RESEARCH ARTICLE

Soil organic matter dynamics as affected by long-term use of organic and inorganic fertilizers under maize–wheat cropping system

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Abstract Understanding the effects of long-term use of fertilizers on soil carbon and nitrogen pools and their activities is essential for sustaining soil productivity. Our objectives were to quantify longterm changes in soil organic carbon (SOC), soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN) and mineralizable C in maize-wheat cropping sequence in fertilized and unfertilized plots (control, N, NP, NPK, and NPK + FYM). Continuous application of fertilizers increased SOC over its initial content. Active fractions of SOC, i.e., water-soluble carbon, hydrolysable carbohydrates, SMBC, SMBN and dehydrogenase activity, improved significantly with an application of NPK and NPK + FYM. A general increase in carbon mineralization with time period was observed throughout the experiment and was maximum in 100% NPK + FYM treated plots. The estimated annual C input value in NPK + FYM treatment was $1.05 \text{ MgC ha}^{-1} \text{ year}^{-1}$. The overall net change in organic carbon was maximum in treatment receiving

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N. S. Dhillon e-mail: ns.dhillon@rediffmail.com FYM along with inorganic fertilizers. Therefore, these results suggest that the integrated use of NPK and FYM is an important nutrient management option for sustaining maize–wheat cropping system.

Keywords Carbon mineralization · Cropping system · Long-term fertilization · Soil organic matter

Introduction

Soil organic matter (SOM) is a key component as it influences soil biological, physical and chemical properties that define soil quality (Doran and Parkin 1994). Recent global concerns over increased atmospheric CO₂, which can potentially alter the earth's climate systems, have resulted in raising interest in studying SOM dynamics and carbon sequestration capacity in various ecosystems (Schlesinger 1999). Agronomic practices such as cultivation, residue management and fertilization regulate microbial activities, which in turn mediate the processes of organic matter turnover and nutrient cycling (Doran and Smith 1987). With changes in the quality and quantity of SOM, the potential of soil to supply or sequester nutrients, especially N, is altered through changes in mineralization-immobilization turnover (Janssen and Persson 1982).

Understanding SOM dynamics is of great importance. It has a profound influence on ecosystem

sustainability, soil fertility and soil structure (Tabatabai 1996). Long-term field experiments have shown that SOM is influenced by management practices and especially by the addition of fertilizers and manures. The content of organic carbon in the soil is controlled by changes in management via the annual input of organic matter and the rate at which it decays (Jenkinson 1988). These changes are not only quantitative, they also affect the quality of SOM in terms of composition. Long-term fertilizer experiments have shown a decline in soil organic carbon (SOC) as a result of continuous application of fertilizer N alone in acid Alfisols of India under maize-wheat cropping system (Sharma et al. 1998). Balanced use of NPK fertilizer either maintained or enhanced the SOC over the initial values. Losses and gains of SOM are influenced by land-management practices such as cropping frequency (Campbell et al. 1995), reduced tillage (Reicosky et al. 1995), fertilizer application (Gregorich et al. 1996), manure application (Sommerfeldt et al. 1988), and also by cultivation of perennial legumes and grasses (Campbell et al. 1991).

Thus, there is a great need to fully quantify the role of SOM fractions, particularly C and N pools, in relation to crop productivity and nutrient availability. Specific objectives of this study were to (1) quantify the changes in soil organic C and N pools, and (2) to assess the mineralization potential and C sequestration.

Materials and methods

Description of field experiment

In order to investigate the long-term effects of organic and inorganic fertilizers, a field experiment with different fertilizer treatments was laid down in 1971 at Punjab Agricultural University research farm, Ludhiana, as part of the All India Coordinated Research Project on long-term fertilizer experiments. The experimental site is located at 75°48′E, 30°54′N at an altitude of about 247 m asl. The maize–wheat–cowpea cropping sequence was selected to study the long-term effects of inorganic fertilizer either alone or in combination with farmyard manure (FYM) for maximizing crop yields and sustaining high soil fertility. Growing of cowpea crop was discontinued from 2000 onwards. Five fertility treatments, i.e.

control (no fertilizer), 100% N, 100% NP, 100% NPK and 100% NPK plus cattle FYM were selected out of ten treatments for the present investigation. The 100% NPK application was equivalent to 120 kg N, 26.2 kg P and 25 kg K ha⁻¹ for both maize and wheat crops. The FYM was applied at 10 Mg ha⁻¹ on dry weight basis before sowing of maize. The soil of the experimental field was sandy loam in texture (Typic Ustochrept). Surface soil (0–15 cm) tested pH 8 (1:2 soil/water), 2.4 g kg⁻¹ total C, 87 kg ha⁻¹ available N, 9 kg ha⁻¹ Olsen P and 88 kg ha⁻¹ NH₄OAc extractable K.

Soil sampling

The soil samples were drawn from experimental plots before sowing of maize crop in May 2004 from five depths, i.e., 0–7.5, 7.5–15, 15–30, 30–45 and 45– 60 cm. An uncropped and unfertilized fallow plot under undisturbed vegetation was also similarly sampled. Soil samples were dried, ground and sieved through a 2-mm sieve and were stored for chemical analysis in a refrigerator. Soil aggregates of undisturbed soil cover (without grinding and sieving) were separated in crushed core samples and organic carbon was determined in each size of the aggregate. For the analysis of active pools of nutrients, fresh soil samples were collected from 0 to 15 cm depth and were stored separately in polythene bags at 4°C for biochemical analysis.

Laboratory analyses

Aggregate size fractions and associated carbon concentration

Soil samples from 0 to 7.5, 7.5 to 15, 15 to 30, 30 to 45 and 45 to 60 cm depth were used for aggregate separation by the wet sieving method (Cambardella and Elliott 1994). For this purpose, soil samples were wet sieved by double stage Yodder's apparatus through a series of three sieves to obtain four size fractions: 2-1, 1-0.5, 0.5-0.25 and 0.25-0.1 mm. Aggregate fractions retained on each sieve were transferred into a container and dried at 65° C in an oven before chemical analysis. Organic carbon in each aggregate-size class was determined by Walkley and Black (1934) rapid titration method.

Soil microbial biomass and enzyme activity

The SMBC was measured by fumigation extraction method (Vance et al. 1987). Two portions of moist soil, each containing 50 g oven dry soil were weighed into 100-ml capacity beakers. One portion served as control and was extracted immediately with 0.5 M K_2SO_4 . Other portion was fumigated. The fumigated soil samples were extracted with 200 ml 0.5 M K_2SO_4 for 30 min. Organic carbon in the soil extracts was measured by dichromate oxidation and SMBC was calculated from:

SMBC = 2.64 EC

where, EC is organic C extracted from fumigated soil minus organic C extracted from unfumigated soil.

Soil dehydrogenase activity (as a measure of microbial activity) was determined by the method of Casida et al. (1964). To 3 g soil sample in a flask, 0.5 ml of 3% triphenly tetrazolium chloride solution and 1.5 ml of water were added. The flasks were tightly stoppered and swirled for few seconds. These samples were incubated for 24 h at 37°C and extracted with 94% methanol. The extract was assayed at 485 nm using spectrophotometer. The unknown values were calculated based on standard curve compared with tri-phenyl formazon (TPF). Enzyme activity was expressed as gm of TPF released μg^{-1} of soil 24 h^{-1} .

Carbon mineralization of soils

Carbon mineralization was estimated by measuring CO_2 evolution from soils for different time intervals (5–90 days) as described by Ladd et al. (1995). Twenty-five grams of moist soil were taken in an erlenmeyer flask. Ten milliliters of 0.5 N NaOH contained in a test tube (10 × 2.5 cm) was placed in each flask and the flasks were sealed airtight with rubber corks at 28°C. The CO_2 evolved was trapped in NaOH and was measured daily by back titration with 0.5 N HCl. The amount of CO_2 evolved was converted to CO_2 –C.

Water-soluble fractions of carbon and nitrogen

Organic C was determined by Walkley and Black's rapid titration method. Water-soluble C was

determined as per the method described by Mc Gill et al. (1986). Five grams of soil with 10 ml of distilled water was centrifuged for 30 min at 10,000 rpm. The supernatant aliquot was filtered. Then 5 ml of filtrate were treated with 5 ml of 0.07 N $K_2Cr_2O_7$, 10 ml of 98% H_2SO_4 and 5 ml of 88% H_3PO_3 and the mixture was digested at 150°C for 30 min. Cooled samples were then titrated with a solution of 0.01 N ferrous ammonium sulphate in 0.4 N H_2SO_4 using diphenylamine as an indicator.

Water-soluble carbohydrates were determined by taking 10 g soil, treating with 24 N H₂SO₄ and then digesting on a hot plate at 100°C for 10–16 h. The filtrate was precipitated with 6N NaOH for neutralization, and to 5 ml aliquot, 10 ml of 0.2% anthrone was added. The intensity of green colour was read at 625 nm on spectrophotometer (Chebhire and Mundie 1966). Water-soluble nitrogen was measured by the method given by Jackson (1973).

Yield trends and statistical analysis

A linear regression analysis of grain yields obtained over the years was done to determine a time trend variable for all treatments. The experiment was conducted in a randomized complete block design with three field replications for each treatment. The statistical significance was evaluated at $p \le 0.05$ and $p \le 0.01$.

Results

Yield trends and soil organic carbon

The grain yield trends over 34 years of multiple cropping with maize–wheat sequence under various fertilizer treatments are presented in Fig. 1a, b. Grain yield of both maize and wheat increased markedly over the years with time in fertilized plots and also in control (unfertilized). Maize grain yield production was the highest where FYM at 10 t ha⁻¹ was applied along with recommended NPK. The residual effect of FYM was also observed on wheat grain yield. The results clearly demonstrated that grain yield of both maize and wheat was the lowest in unfertilized plots and that it improved greatly with successive additions of N, P and K indicating



Fig. 1 Yield trends of (a) maize and (b) wheat crop over the years. * and ** represent significance at $p \le 0.05$ and $p \le 0.01$, respectively.

Equation 1a

Control: $y = 0.001x^3 - 2E - 05x^2 + 0.0738x + 0.999 \quad R^2 = 0.804^{**}$ 100%N: $y = 0.0002x^3 - 0.0047x^2 + 0.0147 + 1.567 \quad R^2 = 0.784^{**}$ 100%NP: $y = 0.0003x^3 - 0.0078x^2 + 0.0413x + 2.003 \quad R^2 = 0.896^{**}$ 100%NPK: $y = 7E - 05x^3 + 0.0035x^2 - 0.1796x + 3.968 \quad R^2 = 0.792^{**}$ 100%NPK + FYM: $y = 0.0003x^3 - 0.0092x^2 + 0.0842x + 3.096 \quad R^2 = 0.765^{**}$ Equation 1b Control: $y = 6E - 05x^3 - 0.003x^2 + 0.0679x + 0.5258 \quad R^2 = 0.732^{**}$ 100% N: $y = -4E - 05x^3 - 0.0033x^2 - 0.0484x + 2.8525 \quad R^2 = 0.326^{*}$ 100% NP: $y = 0.0001x^3 - 0.0086x^2 + 0.1869x + 3.032 \quad R^2 = 0.358^{*}$ 100% NPK: $y = 0.0002x^3 - 0.0126x^2 - 0.2789 + 3.352 \quad R^2 = 0.617^{**}$ 100% NPK + FYM: $y = 8E - 05x^3 - 0.0082x^2 + 0.2293x + 3.453 \quad R^2 = 0.788^{**}$

clearly that balanced fertilization (N + P + K) is necessary for both maize and wheat for obtaining higher yields over the years. Further, addition of FYM in the summer season (over the years) to maize crop ensured the highest production of grain yield and helped in sustaining grain yield production at the highest level under maize–wheat cropping system. Improvement in grain yield of maize and wheat in unfertilized plots over the years may be due to improvement in SOC. It was further noticed that SOC content improved continuously from initial level of 0.20–0.52% with an application of FYM over the years.

Vertical distribution of SOC after 34 years of cropping was highly dependent on the use of various fertilizers (Table 1). The increasing trends were

Date	Depth (cm)	Treatme	CD $(p = 0.05)$					
		Fallow	Control	100% N	100% NP	100% NPK	100% NPK + FYM	
Initial ^a (1971)		-	2.6b	1.8c	1.7a	2.1a	2.0d	-
After 34 years	0–7.5	2.7cd	3.1de	3.5ef	3.5ef	4.2g	5.0h	1.1
	7.5–15	2.2abc	3.1de	3.3e	3.2e	3.5ef	4.0fg	0.7
	15-30	2.0a	2.1a	2.0ab	2.1a	2.2abc	2.6bcd	NS
	30-45	2.0a	2.0ab	2.0a	2.1abc	2.2abc	2.2abc	NS
	45-60	1.9ab	2.0a	2.0a	2.0abc	2.1abc	2.2abc	NS

Table 1 Effect of long-term use of manure and fertilizer application on soil organic carbon (g kg⁻¹) after 34 years of crop rotation

^a Initial soil organic carbon in surface (0-15 cm) soil layer

Within a row, treatment means followed by lowercase letters are significantly different at $p \le 0.05$ by Duncan's multiple range test

observed in SOC content in all fertilized plots as compared to initial value. At 0–7.5 cm depth, SOC was 61.3% greater under treatment receiving 100% NPK + FYM as compared to control plot. In 7.5– 15 cm soil layer, FYM treated plot had maximum organic carbon (4 g kg⁻¹) which was significantly higher than 100% NPK (3.5 g kg⁻¹) and 100% NP (3.2 g kg⁻¹) treated plots. However, in 15–30 cm soil layer the change in organic carbon was not much apparent and it varied between 2 and 2.6 g kg⁻¹ soil in different fertilizer treatments.

Slow pools of soil organic carbon

The content of water stable aggregates organic carbon (WSAC) increased at all the five depths, i.e., 0–7.5, 7.5–15, 15–30, 30–45 and 45–60 cm, with a decrease in the size range of 2–1, 1–0.5, 0.5–0.25 and 0.25–0.1 mm (Table 2), and was maximum between 0.5 and 0.25 mm size range. The highest WSAC content of 4 g kg⁻¹ soil was observed in the plot receiving 100% NPK + FYM at 7.5–15 cm soil depth in 0.5–0.25 mm size range. For 100% NPK and 100% NPK + FYM treatments, the 0.5–0.25-mm-sized aggregates had about 2–33% more SOC than N alone treated plots. The water stable aggregate carbon in different sized fractions declined with increase in soil depth, the highest being in the surface layer.

Active pools of carbon and nitrogen

After 34 years of maize–wheat cropping system, SMBC ranged between 137 and 228 mg kg⁻¹ in 0–15 cm soil depth (Table 3). Compared to the

control (unfertilized plot), SMBC content was 21.1, 34.5, 52.1 and 65.1% more under 100% N, 100% NP, 100% NPK and 100% NPK + FYM treated plots, respectively. The SMBC increased significantly with an application of N fertilizer and comprised 4.5–5.7% of total soil carbon (TOC). The SMBN content of treatment receiving FYM along with inorganic fertilizer NPK was about 14.4–93% more compared to other treatments and correlated significantly with SMBC and C/N ratio of soil microbial biomass (Fig. 2a, b).

On average, water-soluble carbon (WSC) accounted for 0.5–1.1% of TOC and increased significantly with an application of organic fertilizers along with inorganic fertilizers. Water-soluble nitrogen ranged between 2.6 and 11.7 mg kg⁻¹. Water-soluble nitrogen also correlated with water-soluble carbon (r = 0.96). In plots receiving balanced fertilization, water-soluble fractions were sustained better than in plots receiving continuous application of fertilizer N alone. The hydrolysable carbohydrates accounted for 11.9–17.9% of TOC. There was no appreciable change in water-soluble carbohydrates in control and 100% N treatments. However, with an application of K fertilizer its concentration increased.

The dehydrogenase enzyme activity ranged between 22.1 and 76.2 μ g TPF g⁻¹ soil 24 h⁻¹ and increased by 35.1% with an application of 100% NPK + FYM over 100% NPK.

Carbon mineralization in soil

Higher amounts of C mineralization were observed in NPK plus FYM treatment as compared to N, NP and

Depth (cm)	Treatments	Aggrega	CD $(p = 0.05)$			
		2-1	1–0.5	0.5-0.25	0.25-0.1	
0–7.5	Fallow	0.6	0.7	3.0	2.2	0.5
	Control	0.5	0.7	3.5	2.3	0.8
	100% N	0.6	0.7	3.5	2.4	0.2
	100% NP	0.9	1.1	3.5	2.6	0.2
	100% NPK	1.1	1.3	3.5	2.8	0.3
	100% NPK + FYM	1.2	1.7	3.6	3.4	0.3
7.5–15	Fallow	0.7	0.7	2.5	2.3	0.3
	Control	0.5	0.6	2.5	2.7	0.5
	100% N	0.6	0.8	3.2	3.0	0.5
	100% NP	0.8	0.9	3.4	3.1	0.4
	100% NPK	0.9	1.1	3.8	3.1	0.1
	100% NPK + FYM	1.2	1.4	4.0	3.2	0.2
15-30	Fallow	0.7	0.8	2.4	1.7	0.2
	Control	0.6	0.7	2.4	1.5	0.3
	100%N	0.6	1.0	2.5	2.2	0.1
	100% NP	0.8	1.1	2.7	2.5	0.2
	100% NPK	0.8	1.2	2.8	2.5	0.3
	100% NPK + FYM	0.9	1.5	3.0	2.6	0.3
30-45	Fallow	0.7	0.8	1.8	1.4	0.1
	Control	0.7	0.8	1.5	1.4	0.2
	100% N	0.8	0.8	1.8	1.4	0.1
	100% NP	0.8	0.9	1.8	1.5	0.1
	100% NPK	0.9	1.3	2.1	1.6	0.3
	100% NPK + FYM	1.0	1.5	2.2	1.8	0.1
45-60	Fallow	0.6	0.6	1.3	1.2	0.2
	Control	0.5	0.6	1.3	1.2	0.1
	100% N	0.5	0.7	1.4	1.2	0.1
	100% NP	0.6	0.7	1.5	1.3	0.1
	100% NPK	0.7	0.9	1.7	1.3	0.1
	100% NPK + FYM	0.8	1.0	1.9	1.4	0.2

Table 2 Effect of manure and fertilizer application on water stable aggregates organic carbon (g C kg⁻¹) at different depths

NPK treatments. The cumulative amount of mineralized CO₂–C varied between 0.4–5.4 and 0.58–6.5 g CO₂–C kg⁻¹ soil C in the uncropped plots, i.e., outside and control plots, respectively (Fig. 3). In the plots receiving N fertilizer alone, it varied between 1.1 and 7.7 g CO₂–C kg⁻¹ soil C. The amount of mineralized CO₂–C increased further with an application of P along with N fertilizer (1.5–9.1 g CO₂–C kg⁻¹ soil C). In 100% NPK and 100% NPK + FYM plots, cumulative amount of mineralized CO₂–C ranged between 2–10.8 and 2.2–12.7 g CO₂–C kg⁻¹ soil C, respectively.

Carbon balance and its turnover rate

Overall net change in organic C was positive for all the treatments (Table 4). Steady state C content ranged between 5.8 and 9.9 Mg C ha⁻¹. The C addition rate increased significantly in plots receiving 100% NPK and 100% NPK + FYM to 0.73 and 1.05 Mg C ha⁻¹ year⁻¹ as compared to 0.12 Mg C ha⁻¹ year⁻¹ in fallow. The reason may be attributed to increased amount of plant biomass addition. The C loss rate varied between 0.02 and 0.10 Mg C ha⁻¹ year⁻¹/Mg C ha⁻¹ for different treatments.

Treatments	TOC g kg ⁻¹	TN mg kg ⁻¹	SMBC mg kg ⁻¹	SMBC/TOC %	WSC mg kg ⁻¹	WSC/TOC %	WS Carb mg kg ⁻¹	WS Carb /TOC %	SMBN mg kg ⁻¹	SMBN/TN %	WSN mg kg ⁻¹	WSN/TN %	DA ug TPF g ⁻¹ soil 24 h ⁻¹
Fallow	2.4ab	415.0a	136.9a	5.7	15.1ab	0.6	430.7a	17.9	8.0a	1.9	2.6a	0.6	22.1a
Control	3.1b	536.0bc	138.1b	4.5	13.8ab	0.5	469.3b	15.3	8.1a	1.5	4.2b	0.8	24.1b
100% N	3.3b	570.6b	167.2c	5.0	25.6abc	0.8	469.0c	14.0	8.6b	1.5	6.7bc	1.2	35.6b
100% NP	3.4bc	588.0c	185.7d	5.5	33.2bc	1.0	487.3c	14.5	10.9c	1.8	8.3cd	1.4	30.2c
100% NPK	3.8c	657.0c	210.1e	5.5	34.6c	0.9	511.0d	13.3	14.5d	2.2	10.0d	1.5	56.4d
100% NPK + FYM	4.5a	778.0a	228.0a	5.1	49.2a	1.1	534.0a	11.9	16.6a	2.1	11.7a	1.5	76.2a
$CD \ (p = 0.05)$	0.9	9.4	8.7	I	5.4	I	53.3	I	1.1	I	2.1	I	5.0



Fig. 2 Relationships between (**a**) soil microbial biomass N and soil microbial biomass C, and (**b**) soil microbial biomass N and the apparent C/N ratio of soil microbial biomass

Equation 2a y = 9.845x + 68.223 $R^2 = 0.926^{**}$ Equation 2b $y = 23.14e^{-0.0311x}$ $R^2 = 0.800^{**}$

The turnover rate was the highest in fallow plot (48.3 years), which received no fertilizer addition over time, and it decreased with fertilization and manuring. The lowest turnover rate was found in 100% NPK + FYM treated plots (9.5 years) where the highest C input/SMBC ratio (2.06) was recorded due to increased level of microbial activity.

Discussion

Within a column, treatment means followed by lowercase letters are significantly different at $p \leq 0.05$ by Duncan's multiple range test

Yield trends and soil organic carbon

Grain yield of both maize and wheat increased markedly with time in fertilized plots and also in the control (unfertilized). Maize grain yield production was the highest where FYM at 10 t ha^{-1} was applied along with recommended NPK. The residual effect of FYM was also observed on wheat grain yield. The



Fig. 3 Carbon mineralization as affected by application of organic manure and inorganic fertilizers in surface soil

Outside:
$$y = -0.020 + 0.071x$$
 ($R^2 = 0.987^{**}$)
Control: $y = 0.0750 + 0.078x$ ($R^2 = 0.967^{**}$)
100% N: $y = 0.989 + 0.090x$ ($R^2 = 0.946^{**}$)
100% NP: $y = 1.040 + 0.1089x$ ($R^2 = 0.968^{**}$)
100% NPK: $y = 1.611 + 0.121x$ ($R^2 = 0.979^{**}$)
100% NPK + FYM: $y = 2.14 + 0.137x$ ($R^2 = 0.954^{**}$)

results clearly demonstrated the improvement in grain yield of both maize and wheat due to the increased OC content of soil with time. Grain yield of both maize and wheat correlated significantly with OC content of FYM fertilized plots. The R^2 value was of higher magnitude (0.674) in case of maize as compared to wheat ($R^2 = 0.453$), as presented in Fig. 4a, b.

Organic carbon content of all the fertilizer treatments increased as compared to their initial values. The substantial build up in SOC was due to added source of carbon through FYM and addition of root biomass and crop residues for 34 years. Bhriguvanshi (1998) also reported that organic carbon status of soil was significantly improved by the application of FYM alone or in conjunction with nitrogenous fertilizers. Build up of organic carbon is more in surface layers as compared to lower layers, due to more addition of roots and plant biomass in surface layers and lack of nutrient and biological activity in deeper soil layers, which ultimately constrains the rooting depth (Ingram and Fernandes 2001; Tiwari et al. 1995; Sharma et al. 1992).

Slow pools of soil organic carbon

The results revealed that soil aggregates organic carbon increased with decrease in size, and its content was the highest in aggregate size range 0.5-0.25 mm at all the soil depths under permanent cropping and manuring programme for the last 34 years. On the destruction of soil aggregates, much of the SOC is redistributed in finer soil particle fractions associated primarily with silt and clay fractions. This clay fraction provides protection for SOM against microbial and enzymatic degradation (Leinweber and Reuter 1992; Singh and Singh 1996). Carbon content in various particle soil fraction was higher in NPK + FYM followed by NPK, NP, N and control. The low content of WSAC in N treated plots corroborates the deleterious effects of nitrogenous fertilizers on soil quality in the present study.

Active pools of carbon and nitrogen

Cropped soils support significantly higher microbial populations than fallow indicating a strong

Table 4 Initial value, steady-state value, overall rate of loss, rate of addition, period of turnover, soil biological activity in long-termfertilizer and manuring under maize-wheat cropping system

Treatments	Initial soil C MgC ha ⁻¹	Steady state C	Net change in SOC	SMBC	C loss rate MgC ha ⁻¹ year ⁻¹ /MgC ha ⁻¹	C addition rate MgC ha ⁻¹ year ⁻¹	Turnover rate years	C input/SMBC ratio
Fallow	_	5.8	_	0.31	0.02	0.12	48.3	0.40
Control	5.8	6.8	+1.0	0.31	0.05	0.32	21.2	1.05
100% N	4.0	7.5	+3.5	0.37	0.04	0.34	22.0	0.91
100% NP	3.8	7.5	+3.7	0.42	0.06	0.46	16.3	1.11
100% NPK	4.7	8.6	+3.9	0.47	0.08	0.73	11.7	1.56
100% NPK + FYM	4.5	9.9	+5.4	0.57	0.10	1.05	9.5	2.06



Fig. 4 Relationship between (a) organic carbon content of soil and maize yield and (b) organic carbon content of soil and wheat yield over the years

Equation 4a

$y = 1387.4x^3 - 1063.2x^2 + 235.21x + 19.356$	$R^2 = 0.674^{**}$
Equation 4b	
$y = 1948.4x^3 - 2378.7x^2 + 938.35x - 67.039$	$R^2 = 0.453^{**}$

rhizosphere effect promoted by the presence of roots. The supply and availability of additional mineralizable and readily hydrolysable carbon due to manure application resulted in higher microbial activity and microbial biomass. Higher SMBC was observed with integrated use of FYM and inorganic fertilizers due to addition of fresh organic matter every year (Collins et al. 1992). Greater organic carbon as SMBC has been suggested as an indicator of an enlarging pool of organic matter (Powlson et al. 1987).

Application of N alone over the years had shown significant decline in SMBC content over the rest of the fertilized treatments. The decrease in microbial biomass could be due to the acidifying effect of N fertilizers applied in the form of urea alone, which probably resulted in the appearance of unfavorable conditions to many types of microorganisms, i.e., bacteria and actinomycetes. Negative effect of acid-ification on soil microbial biomass has also been observed by Kowalenko (1978) and Vance et al. (1987).

High soil carbon content, more root proliferation and additional supply of N by FYM to microorganisms is responsible for increasing the level of SMBN in organically amended plots. The apparent C/N ratio of soil microbial biomass increased in a nonlinear manner with decreasing SMBN. Unusually large C/N ratios of soil microbial biomass at low levels of SMBN may be indicative of (1) a biophysical response to decreased N availability or slower decomposition in relatively undisturbed soil horizons or more likely, and (2) a result of re-immobilization of N during the incubation period following fumigation. It has been suggested that fungi are capable of re-immobilizing N from less active to more active tissues to maintain growth (Cowling and Merrill 1966) such that the C/N ratio of less active tissue increases.

The water-soluble fractions (WSC and WSN) are considered the most active component of SOM. Although they comprise a small fraction of SOM, they act as buffering agents in replenishment mechanisms like desorption from soil colloids, dissolution from litter, and exudation from plant roots (Six et al. 2000). They appear to be an immediate substrate for the soil organisms. The WSC content increased with increasing level of fertilizers. The results are in agreement with Yagi et al. (2005), who attributed the same to the priming effect of application of inorganic N or fresh organic material to the soil, which stimulates the microbial activity and mineralization of N present in SOM helping decomposition of SOM with rapid release of the WSC fraction. Soil carbohydrates were in proportion to organic matter content. Contribution of water soluble carbohydrates in inorganic fertilizer treatment was less as compared to organic fertilizers because above ground biomass did not return to the soil in any form.

Carbon mineralization in soil

Mineralizable C can provide an assessment of soil organic matter changes induced by tillage or other management practices (Carter and Rennie 1982). The regular addition of manure-enhanced water-soluble fraction of carbon acted as an important source of bioenergy as compared to inorganic alone. The differences in the rate of C mineralized are indicative of variable amounts of labile organic C accumulated in response to crop rotation (Campbell et al. 1992). Therefore, balanced plant nutrition (fertilizer in combination with manure) every year may contribute more labile C substrates to sustain the mineralization process (Curtin and Wen 1999).

Carbon balance and its turnover rate

The increase in C sequestration was due to an improved physico-chemical and biological environment suitable for crop growth resulting in more addition of root biomass carbon to the soil with passage of time. Increased levels of long-term stabilized humic material in organically amended plots and high content of soil carbohydrates in fertilized and FYM treated plots have played a crucial role in building SOC stock over the years. This corroborated the results of Kruell et al. (2004).

Although the rate of decomposition was higher in NPK + FYM than inorganic fertilizerd plots, the higher value of C input was because of relatively greater amounts of organic inputs in the system. Furthermore, many studies reported that materials with higher lignin content (FYM) result in a greater accumulation per unit of C input than that of low-lignin residue amendments (Paustian et al. 1992; Stevenson 1982).

Conclusions

Long-term use of organic and inorganic fertilizers significantly affected the depth distribution of SOC and distribution of SMBC, SMBN and mineralizable C. Application of balanced fertilizer NPK either alone or in combination with FYM maintained active and slow pools of C and N in the surface soil layer (0–15 cm depth). This indicated that organic pools of C-associated nutrients, particularly N, may be maintained in the rhizosphere zone and thereby sustaining soil quality and productivity. Improvement of WSC and carbohydrates helped in improving soil nutrient dynamics and transformation through biological means. The pronounced effect of integrated plant and nutrient supply system on the distribution of organic matter among slow pools is an indicator of greater impact on soil fertility improvement. More intensive crop management systems that maintained residue cover provided the greatest benefit towards increasing the quantity of mineralizable nutrients within the active fraction of SOM, as well as increasing C sequestration as SOM. Moreover, increase in grain yields of both maize and wheat crops over the years was directly due to improvement in soil organic matter and its different fractions. Thus, it is clear from this study that a more efficient and integrated nutrient supply strategy is necessary to sustain long-term productivity and soil quality.

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