil layers as influenced by long-term fertilization under irrigated soybean-wheat rotation.

Treatments	FDA hydrolysis (μg fluorescein g^{-1} h^{-1})		MBC (mg kg^{-1})		EEGRSP (mg g^{-1})		TGRSP (mg g^{-1})	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Control	3.66 ± 0.15d	3.06 ± 0.16c	350 ± 7.65[f]	260 ± 11.92[d]	0.10 ± 0.009c	0.08 ± 0.007b	5.12 ± 0.03c	4.29 ± 0.02c
N	3.62 ± 0.17d	3.10 ± 0.17c	380 ± 7.23[e]	290 ± 16.75[cd]	0.12 ± 0.007c	0.08 ± 0.005b	4.99 ± 0.11bc	4.18 ± 0.12d
NPK	4.26 ± 0.11c	3.79 ± 0.12b	410 ± 6.48[d]	330 ± 11.58[c]	0.14 ± 0.009b	0.10 ± 0.009ab	5.16 ± 0.03abc	4.29 ± 0.13c
FYM	5.35 ± 0.19b	4.17 ± 0.14b	430 ± 10.64[c]	370 ± 13.30[bc]	0.15 ± 0.005b	0.12 ± 0.019ab	5.19 ± 0.16abc	4.34 ± 0.20bc
N + FYM	5.42 ± 0.13b	4.34 ± 0.19b	470 ± 7.74[b]	420 ± 12.94[b]	0.17 ± 0.011b	0.12 ± 0.012ab	5.29 ± 0.09ab	4.40 ± 0.09ab
NPK + FYM	5.87 ± 0.22a	5.18 ± 0.21a	560 ± 6.27[a]	480 ± 20.18[a]	0.19 ± 0.008a	0.14 ± 0.016a	5.42 ± 0.04a	4.45 ± 0.16a
Mean	4.70	3.94	440	360	0.14	0.10	5.20	4.33

Different letters in the same column indicate the significant difference at $p < 0.05$ according to Duncan Multiple Range Test for separation of means (values are mean ± SE).

layers, respectively (Suppl. Table 4).

Results showed that the highest value of MP as 1.45 and 1.12 μg TPF h^{-1} μg C^{-1} was recorded under NPK + FYM treatment and the lowest value was under control as 0.87 and 0.51 μg TPF h^{-1} μg C^{-1} in 0–15 and 15–30 cm soil layers, respectively (Table 1). This represented that balanced fertilization along with manure improved labile fractions of carbon to microbes and ultimately enhanced soil metabolism. In 0–15 cm soil layer, there was no significant difference among treatments of NPK + FYM, N + FYM and FYM but found significant under 15–30 cm soil layer. FYM treatment with and without mineral fertilizer had higher value of MP than N, NPK and control (Bhattacharjya et al., 2017).

3.3. Microbial traits

Table 2 showed that plot under NPK had significantly ~16 and 24% higher FDA activity as compared to without fertilization (control) and ~18 and 22% higher than N treatment in 0–15 and 15–30 cm soil layers, respectively. Balanced application of fertilizer provides additional nutrients to microbes which increased FDA activity. At both soil layers, significantly higher FDA activity ($p < 0.05$) was recorded in plot with NPK + FYM as compared to rest of the treatments. FDA had significant strong positive correlation with SOC ($r = 0.83$ and 0.77; $p < 0.01$), MBC ($r = 0.75$ and 0.70; $p < 0.01$) and different soil enzyme in 0–15 and 15–30 cm soil layers (Suppl. Table 4). Result obtained in current investigation agreed with the findings of Basak et al. (2016) and Ghosh et al. (2019).

Results showed that application of NPK + FYM could enhance MBC by 60 and 84% in 0–15 and 15–30 cm soil layer, respectively as compared to the control plots (Table 2). There was no significant difference in N + FYM and FYM treatment in 0–15 cm layer, and N and control in 15–30 cm soil layers. Results revealed that MBC content declined with increase in soil depth (0–15 to 15–30 cm) which might be due to the availability of less carbon (Qiu et al., 2016). Plots with the NPK + FYM application contributed higher MBC substrates to sustain the mineralization process, although the aboveground residues were removed (Li and Han, 2016). Soil enzymes, like DHA ($r = 0.68$ and $r = 0.67$; $p < 0.01$), β-GA ($r = 0.89$ and 0.65; $p < 0.01$), IA ($r = 0.93$ and 0.88; $p < 0.01$), ASA ($r = 0.76$ and 0.93; $p < 0.01$) and UA ($r = 0.84$; $p < 0.01$ and 0.48; $p < 0.05$) had significant and positively correlation with MBC in 0–15 and 15–30 cm soil layers (Figs. 2 and 3; Suppl. Table 4). Similar outcomes were also perceived by some researchers (Luo et al., 2015; Siwik-Ziomek et al., 2016).

Results showed application of NPK + FYM significantly enhanced EEGRSP and TGRSP content over NPK in both soil layers. Results revealed that the EEGRSP was ~36 and 40% higher compared to NPK and ~90 and 75% higher compared to control treatment in 0–15 and 15–30 cm soil layers, respectively. Plots under mineral fertilizer along with organic manure (FYM, N + FYM and NPK + FYM) had higher EEGRSP content than that under mineral fertilizer and control (Table 2). The highest TGRSP (5.42 and 4.45 mg g^{-1}) was recorded under NPK + FYM treated plot and the lowest (4.99 and 4.18 mg g^{-1} in 0–15 and 15–30 cm soil layer, respectively) under N plot. It might be due to the organic amendments along with mineral fertilizer improved the development of AMF in soil system (Bhattacharjya et al., 2017). Researchers testified that TGRSP and EEGRSP were increased by organic amendments (Zhang et al., 2019). EEGRSP were significantly ($p < 0.01$) positive correlation with SOC, MBC, FDA, DHA, β-GA, IA, Acid PA, Alkaline PA, ASA, UA except qCO_2(-0.59; $p < 0.01$), while TGRSP also had positive significant correlation (Suppl. Table 4 and Fig. 4a, b).

3.4. Enzymatic activities (EA)

3.4.1. Dehydrogenase activity (DHA)

Results showed that balanced use of mineral fertilizer significantly improved DHA activity due to increased SOC and MBC in soil. The significant and positive correlation (r = 0.68 and r = 0.67; $p < 0.01$ in 0–15 and 15–30 cm soil layers, respectively) was also recorded between MBC and DHA (Luo et al., 2015) (Suppl. Table 4 and Fig. 3a, b). DHA also exposed substantial relationship with FDA ($r = 0.92$ and 0.88; $p < 0.01$) and the entire enzyme assayed. Our results consisted with Chinnadurai et al. (2014) and Balachandar et al. (2016). Plot received NPK + FYM had the highest DHA and the lowest under control. Positive and significant effect of combined application of NPK + FYM on DHA it may be due to the greater biological activity was accordance with Srinivas et al. (2015) and Luo et al. (2015).

3.4.2. β-Glucosidase activity (β-GA)

Significantly the highest β-GA as 458 and 419 mg PNP g^{-1} h^{-1} in 0–15 and 15–30 cm soil layers, respectively was recorded under NPK + FYM plots and lowest under control as 320 and 284 mg PNP g^{-1} h^{-1} in 0–15 and 15–30 cm soil layers, respectively (Table 3). β-Glycosidase activity was ~43 and 47% higher than control and 23 and 29% higher than that in NPK treatment in 0–15 and 15–30 cm soil layers, respectively. Addition of organic manure along with mineral fertilization induced soil microbial properties and enzymatic activities by utilization of easily available carbon and energy source (Srinivas et al., 2015). β-GA had significant positive correlation with MBC ($r = 0.89$ and 0.65; $p < 0.01$), FDA ($r = 0.65$ and 0.64; $p < 0.01$), and other enzymatic activities.

3.4.3. Invertase activity (IA)

Table 3 showed that plot received NPK + FYM had the highest IA (132 mg glucose eq. g^{-1} h^{-1}) and the lowest under N treatment (67 mg glucose eq. g^{-1} h^{-1}) in 0–15 cm soil layer. In case of 15–30 cm soil layer, similarly the highest IA (115 mg glucose eq. g^{-1} h^{-1}) was recorded under NPK + FYM treatment, while the lowest under control (48 mg glucose eq. g^{-1} h^{-1}). Treatments N + FYM and FYM were at par under both soil layers. Results suggested that use of NPK + FYM

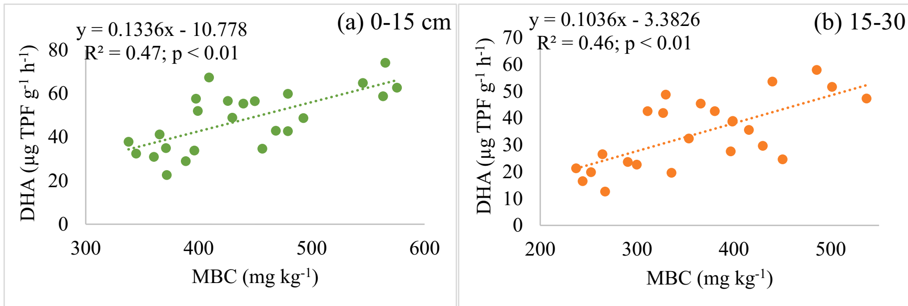

Fig. 2. Relationship between DHA and MBC under 0–15 (a) and 15–30 cm (b) soil layers as influenced by long-term mineral fertilizer and organic manure under irrigated soybean-wheat rotation.

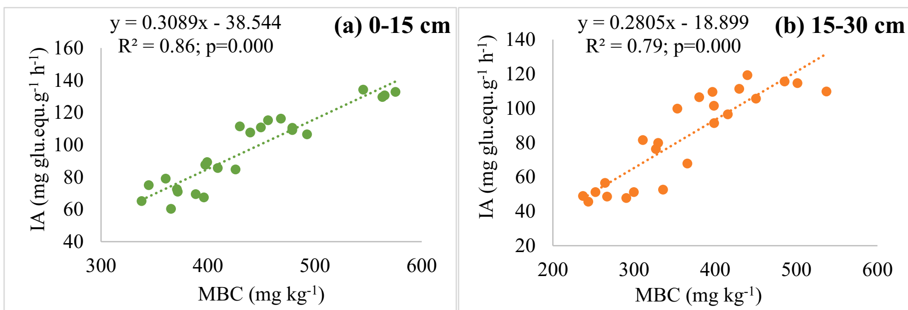

Fig. 3. Relationship between IA and MBC under 0–15 (a) and 15–30 cm (b) as influenced by long term application of mineral fertilizer and organic manure under irrigated soybean-wheat rotation.

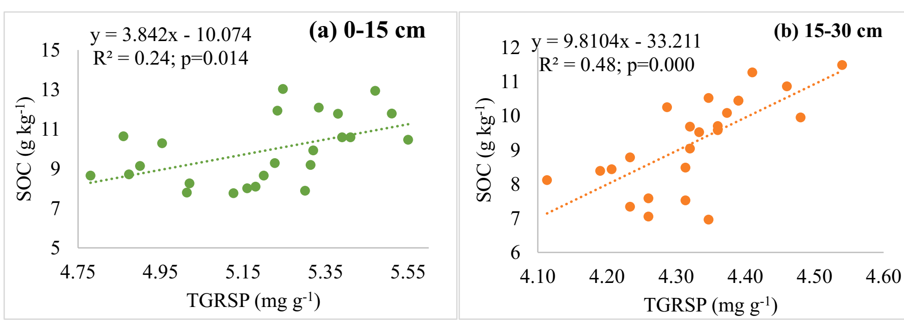

Fig. 4. Relationship between SOC and TGRSP under 0–15 (a) and 15–30 cm (b) soil layers as influenced by long-term mineral fertilizer and organic manure under irrigated soybean-wheat rotation.

Table 3
Dehydrogenase (DHA), β-glucosidase (β-GA) and invertase (IA) under different soil layers as influenced by long-term fertilization under irrigated soybean-wheat rotation.

Treatments	DHA(µg TPF g^{-1} h^{-1})		β-GA (mg PNP g^{-1} h^{-1})		IA (mg glu·equ·g^{-1} h^{-1})	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Control	31 ± 3.17d	20 ± 1.50e	320 ± 6.98d	284 ± 8.33d	73 ± 4.94d	48 ± 3.95e
N	35 ± 2.53 cd	20 ± 2.90e	328 ± 6.95d	287 ± 4.80d	67 ± 5.57d	52 ± 2.87e
NPK	42 ± 2.90c	30 ± 3.00d	371 ± 8.58c	326 ± 5.40c	87 ± 7.01c	76 ± 4.06d
FYM	55 ± 2.28b	37 ± 2.20c	428 ± 9.99b	389 ± 5.37b	110 ± 6.85b	99 ± 3.69c
N + FYM	58 ± 3.24ab	45 ± 1.50b	441 ± 7.82ab	402 ± 7.20ab	112 ± 3.25b	107 ± 4.56b
NPK + FYM	65 ± 3.25a	53 ± 2.20a	458 ± 4.50a	419 ± 4.85a	132 ± 5.03a	115 ± 6.98a
Mean	48	34	392	351	97	83

Different letters in the same column indicate the significant difference at $p < 0.05$ according to Duncan Multiple Range Test for separation of means (values are mean ± SE).

significantly improved (Suppl. Table 4 and Fig. 4a, b) SOC and microbial activity in soil and invertase activity direct linked to microbial biomass carbon (r = 0.93 and 0.88; $p < 0.01$) have been confirmed by several researchers (Liang et al., 2014).

3.4.4. Phosphatases activity (PA)

Application of NPK + FYM had the highest AcidPA as 531 and 473 mg PNP g^{-1} h^{-1} in 0–15 and 15–30 cm soil layers, respectively; the corresponding values for AlkalinePA was 260 and 236 mg PNP g^{-1} h^{-1} in 0–15 and 15–30 cm soil layers, respectively

Table 4
Acid phosphatase (AcidPA), alkaline phosphatase (AlkalinePA), arylsulphatase (ASA) and urease enzymatic activity (UA) under different soil layers as influenced by long-term fertilization under irrigated soybean-wheat rotation.

Treatments	A_{cid}PA (mg PNP g^{-1} h^{-1})		$A_{lkaline}$PA (mg PNP g^{-1} h^{-1})		ASA (mg PNP g^{-1} h^{-1})		UA (μg urea hydrolysed g^{-1} h^{-1})	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Control	351 ± 8.19f	306 ± 9.45d	147 ± 7.64d	120 ± 9.97e	297 ± 12.7d	219 ± 16.2e	38 ± 3.56c	36 ± 4.82b
N	325 ± 5.29e	297 ± 15.6d	152 ± 8.94d	128 ± 5.41e	306 ± 15.8d	244 ± 14.4d	35 ± 2.74c	33 ± 4.44b
NPK	409 ± 5.18d	367 ± 11.3c	191 ± 7.33c	151 ± 10.56d	337 ± 12.0c	267 ± 16.5c	41 ± 3.98c	40 ± 3.12ab
FYM	482 ± 4.79c	403 ± 9.80b	220 ± 9.89b	171 ± 9.78c	383 ± 16.0b	281 ± 15.1c	52 ± 5.41b	42 ± 6.15ab
N + FYM	498 ± 6.09b	415 ± 12.7b	225 ± 11.6b	197 ± 8.70b	395 ± 15.3b	310 ± 19.6b	58 ± 5.26b	48 ± 5.93ab
NPK + FYM	531 ± 7.33a	473 ± 10.7a	260 ± 9.78a	236 ± 14.7a	436 ± 13.7a	358 ± 11.9a	68 ± 4.78a	55 ± 7.25a
Mean	433	377	199	167	359	280	49	42

Different letters in the same column indicate the significant difference at $p < 0.05$ according to Duncan Multiple Range Test for separation of means (values are mean ± SE).

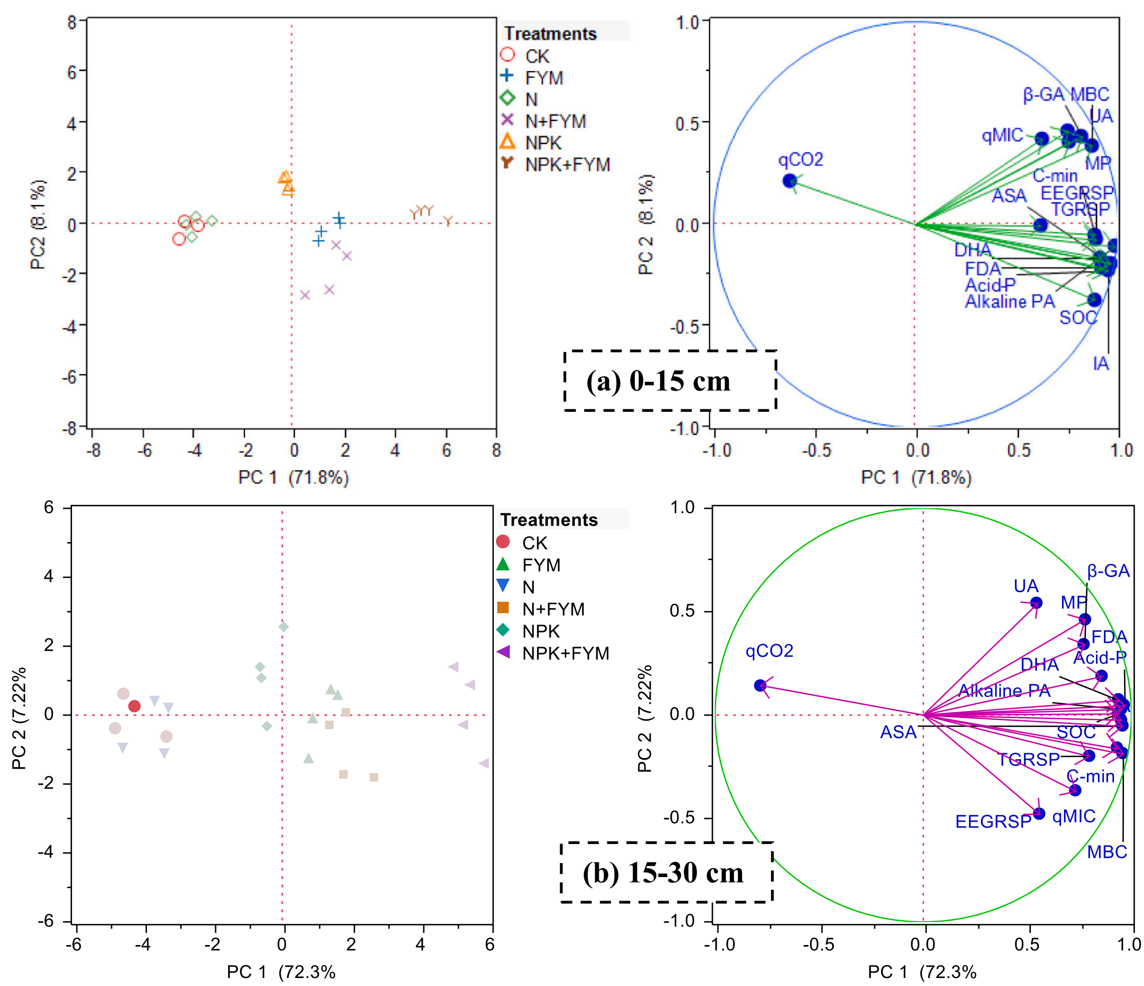

Fig. 5. Score plots (left) and loading plots (right) in 0–15 (a) and 15–30 cm (b) of principal components analysis (PCA) on soil biochemical properties and enzymatic activities. Control (no fertilizer), N (120 kg N ha^{-1}); NPK (120–26–33 kg ha^{-1}); FYM (10 Mg ha^{-1}); N + FYM (120 kg ha^{-1} + 10 Mg ha^{-1}); NPK + FYM (120–26–33 kg ha^{-1} + 10 Mg ha^{-1}). SOC: soil organic carbon, MBC: microbial biomass carbon, *Cmin*: carbon mineralization, FDA: fluorescein diacetate hydrolysis, *q*MIC: microbial quotient, qCO_2: microbial metabolic quotient, *MP*: metabolic potential, EEGRSP: easily extractable glomalin related soil protein, TGRSP: total glomalin related soil protein, DHA: Dehydrogenase, β-GA: β-Glycosidase, IA: invertase, A_{cid}PA: acid phosphatase, $A_{lkaline}$PA: alkaline phosphatase, ASA: arylsulphatase, UA: urease activity, MP: metabolic potential.

(Table 4). Balanced fertilization (NPK) provided significantly higher AcidPA by ~17 and 20% in 0–15 and 15–30 cm soil layers, respectively. In case of AlkalinePA was increased by ~30 and 26%, in 0–15 and 15–30 cm soil layers, respectively as compared to control. Use of long-term mineral fertilizer prompted soil acidification, which in turn decreased phosphorus availability and raises the N:P ratio, increases P demand in soil as compared to sole application of FYM (Frossard et al., 2016). Correlation matrix (Suppl. Table 4) shows significant and strong correlation among the different soil biological characteristics. AcidPA and AlkalinePA had positive strong significant relationship with MBC FDA, IA, ASA and DHA (Bowles et al., 2014).

3.4.5. Arylsulphatase activity (ASA)

Results showed that ASA was significantly greater in NPK as compared to N and control plots in both soil layers. The highest ASA was recorded under NPK + FYM treatment and the lowest was under control in both soil layers (Bhatt et al., 2016). ASA had significant and strong correlation with SOC (r = 0.92 and 0.89; $p < 0.01$), MBC (r = 0.76 and 0.93; $p < 0.01$) in 0–15 and 15–30 cm soil layers, respectively (Suppl. Table 4). This corroborates with the conclusions of Iovieno et al. (2009) and Siwik-Ziomek et al. (2016).

3.4.6. Urease activity (UA)

Results showed that the plots under organic manure with and without mineral fertilizer significantly improved UA as compared to that plots received control, N and NPK in 0-15 cm soil layers (Table 4). In 0-15 cm soil layer, across the treatments, mean UA was ~ 17% higher as compared to that in the 15-30 cm soil layer. Although significantly highest UA was recorded in NPK + FYM plots and lowest in N treatments in surface soil. The significantly higher UA was observed in NPK + FYM treatment, it might be due to continuous cropping under organic manure fertilization, stimulates heterotrophic microbes, which are decomposing organic substrates, which supplied carbon and energy for heterotrophy (Liu et al., 2018; Zhang et al., 2019; Chen et al., 2020). A significant and positive relationship was witnessed between UA and MBC (r = 0.84; $p < 0.01$ and 0.48; $p < 0.05$) in 0-15 and 15-30 cm layers, respectively (Suppl. Table 4).

3.5. Correlation matrix

The pearson correlation matrix naked that all the bio-chemical parameters were positively simultaneous with SOC and MBC contents (except qMIC and qCO_2) in the 0–15 and 15–30 cm soil layers (Suppl. Table 4). Correlation showed that SOC and MBC are the major determinant, which governed a number of biological processes in soil (Liang et al., 2014). Cmin, FDA, EEGRSP, TGRSP, DHA, IA, β-GA, AcidPA, AlkalinePA, ASA, and UA were significant and positively associated with each other in the both layers (Suppl. Table 4). Our results were established by principal component analysis (PCA) of all soil biochemical properties and enzymatic activities, which showed significant effect of mineral and organic manure fertilization through C1 and C2 (71.8% and 8.10% of total variance) in 0–15 cm layer, respectively. In case of 15–30 cm layer, C1 was 72.3% and C2 was 7.22% of total variance (Fig. 5). The split-ups via PC1 were linked with most of the selected parameters, while the separations via PC2 were associated only with qCO2 in both soil layers (Li and Han, 2016).

4. Conclusions

A long-term (21 years) experiment was conducted to assess the effect of various nutrient management practices on soil biological indicators in soybean-wheat rotation. Result reveals that continuous application of balanced fertilization along with organic manure (NPK + FYM) significantly enhanced carbon mineralization, glomalin related soil protein (EEGRSP and TGRSP), soil microbial indices, viz. qMIC, MP (except qCO_2) and soil enzymatic activities, i.e. FDA, DHA, IA, β-GA, AcidPA, AlkalinePA, ASA, and UA as compared to N + FYM, FYM, NPK, N and control treatments in irrigated soybean-wheat rotation of the Indian mid-Himalaya. Plots received NPK or FYM showed superior biological parameters than that in N and control plots. Soil microbial indices and enzymatic activities were meaningfully and simultaneous with each other in the both layers. We established that long-term use of NPK + FYM is the best nutrient management practices for improving soil microbial functioning for sustainable production.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103754.

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