Published online March 12, 2007

Organic Amendments Affect Soil Parameters in Two Long-Term Rice-Wheat Experiments

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The impacts of continuous applications of an organic manure (farmyard manure [FYM], green manure [GM], and wheat straw [WS]) combined with inorganic fertilizers (N, P, and K) on soil parameters and productivity of rice (*Oryza sativa* L.)–wheat (*Triticum aestivum* L.) systems were investigated in two long-term experiments under conventional tillage in Ludhiana, India, and Bhairahawa, Nepal. Changes in total and labile soil C and N, and microbiological parameters relative to unfertilized and inorganically fertilized controls were measured. Organic amendments had positive but variable effects. In Ludhiana, FYM application increased total C and N, permanganate-oxidizable C, and hot-water-extractable C (HWEC) by 40 to 70% relative to the control after 20 yr and maintained HWEC and total N with time. In the other treatments, HWEC and total N showed declining trends with time, whereas total C increased by 17% on average. In Bhairahawa, although total organic C and N increased with organic amendments after 15 yr, HWEC did not. Increases in C and N, respectively, as fractions of the applied organic fertilizers were 11 to 23 and 37 to 39% from FYM, 4 to 21 and 19 to 41% from GM, and 3 and 24% from WS. The FYM improved available P, cation exchange capacity, potential mineralizable N, and dehydrogenase activity, but microbial biomass C, basal respiration, flush of CO_2 after rewetting dried soil, and metabolic quotient remained unchanged. The current practice of inorganic fertilization alone cannot maintain the soil quality needed to sustain crop productivity. Amounts of organic manures to supplement inorganic fertilizers must be optimized to increase C and N accumulations in the soil without negative effects on crop yield.

Abbreviations: FYM, farmyard manure; GM, green manure; HWEC, hot-water-extractable carbon; LTE, long-term experiment; MBC, microbial biomass carbon; MnOC, permanganate-oxidizable carbon; PMN, potentially mineralizable nitrogen; qCO_2, metabolic quotient; SOM, soil organic matter; WS, wheat straw.

Developing and implementing management strategies that maintain the quality of soil are essential to enhance the performance and sustainability of an agroecosystem. Carbon is the key attribute of soil quality (Christensen and Johnston, 1997; Carter, 2002) because it influences nutrient cycling, soil structure, water availability, and other important soil properties (Doran et al., 1998; Arshad and Coen, 1992; Yakovchenko et al., 1998). Increasing soil organic matter has a significant impact on the mineralization and recycling of C and N (Sanchez et al., 2004).

Soil Sci. Soc. Am. J. 71:442–452
doi:10.2136/sssaj2006.0141
Received 7 Apr. 2006.
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Soil C may be divided into an inert fraction that has no considerable share in soil transformation processes and a labile fraction that has an active part in soil transformation processes and can be influenced by management systems (Schulz and Körschens, 1998). Among the labile pools that are highly responsive to changes in soil management and the environment are microbial biomass C (MBC) and HWEC. The chloroform–fumigation–incubation and chloroform-extraction techniques for measuring MBC are widely used (Jenkinson et al., 2004). On the other hand, Schulz and Körschens (1998) have shown that HWEC is a reliable parameter for estimating the labile part of soil organic matter (SOM). The chemical composition of the HWEC is still not well defined but it contains large parts of soil microbial biomass and simple organic compounds that dehydrolyze or depolymerize under the given extraction conditions and it represents a very easily decomposable part of the active SOM (Schulz and Körschens, 1998).

A flush of CO_2 following rewetting of dried soil under laboratory conditions has also been reported to relate to the active organic matter fraction (Franzluebbers et al., 2000). It was shown that the flush of CO_2 during the first day following rewetting of dried soil was related to both soil MBC and potentially mineralizable C and N (Franzluebbers et al., 1996).

In terms of soil quality assessments, the metabolic quotient, or qCO_2, which is calculated from basal soil respiration (CO_2–C h^{-1}) per unit microbial biomass C may provide a method to explain both the size and activity of the microbial biomass. Anderson and Domsch (1985, 1990) found qCO_2 to be a sensitive indicator of soil microbial reactions to cropping systems (monoculture vs. crop rotation) and temperature regimes. Soils under crop rotation showed a lower qCO_2, suggesting the presence of a C-use-efficient microbiota.

Enzyme activity assays (dehydrogenase, amidohydrolases, glycosidases, phosphatases, and arylsulfatase) have been done to determine a soil's potential in carrying out important processes in SOM degradation and nutrient cycling. Specifically, dehydrogenases are involved in the oxidation of SOM. Enzyme activities have also been shown to be sensitive discriminators of soil management effects (Dick, 1994).

Several researchers have conducted long-term cropping system experiments (LTE) in Asia to evaluate various agronomic and cultural practices. Mostly assessments were made based on crop productivity trends, and sometimes changes in soil total C and various nutrients (e.g., Nambiar, 1994; Yadav et al., 1998, 2000; Dawe et al., 2000; Duxbury et al., 2000; Tsuchiya et al., 2000; Bhandari et al., 2002; Regmi et al., 2002). It is equally important, however, to study the changes in the readily decomposable C and N fractions (Schulz and Körschens, 1998) and soil microbiological properties in response to agronomic interventions. Two LTE with 15- to 20-yr rice–wheat rotation in India and Nepal, having various combinations of inorganic and organic sources of nutrients, were used to extend earlier research (Bhandari et al., 2002; Regmi et al., 2002). These two LTE also provided an opportunity to analyze archived soils that were sampled periodically over the years.

In this study, we assessed the (i) cumulative effects of selected combinations of inorganic and organic sources on various chemical and microbiological soil properties with emphasis on labile C and N fractions, and (ii) time trends of a few key soil parameters associated with C and N dynamics.

MATERIALS AND METHODS

Long-Term Experiments

Two ongoing long-term rice–wheat experiments in Ludhiana, India (Bhandari et al., 2002), and Bhairahawa, Nepal (Regmi et al., 2002), were used for this study. The Ludhiana experiment began in June 1983 on a Typic Ustochrept (Tolewal loamy sand) at the experimental farm of the Punjab Agricultural University. Under average climatic conditions, the area receives 800 mm of annual rainfall, about 80% of which occurs from June to September. The mean maximum and minimum temperatures during rice cultivation (July–October) were 35 and 18°C, whereas during wheat cultivation (November–April), the temperatures were 22.6 and 6.7°C, respectively. The soils are well drained, with the groundwater table at 6.6 and 10 m deep during the rainy and summer seasons, respectively. Before the experiment, the field had been under maize (*Zea mays* L.)–wheat cropping for several years.

The experiment included two crops per year, rice (July–October) and wheat (November–April), with 11 treatments. The rice cultivar used from Years 1 to 9 was PR106, and PR110 from Year 10 onward; wheat cultivars used were PBW 12 for Years 1 to 3, PBW 120 for Years 4 to 6, PBW 154 for Years 7 to 12, and WH 542 from Year 13 onward. The treatments represented different combinations of inorganic and organic sources of nutrients to rice and wheat. In rice, the full recommended levels of N, P, and K (120:26:25) were supplemented with N through FYM, WS, and sesbania (*Sesbania cannabina* Linn. & Merrill), a leguminous green manure (GM) so that the 100% recommended N dose was available to the rice crop. The wheat did not receive an organic amendment but received N–P–K fertilizer (120:26:25). Of the 11 treatments in Ludhiana, five were selected for the present study (Table 1).

Crop residue, if any, of the previous crop was removed from the field in April every year. During June and July, the land was plowed, puddled, and leveled. Two rice seedlings (5 wk old) were transplanted in the puddled lowland field at 20- by 15-cm spacing. All the plots were normally flooded (2–4 cm) until 2 wk before rice harvest. Sesbania was grown in situ for 6 wk before the rice crop in the GM treatment. An appropriate amount of aboveground biomass of sesbania was chopped into 5- to 10-cm pieces, uniformly spread into the plots following flooding of the field, and incorporated into the soil with an offset disk harrow while the soil was being puddled for transplanting of rice. Sesbania was incorporated into the soil a day before transplanting; however, FYM and WS were incorporated into the moist soil 2 wk before transplanting of rice.

Wheat (100 kg seed ha^{-1}) was sown in rows 22.5 cm apart in November every year. Before seeding, the land was plowed two times to about a 20-cm depth with a cattle (*Bos taurus*)-drawn plow. After seeding, a plank was dragged over the field to cover the seed. In wheat, three to four irrigations were applied at sowing, crown root initiation, maximum tillering, and flowering. All P and K and a half dose of N were drilled at sowing. The remaining N was topdressed 21 d after sowing with irrigation. Once in every 3 yr, $ZnSO_4$ (60 kg ha^{-1}) was applied to all plots before the rice crop. Irrigation water was applied from a deep tube well and also by canal. Weeds, pests, and diseases were controlled as needed.

The Bhairahawa experiment began in June 1988 on a Typic Haplaquept (calcareous) at the Agricultural Research Station, Bhairhawa, Nepal. The area has a subtropical climate highly influenced by the southwestern monsoon. The average annual rainfall is around 1687 mm. More than 85% of the rainfall occurs from mid-June to the end of September. November and December are the driest months and light precipitation may be expected in January and February. Mean monthly temperature is lowest (8.5°C) in January and highest (36.2°C) in May. The source of irrigation water is the groundwater pumped from a deep (200-m) tube well.

The experiment also included two crops per year: rice (July–October) and wheat (November–March). There were six nutrient treatments comprising different combinations of N, P, FYM, and GM grown in situ (Table 1). All the P as $(NH_4)_2HPO_4$ and K as KCl were applied as basal treatments. Nitrogen was applied in two splits, 50% at transplanting or sowing as $(NH_4)_2HPO_4$ + urea and the remaining 50% topdressed as urea at 30 to 35 d after transplanting rice and at 21 to 25 d after sowing wheat. The FYM consisting mainly of cattle-shed wastes and cattle dung was applied manually just before sowing of wheat and incorporated using a Chinese two-wheel tractor. Sesbania was grown in situ for 8 wk before rice and incorporated into the soil with an offset disk harrow 1 to 7 d before rice transplanting.

Aboveground crop residue, if any, of the previous crop was removed from the field in April and October every year before planting rice and wheat, respectively. The land was plowed, puddled, and leveled and two rice seedlings (30 d old) were transplanted in the puddled lowland field at 20- by 20-cm spacing from 9 June to 19 July every year. The cultivars of rice used were Janaki from Years 1 to 5 and Sabitri from Year 6 onward. All the plots were flooded (2–4 cm) until 2 wk before the rice harvest. Wheat (120 kg seed ha^{-1}) was sown in rows 20 cm apart from 23 November to 29 December. The wheat cultivar used was NL 297. Three irrigations were applied: at crown root initiation, maximum tillering, and flowering stages of wheat. Hand weeding was done to manage the weeds and plant protection measures were applied to both crops, as needed, to control pests.

Table 1. Selected treatments from the Ludhiana and Bhairahawa long-term experiments.

Treatment†	Rice N I‡	Rice N O§	Rice P I	Rice P O	Rice K I	Rice K O	Wheat N I	Wheat N O	Wheat P I	Wheat P O	Wheat K I	Wheat K O	Rice+Wheat N (I + O)	Rice+Wheat P (I + O)	Rice+Wheat K (I + O)
	kg ha^{-1}														
Ludhiana															
Unfertilized control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100% N–P–K in R & W (NPK) ¶	120	0	26	0	25	0	120	0	26	0	25	0	240	52	50
50% N–P–K + 6 Mg ha^{-1} FYM in R, 100% N–P–K in W (FYM+NPK)	60	60	13	18	13	30	120	0	26	0	25	0	240	57	68
75% N + 50% P–K + 6 Mg ha^{-1} WS in R, 100% N–P–K in W (WS+NPK)	90	30	13	6	13	18	120	0	26	0	25	0	240	45	56
50% N–P–K + 50% N with GM in R, 100% N–P–K in W (GM+NPK)	60	60	13	6	13	54	120	0	26	0	25	0	240	45	92
Bhairahawa															
Inorganic N in R & W (N)	100	0	0	0	0	0	100	0	0	0	0	0	200	0	0
Inorganic N and P in R&W (NP)	100	0	26	0	0	0	100	0	26	0	0	0	200	52	0
Inorganic N in R & W and GM in R (GM+N)	100	33	0	3.3	0	30	100	0	0	0	0	0	239	3.3	30
Inorganic N and P in R & W and GM in R (GM+NP)	100	60	26	6	0	54	100	0	26	0	0	0	260	58	54
Inorganic N in R & W and FYM in W (FYM+N)	100	0	0	0	0	0	100	80	0	20	0	40	280	20	40
Inorganic N and P in R & W and FYM in W (FYM+NP)	100	0	26	0	0	0	100	80	26	20	0	40	280	72	40

† R = rice, W = wheat, FYM = farmyard manure, WS = wheat cropped straw, GM = green manure (Sesbania aculeata).

‡ I = inorganic nutrient.

§ O = organic nutrient.

¶ 100% recommended N, P, and K for rice and wheat were 120, 26.2, and 25 kg ha^{-1}, respectively. Beginning in 1992, application of P was reduced to 13.1 kg ha^{-1} in rice.

Biochemical and Physical Analyses of Air-Dried Soils

Air-dried soils collected after the rice harvest in 2003, 20 and 15 yr after continuous applications of organic and inorganic fertilizers in Ludhiana and Bhairahawa, respectively, were analyzed for particle size, pH, cation exchange capacity, available P and K, HWEC, MnOC, total C and N, and dehydrogenase activity. The HWEC, MnOC, total C and N, and dehydrogenase activity were also analyzed in archived soil samples collected after the rice harvest during earlier years (starting from the 5th yr in Ludhiana and 3rd yr in Bhairahawa) to determine time trends.

Particle size was analyzed by the pipette method and pH was measured in a 1:1 soil/water suspension. Total C and N were determined by automated combustion using a PerkinElmer 2400 Elemental CHN analyzer (PerkinElmer Corp., Norwalk, CT; Jimenez and Ladha, 1993). Organic C was measured by adding one to two drops of 15% HCl to a 60-mg soil sample in a silver capsule to convert carbonates to CO_2. The sample in the silver capsule was then dried in an oven at 80°C for about 2 h. The sample was sealed in the silver capsule and analyzed for C using the PerkinElmer 2400 CHN analyzer. Available P and K were analyzed by the methods described in Olsen et al. (1954) and Knudsen et al. (1982), respectively. Cation exchange capacity was measured using NH_4OAc at pH 7.

To obtain HWEC, 100 mL of deionized–distilled water was added to 20 g of dry soil and the mixture was boiled under reflux for 1 h. The mixture was centrifuged at 2600 × *g* for 10 min to obtain a clear centrifugate, after which the water extract was analyzed directly for total and organic C using a 1020A combustion total organic C analyzer (Oceanography Int. Corp., College Station, TX).

Permanganate-oxidizable C was determined by shaking soil samples with 33 m*M* $KMnO_4$ for 6 h following the procedure described in Tirol-Padre and Ladha (2004), while dehydrogenase activity was determined using 6 g of air-dried soil following the method of Tabatabai (1982). All soil samples were analyzed in triplicate.

Soil Incubation Experiments

Fifteen-gram portions of air-dried soil sampled in 2003 from each of the different treatments were incubated under aerobic and flooded conditions. For aerobic incubation, soils were placed in 4-cm-diameter, 60-mL screw-capped bottles, and water was added to 50% water-filled pore space (Franzluebbers et al., 2000). For flooded conditions, soils were placed in 2.8-cm-diameter, 60-mL tubes and then flooded with 15 mL of distilled water. Trapped air was removed by lightly tapping the tubes. Soils were incubated at 28°C for 31 d.

To measure the flush of CO_2, a microcentrifuge tube containing 0.5 mL of 5 *M* NaOH was placed inside each bottle with aerobic soil, which was then tightly capped. For the flooded soils, 0.5 mL of 5 *M* NaOH was placed inside a closed glass tube with a hole bored in the side and hanging from the center of a rubber stopper covering the 60-mL tube with the flooded soil. After 3 d, the NaOH was transferred into a partially evacuated tube covered with a serum cap using a 1-mL syringe and acidified with excess HCl to release the trapped CO_2. The CO_2 evolved was then analyzed using a Hitachi gas chromatograph with a thermal conductivity detector. The column was packed with Porapak QS maintained at 50°C. Helium was used as the carrier gas. A standard 10% CO_2 gas in He was used for calibration.

After 30 d of soil incubation, NaOH was again placed inside the soil containers to measure basal soil respiration for 24 h. The same procedure as above was followed for measuring the CO_2 evolved.

After 31 d, MBC was measured in the aerobic and flooded soils by the fumigation–extraction method following the procedure used by Inubushi et al. (1991), except that the organic C in the fumigated and unfumigated K_2SO_4 extracts was measured using the 1020A combustion total organic C analyzer.

Potential mineralizable N (NH_4^+ + NO_3^-) was determined 31 d after soil incubation from the K_2SO_4 soil extract after subtracting available N from the initial soil sample. Ammonium-N was determined colorimetrically by the salicylate method (Kempers and Zweers, 1986) and NO_3–N by the copperized Cd reduction method (Page et al., 1982).

Statistical Analyses

A single-factor ANOVA for a randomized complete block design with three replications was done for Ludhiana data using SigmaStat for Windows, Version 2.0 (Systat Software, San Jose, CA). A two-factor (organic fertilizer and P) ANOVA was done for Bhairahawa data using Proc GLM in SAS 9.1 for Windows (SAS Institute, Cary, NC). When the organic fertilizer × P interaction was significant, organic fertilizer × P means were compared; otherwise, only the organic fertilizer means or P means were compared using Tukey's test. The ANOVA among treatments was done within a year (Tables 2 and 3) or across years for some soil parameters (total C, HWEC, MnOC, PMN, and dehydrogenase activity). When treatment × year interaction was not significant at the 0.05 level, a comparison of year means across all treatments or treatment means across all years was done (total C and HWEC in Bhairahawa, and PMN in Ludhiana). On the other hand, when the treatment × year interaction was significant at the 0.05 level, mean comparison across years was done for each treatment or mean comparison among treatments was done for each year (HWEC and dehydrogenase activity). Tukey's test was used for mean comparison and differences were considered significant at $P \leq 0.05$. Linear or quadratic regression analysis (whichever showed the best fit) was done using Sigmaplot Version 8 for Windows (Systat Software) to show the time trends and relationships among soil parameters. Regression lines

Table 2. Effects of organic and inorganic fertilization on soil properties† measured from air-dried soil.

Treatment‡	pH	CEC	Olsen P	Total C	Organic C	MnOC	HWEC	Total N	Min N	DA
	1:1 (H_2O)	cmol kg^{-1}	mg kg^{-1}	g kg^{-1}					mg kg^{-1}	mg TPF kg^{-1} soil
					Ludhiana					
Control	7.4 a§	5.8	4 b	4.53 b	4.53 b	0.73 b	0.18 b	0.52 b	8.07 b	46.5 b
NPK	7.0 ab	6.3	9 b	4.78 ab	4.78 ab	0.81 b	0.17 b	0.54 b	14.15 b	39.8 b
FYM+NPK	7.1 ab	6.8	52 a	6.48 a	6.00 a	1.24 a	0.25 a	0.73 a	32.85 a	69.7 a
WS+NPK	6.8 ab	6.0	7 b	5.37 ab	5.28 ab	0.94 b	0.21 ab	0.59 ab	13.84 b	50.2 b
GM+NPK	6.5 b	6.4	11 b	5.37 ab	4.94 ab	0.94 b	0.22 ab	0.62 ab	23.14 ab	45.7 b
					Bhairahawa					
Organic fertilizer · P means										
N	8.0	7.7 b	2	8.44 bc	7.44 b	1.34	0.29	0.89 bc	10.08	95.8
GM+N	8.0	7.5 b	2	7.88 c	6.76 c	1.35	0.27	0.82 c	12.10	83.1
FYM+N	7.9	8.5 a	5	9.68 ab	9.41 a	1.68	0.31	1.00 ab	13.25	115.2
NP	7.8	8.1 ab	21	9.08 abc	8.03 b	1.38	0.28	0.96	10.95	153.6
GM+NP	7.8	8.7 a	18	10.21 a	9.35 a	1.60	0.33	1.07 a	11.00	168.2
FYM+NP	7.8	8.6 a	28	10.33 a	9.80 a	1.90	0.30	1.11 a	12.39	176.4
Organic fertilizer means										
N/NP	7.9	7.9 b	12 b	8.76	7.73	1.36 b	0.29	0.93	10.52	124.7
GM	7.9	8.1 b	10 b	9.05	8.06	1.48 ab	0.30	0.94	11.56	125.7
FYM	7.8	8.6 a	16 a	10.00	9.61	1.79 a	0.31	1.05	16.48	145.8
P means										
−P	8.0 a	7.9 b	3 b	8.67	7.87	1.46 a	0.33	0.90	14.25	98.0 b
+P	7.8 b	8.5 a	22 a	9.87	9.06	1.62 a	0.33	1.04	11.45	166.1 a

† CEC, cation exchange capacity; MnOC, permanganate-oxidizable C; HWEC, hot-water-extractable C; TPF, triphenyl formazan; DA, dehydrogenase activity.

‡ NPK, inorganic N, P, and K fertilizer; GM, green manure; FYM, farmyard manure; WS, wheat straw.

§ Comparison of means is given where *F* values from analysis of variance are significant. In a column, means followed by a common letter are not significantly different from each other at the 0.05 level by Tukey's test.

were shown when the regression coefficient was significant at $P \leq 0.05$ except in HWEC in Bhairahawa, where only the regression trends for the FYM and NPK treatments are shown to emphasize the difference between the FYM and NPK treatments, as the other treatments followed trends similar to the NPK treatment. Regression analysis was not done for Bhairahawa, as only four data points were available for each treatment.

RESULTS

Chemical and Microbiological Soil Properties

The soil texture remained unchanged with long-term treatments including organic amendments in the two soils (data not shown). Soil pH did not change with various organic and nutrient amendments at either site, except with GM+NPK, in which soil pH decreased by 0.3 to 0.9 units compared with the other treatments at Ludhiana. At Bhairahawa, soil pH increased by 0.2 units on average with the addition of P but it was not affected by organic treatments (Table 2). Cation exchange capacity remained unchanged at Ludhiana, but at Bhairahawa, FYM increased cation exchange capacity 0.5 to 0.7 cmol kg^{-1} compared with inorganic N and GM treatments. The addition of P increased cation exchange capacity by 0.6 cmol kg^{-1}, while FYM amendments increased Olsen soil P at Ludhiana by 477% (Table 2). At Bhairahawa, the averages of treatments with and without P showed a significant P increase of 33% due to FYM (Table 2). Results show that there was a greater accumulation of P at Ludhiana than at Bhairahawa, although more P (inorganic and organic) was being added at Bhairahawa than at Ludhiana. It should be noted that, at Ludhiana, FYM was added to rice, while at Bhairahawa it was added to wheat. Available K, which was <30 mg kg^{-1} at both sites, remained unchanged (data not shown).

At Ludhiana, FYM amendment compared with the control increased total organic C and MnOC fractions by 32 and 70%, respectively, but the GM and WS did not change significantly (Table 2). At Bhairahawa, total organic C with GM+NP and FYM+NP were 26 to 32 and 16 to 24% higher than those with only N and NP, respectively, while that with GM+N was even lower by 9% than with N only (Table 2). The low soil C without P was due to the incorporation of a suboptimal amount of GM biomass compared with that in the GM+NP treatment, where P addition enhanced biomass production.

The soil MnOC fraction was higher in the FYM+NPK treatment than in any of the other treatments at Ludhiana. Farmyard manure was earlier reported to contain higher MnOC and lignin content than GM (Tirol-Padre and Ladha, 2004). The HWEC had treatment differences similar to those of total C at Ludhiana but not at Bhairahawa, where no treatment differences were observed (Table 2). At both sites, organic manures did not show a significant positive effect on MBC (Table 3).

Farmyard manure increased total soil N by 40 and 14% at Ludhiana and Bhairahawa, respectively. Similarly, total mineralized N measured from air-dried soil was highest with FYM+NPK at Ludhiana (Table 2), but it did not vary among

Table 3. Effects of organic and inorganic fertilization on soil properties† measured from incubated soil.

Treatment‡	MBC 50% WFPS	MBC flooded	Basal soil respiration 50% WFPS	Basal soil respiration flooded	qCO_2 50% WFPS	qCO_2 flooded	3-d CO_2 50% WFPS	3-d CO_2 flooded	PMN 50% WFPS	PMN flooded
	g C kg^{-1}soil		mg C kg^{-1}soil d^{-1}		mg C kg^{-1} MBC h^{-1}		mg C kg^{-1}soil d^{-1}		mg N kg^{-1} soil	
Ludhiana										
Control	0.22 a§	0.23	13.14	10.09	2.67 ab	4.40	55.98	10.20 ab	17.26 b	2.56 b
NPK	0.22 a	0.31	9.97	10.56	2.01 b	3.27	54.27	8.54 b	16.52 b	2.03 b
FYM+NPK	0.20 ab	0.37	9.18	13.67	1.98 b	3.45	67.81	11.63 ab	25.01 ab	3.85 ab
WS+NPK	0.11 ab	0.27	10.12	12.08	3.98 ab	4.23	65.97	12.93 ab	25.62 ab	6.26 a
GM+NPK	0.10 b	0.35	8.77	17.30	6.11 a	4.67	62.75	17.83 a	29.74 a	5.10 ab
Bhairhawa										
Organic fertilizer • P means										
N	0.34	0.38	15.19	12.02	1.83	1.33	81.33	9.63	31.19	8.31
GM+N	0.33	0.31	14.84	12.08	1.92	1.65	71.67	8.08	27.64	10.19
FYM+N	0.27	0.30	14.34	14.78	2.82	2.20	84.18	9.66	33.74	12.11
NP	0.30	0.37	15.63	15.89	2.20	1.80	74.53	12.39	33.57	10.17
GM+NP	0.29	0.33	14.84	13.66	2.47	1.81	76.43	9.18	34.18	12.32
FYM+NP	0.27	0.28	15.22	13.25	2.64	2.25	89.62	12.99	38.63	14.43
Organic fertilizer means										
N/NP	0.32	0.38	15.34	13.96	2.02	1.56	77.93 ab	11.01	32.38	9.24 b
GM	0.31	0.32	15.34	13.96	2.19	1.73	74.05 b	8.63	30.91	11.26 ab
FYM	0.27	0.29	14.84	12.87	2.73	2.23	86.90 a	11.33	36.19	13.27 a
P means										
−P	0.32	0.33	14.80	13.00	2.20	1.70	79.10	9.10	30.90	10.20 b
+P	0.29	0.33	15.00	14.30	2.40	2.00	80.20	11.50	35.50	12.30 a

† MBC, microbial biomass C; qCO_2, metabolic quotient; PMN, potential mineralizable N; WFPS, water-filled pore space.

‡ NPK, inorganic N, P, and K fertilizer; GM, green manure; FYM, farmyard manure; WS, wheat straw.

§ Comparison of means is given where *F* values from analysis of variance are significant. In a column, means followed by a common letter are not significantly diffrerent from each other at the 0.05 level by Tukey's test.

Table 4. Total amounts of C accumulated in the soil from continuous application of organic fertilizer.

Treatment†	C content of organic fertilizer	Rate of application of organic fertilizer	C/N (molar ratio)	Total C applied per year	Years applied	Total C applied	Increase in organic C relative to the control‡	C accumulation as a fraction of total organic C applied
	g kg^{-1}	Mg ha^{-1}		kg ha^{-1}	no.	—— kg ha^{-1} ——		%
				Ludhiana, India				
GM+NPK	376	2.86	21	1075	20	21507	855	4.0
FYM+NPK	243	6	28	1458	20	29160	3233	11.1
WS+NPK	404	6	94	2424	20	48480	1620	3.3
				Bhairahawa, Nepal				
GM+NP	450	3	26	1287	15	19305	4017	20.8
FYM+NP	365	4	21	1460	15	21900	4956	22.6

† NPK, inorganic N, P, and K fertilizer; GM, green manure; FYM, farmyard manure; WS, wheat straw.

‡ Increase in soil C was measured relative to an unfertilized control at Ludhiana and relative to an N-fertilized control at Bhairahawa.

treatments at Bhairahawa. On the other hand, potentially mineralizable N (PMN) had a variable response. At Ludhiana, it was highest with GM+NPK under aerobic incubation and with WS+NPK under flooding (Table 3). At Bhairahawa, P application increased PMN under both incubations. In addition, compared with the control, PMN with FYM was 44% higher when soil was incubated under flooded conditions (Table 3).

An organic source (FYM) increased dehydrogenase activity measured from air-dried soil at Ludhiana but had no effect at Bhairahawa, although long-term addition of P had a stimulatory effect at the latter site (Table 2). The other treatments at Ludhiana did not show a significant effect in terms of dehydrogenase activity. No differences in basal soil respiration were observed among treatments at either site (Table 3). At Ludhiana, however, compared with NPK, the GM+NPK treatment had a twofold increase in the 3-d flush of CO_2 following rewetting of dried soil. The metabolic quotient (qCO_2 = basal respiration/MBC), which is inversely related to metabolic efficiency, was not different among treatments under flooded conditions. But qCO_2 increased three times in the GM+NPK treatment relative to the NPK and FYM+NPK treatments under aerobic conditions. At Bhairahawa, the 3-d flush of CO_2 after rewetting dried soil was highest with FYM and lowest with GM under aerobic conditions (Table 3). No differences among treatments were observed under flooded conditions.

Accumulations of soil C and N from organic amendments were estimated from the difference between unfertilized and fertilized treatments. At Ludhiana, the average accumulation of C as a fraction of the total organic C applied was three times higher with FYM than with WS and GM (Table 4). Similarly, the accumulation of N as a fraction of the total organic N applied was about two times higher with FYM than with GM and WS (Table 5). At Bhairahawa, increases in C over the N-fertilized and NP-fertilized controls were significant in both the GM+NP and FYM+NP treatments (Table 2) and the estimated C accumulation as a fraction of C applied was similar in the two treatments (Table 4). Although the increase in soil N with FYM+NP over the N treatment was slightly higher than that with GM+NP, the N accumulation as a fraction of the organic N applied was higher with GM+NP due to the lower amount of N applied from GM (Table 5). These estimates, however, do not account for C and N added as root biomass.

Time Trends of Key Soil Parameters as Affected by Inorganic and Organic Amendments

At Ludhiana, ANOVA with time showed a significant effect of year and treatment on total soil C and a significant treatment × year interaction. An earlier study by Bhandari et al. (2002) showed an increasing linear trend of total C only in the FYM+NPK treatment up to the 15th yr. This study shows that total C continued to increase up to the 20th yr with FYM+NPK. Total C in the NPK, WS+NPK, and GM+NPK treatments displayed quadratic trends, which increased until about 15 yr but declined or stabilized thereafter (Fig. 1a). At

Table 5. Total amounts of N accumulated in the soil from continuous application of organic fertilizer.

Treatment†	N content of organic fertilizer	Rate of application	C/N (molar ratio)	Organic N applied per year	Years applied	Total organic N applied	Increase in soil N relative to the control‡	N accumulation as a fraction of organic N applied
	g kg^{-1}	Mg ha^{-1}			no.	——— kg ha^{-1} ———		%
				Ludhiana, India				
GM+NPK	21	2.86	21	60	20	1200	225	18.8
FYM+NPK	10	6	28	60	20	1200	466	38.8
WS+NPK	5	6	94	30	20	600	144	24.0
				Bhairahawa, Nepal				
GM+NP	20	3	26	60	15	900	365	40.6
FYM+NP	20	4	21	80	15	1200	449	37.4

† NPK, inorganic N, P, and K fertilizer; GM, green manure; FYM, farmyard manure; WS, wheat straw.

‡ Increase in soil N was measured relative to an unfertilized control at Ludhiana and relative to an N-fertilized control at Bhairahawa.

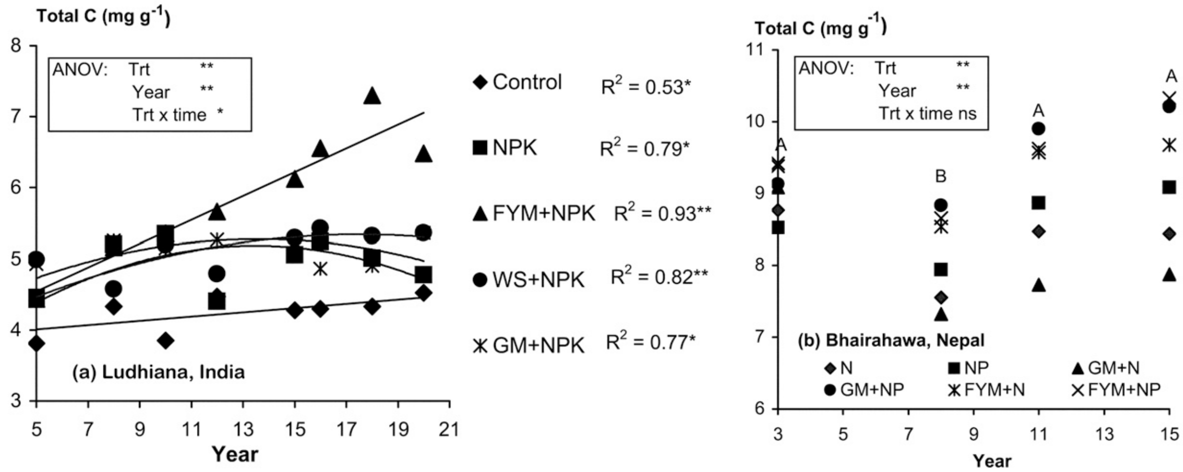

Fig. 1. Changes in soil total C as affected by organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) fertilization at (a) Ludhiana and (b) Bhairahawa. For Bhairahawa, uppercase letters indicate mean comparison among years averaged across treatments while lowercase letters indicate mean comparison among treatments averaged across years. Means with a common letter are not significantly different from each other at the 0.05 level by Tukey's test.

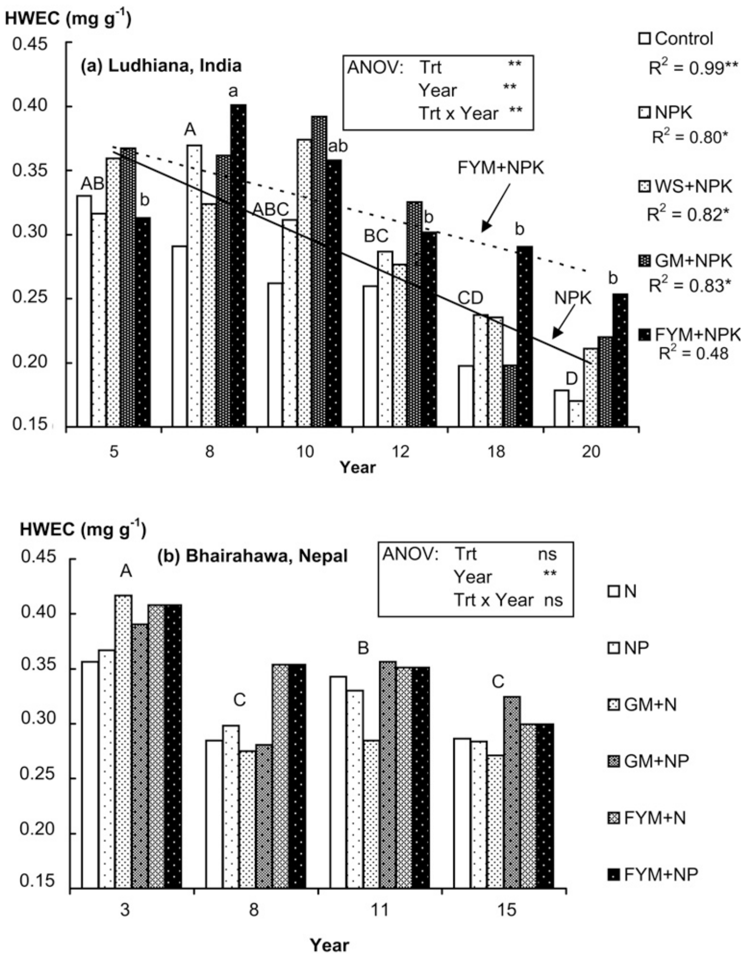

Fig. 2. Changes in hot-water-extractable C (HWEC) as affected by organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) fertilization at (a) Ludhiana and (b) Bhairahawa. In (a), uppercase letters indicate mean comparison among years within inorganic fertilizer (NPK) treatment while lowercase letters indicate mean comparison among years within farmyard manure (FYM) + NPK treatment. Regression lines are for NPK (solid line) and FYM (broken line). In (b), letters indicate mean comparison among years averaged across treatments. Means with a common letter are not significantly different from each other at the 0.05 level by Tukey's test.

Bhairahawa, total soil C decreased from the 3rd to the 8th yr but increased from the 8th to the 11th and 15th year (Fig. 1b).

On the other hand, ANOVA and regression showed that HWEC declined significantly by 46 to 63% from the 5th to the 20th year in all except the FYM treatment (Fig. 2a). At Bhairahawa, ANOVA also showed that HWEC declined by 25% on average from the 5th to the 15th year, but there was no year × treatment interaction (Fig. 2b).

The time trend of the MnOC fraction at Ludhiana was similar to that of total C but opposite to that of HWEC. The MnOC showed an increasing trend up to the 15th year only in the FYM+NPK treatment. (Fig. 3). The observed opposite trends of MnOC and HWEC are understandable, as lignin, which is related to MnOC, would tend to accumulate with organic manure incorporation, whereas HWEC would be readily mineralized.

Earlier reports (15 yr of data) from the LTE at Ludhiana have shown significant declining trends in total soil N in the control, GM, and NPK treatments but not in treatments where FYM was applied (Bhandari et al., 2002). The present analysis (20 yr of data) shows that total soil N has continued to decline in all treatments except the FYM+NPK treatment (Fig. 4a). Soil N decreased by 7 to 22% in all treatments except FYM+NPK. At Bhairahawa, the FYM+NP treatment showed an increasing soil N content, whereas the N and GM+N treatments showed declining trends with time. Due to the limited number of data points, however, only the GM+N treatment gave a significant regression coefficient (Fig. 4b). The PMN from archived soil samples at Ludhiana showed a decrease of 73 to 91% in all treatments from the 5th to the 20th year (Fig. 5). This decline in PMN may be related to the decrease in the labile organic matter, as shown by the high correlation between PMN and HWEC across years (Fig. 6). An accumulation of more stable organic matter over the years may have led to a slower release of mineralizable N.

Dehydrogenase activity from archived soil samples at the Ludhiana LTE showed increasing trends start-

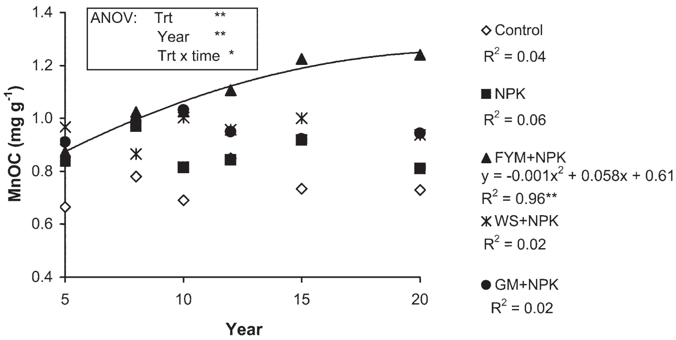

Fig. 3. Changes in permanganate-oxidizable C (MnOC) with time as affected by various organic(green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) amendments (Ludhiana long-term experiment).

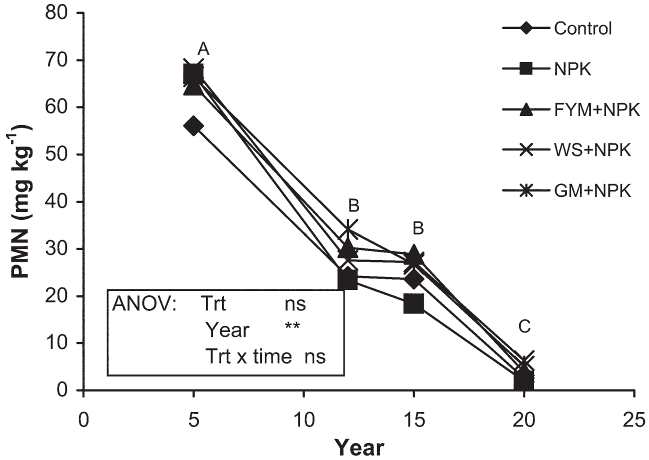

Fig. 5. Changes in potentially mineralizable N (PMN) across years as affected by various organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) amendments. Uppercase letters above the points indicate comparison among year means. Means with a common letter are not significantly different at the 0.05 level by Tukey's test.

ing from the 5th yr in all treatments except for the GM+NPK treatment, which already showed a significantly higher activity than the control in the 5th yr (Fig. 7). The FYM+NPK treatment, however, increased by 488% over the control and showed the highest activity in the 15th yr. Only the FYM+NPK treatments had an increased dehydrogenase activity over the control after 20 yr, which is also an indication of enhanced biological activity in the long term. On the other hand, GM exhibited a more immediate effect on dehydrogenase activity. These results show the accumulation of dehydrogenase even in the control, most probably because of the accumulation of root residues. Dehydrogenase is involved in the oxidation of organic matter in soils. Thus, it was expected that the FYM treatment, which had the highest soil organic C, also exhibited the highest dehydrogenase activity in the long term.

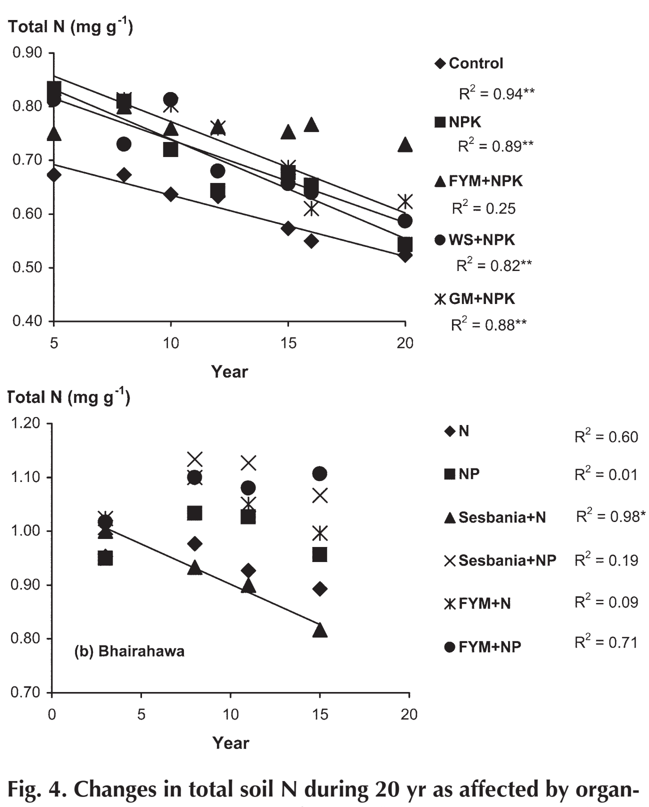

Fig. 4. Changes in total soil N during 20 yr as affected by organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) fertilizer treatments (Ludhiana long-term experiment).

Yield trends were analyzed to extend until the 20th yr in Ludhiana and similar declining trends, as was reported earlier (Bhandari et al., 2002), were observed in all treatments (Fig. 10a). At Bhairahawa, all treatments except the NP and NP+FYM treatments showed declining trends until the 15th yr (Fig. 10b), as reported by Regmi et al. (2002).

DISCUSSION

Yield declines with continuous rice–wheat cultivation in soils with various organic and inorganic amendments have been observed from the two LTE at Ludhiana (Bhandari et al., 2002) and Bhairahawa (Regmi et al., 2002) but these have not been associated with changes in the SOM content since total soil organic C was maintained. This study has shown that soil HWEC or labile C was decreasing with the application of inorganic fertilizer alone or in combination with GM and WS but not with FYM. At Ludhiana, the magnitude of yield

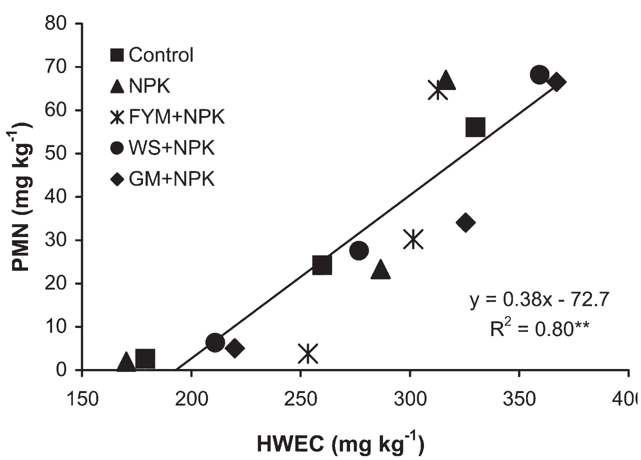

Fig. 6. Relationship between potentially mineralizable N (PMN) and hot-water-extractable C (HWEC) as affected by organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) amendments across years at Ludhiana, India.

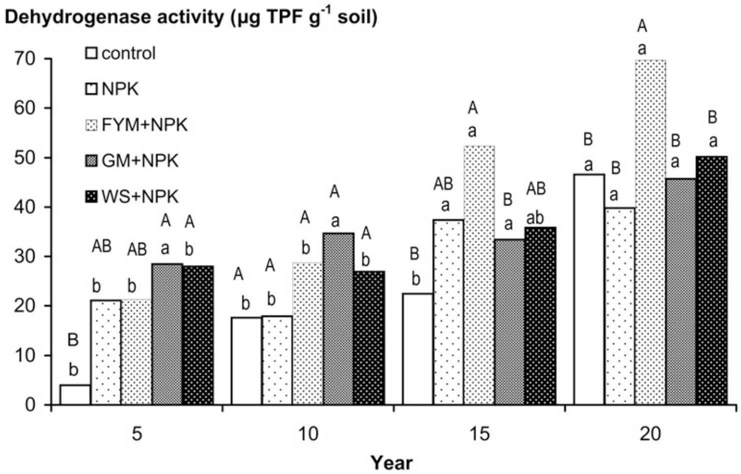

Fig. 7. Changes in soil dehydrogenase activity as affected by long-term organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) treatments in a rice–wheat system at Ludhiana, India. Means within each year with a common uppercase letter and means across years within each treatment with a common lowercase letter are not significantly different at the 0.05 level by Tukey's test. TPF is triphenylformazan.

decline was least with FYM+NPK among the organic matter treatments (Fig. 10a). Moreover, an integrated analysis of yield trends in 19 LTE at various South Asian sites revealed a positive yield trend in the FYM+NPK treatment that was not observed with the NPK treatment alone (Tirol-Padre and Ladha, 2006). Thus, rice grain yields across years at Ludhiana correlated with HWEC but not with total C (Fig. 8). Rice yields were lower with FYM+NPK than with NPK and with GM+NPK for the first 5 yr and this may be related to the faster N mineralization in GM than in FYM. From the 6th yr, however, yields in the NPK, FYM+NPK, and GM+NPK treatments were not significantly different from each other (Bhandari et al., 2002) and only the treatment with FYM showed a significantly higher total C, MnOC, HWEC, and total N in the soil than the unfertilized control during the 20th year. These observations indicate a greater impact of FYM in terms of C and N sequestration than in terms of productivity.

At Bhairahawa, total and organic soil C in 2003 was highest in the FYM+NP treatment, following a trend similar to that of rice grain yield (Fig. 10b). The HWEC and MnOC were not different among treatments in 2003, but rice grain yield correlated with both total C and HWEC across years at Bhairahawa (Fig. 9). At Bhairahawa, where the total organic C was higher than at Ludhiana, rice grain yield was being maintained even in treatments with only inorganic fertilizer. In this LTE, P and not FYM has mitigated the rice yield decline. Regmi et al. (2002) have also suggested that the K in FYM mitigated the yield decline in wheat. A significant decline in HWEC, however, was also observed over the years at Bhairahawa, although there was no significant treatment × year interaction. Continuous monitoring of labile C with and without organic amendments in this LTE would provide valuable information for maintaining the soil fertility of rice–wheat systems in this area. Moreover, although there were no significant differences in HWEC among treatments after 15 yr, total C and MnOC, which may represent the less labile C fraction, were significantly higher in the FYM treatment than in the control.

Aside from showing significant increases in total organic C and MnOC over the inorganically fertilized control, and preventing a decline in HWEC and total N, the FYM treatment significantly increased cation exchange capacity, PMN, and available P over the control at one or both of the LTE. The FYM amendments showed a remarkable increase in Olsen soil P at Ludhiana, as the amount of organic P added from FYM at Ludhiana was greater than the amount of inorganic P added (Table 1). The increase in MnOC in the FYM treatment (Fig. 3) at Ludhiana suggests an accumulation of lignin, which is being oxidized by $KMnO_4$ (Tirol-Padre and Ladha 2004). As manures reached a more advanced state of decomposition, lignin and humic substances that are more resistant to decomposition and less utilized by microorganisms, may have been formed. Consequently, microorganisms may have consumed the more labile C pools more quickly, resulting in declining trends in HWEC and PMN. Matsuzaki (1977) showed that composting cattle manure for 1 or 2 wk reduced the amount of easily decomposable organic matter it contained.

The cumulative effects of organic amendments on MBC and soil respiration were not yet clearly manifested after 15 and 20 yr of continuous cropping at Bhairahawa and Ludhiana. Nevertheless, an enhanced biological activity due to organic amendments based on increases in dehydrogenase activity was observed at Ludhiana. Since air-dried soils were used, these results can be used as indicators of long-term biological activity as measured from the activity of accumulated enzymes complexed in the soil matrix. Freshly collected soils, on the other hand, would show enzyme activity stimulated by recent soil amendments (Tabatabai, 1982). Microbial biomass C is usually measured in freshly collected soil samples when monitoring short-term changes or seasonal changes. In this study, however, MBC was measured from air-dried soil samples after incubation under aerobic and flooded conditions. Although drying and rewetting are expected to change the amount of MBC from field-moist samples, incubation under standardized conditions could provide relative estimates of the potential soil microbial biomass with various organic amendments. The 40-yr rice–wheat LTE

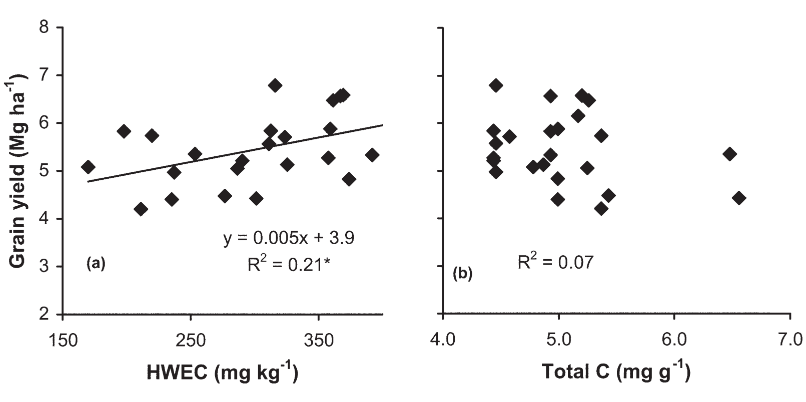

Fig. 8. Relationships of grain yield with hot-water-extractable C (HWEC) and total C across years (Ludhiana, India, long-term experiment).

at Fukuoka, Japan, showed significant increases in MBC measured from incubated soils due to residue treatments (Tirol-Padre et al., 2005), unlike the 20- and 15-yr-old LTE at Ludhiana and Bhairahawa. The amount of organic C incorporated as rice and wheat straw in the Fukuoka LTE was more than twice that at Ludhiana and Bhairahawa.

The difference in texture between the two soils (loamy sand at Ludhiana and silty loam at Bhairahawa) provided an opportunity to assess changes in soil C status in relation to texture, as a major portion of soil organic C is retained through clay–organic matter interactions. Carbon accumulation as a fraction of total organic C applied was greater in the silty loam soil of Bhairahawa than in the sandy loam soil of Ludhiana (Table 4) with a clay content of only about half that at Bhairahawa. Moreover, the accumulation of C and N from applied FYM at Ludhiana was greater than that from GM and WS, although the total amount of C applied from WS and its C/N ratio was much higher than that of the FYM, indicating that the ability of C and N to accumulate in soils does not depend only on the C/N ratio and quantity of organic matter but also on the quality of the organic matter applied (Shiga et al., 1985). Manures are generally more resistant to decomposition than plant residues since they are somewhat partially decomposed. For this reason, animal manures can be considered a slow-release fertilizer source (Horwath, 2005). Further, the abilities of organic materials to supply N, increase soil fertility, and accumulate in soils differ according to their origin and processing.

The high qCO_2 values in the GM treatment relative to the NPK treatment under aerobic condition at Ludhiana may indicate that, during C mineralization of organic matter, microbes divert more C to respiration than to new microbial biomass, causing more C loss. On the other hand, a decline in qCO_2 may indicate the presence of microbial populations that are more efficient in incorporating C compounds into microbial cells, or the availability of less labile organic residues. With an FYM application rate of 200 Mg ha^{-1} yr^{-1}, which is about 40 times that at Ludhiana and Bhairahawa, a 35% accumulation of the C applied was estimated in European LTE (Körschens et al., 1998) vs. 11 to 23% C accumulation at Ludhiana and Bhairahawa. Further studies should be done at the farm level to improve the quality and optimize the rate of FYM and other organic materials applied to rice–wheat soils. The quality of manures may be improved by composting, which reduces nutrient availability (Castellanos and Pratt, 1981; Hadas et al., 1996) but increases C and N accumulation (Tirol-Padre et al., 2005), leading to enhanced soil fertility. Studies on the mineralization–immobilization patterns of organic manures would determine the appropriate timing of their application as fertilizer. The timing and method of manure application affect both soil organic matter maintenance and nutrient availability. Amounts of organic manures must be optimized to increase C and N accumulations in the soil without negative effects on crop yield. Measurements of gaseous and aqueous C and N losses would also indicate the efficiency of soil C and N storage. Use of organic farm waste materials to supplement inorganic fertilizers could alleviate the yield decline and sustain the environment.

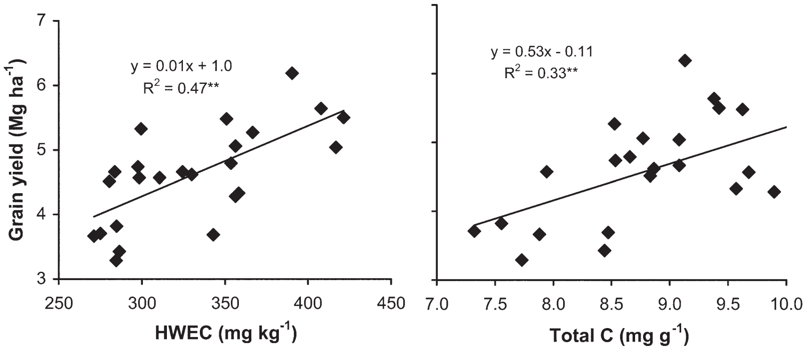

Fig. 9. Relationships of grain yield with hot-water-extractable C (HWEC)and total C across years (Bhairahawa, Nepal, long-term experiment).

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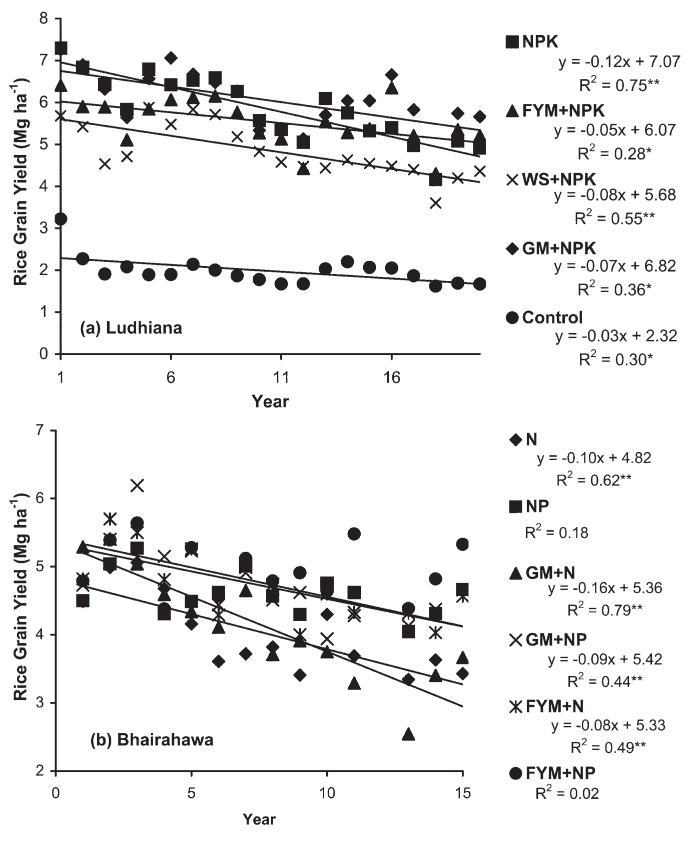

Fig. 10. Rice grain yield trends at (a) Ludhiana and (b) Bhairahawa with various organic (green manure [GM], farmyard manure [FYM] or wheat straw [WS]) and inorganic (NPK) treatments.

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