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Greenhouse gas emission in relation to labile soil C, N pools and functional microbial diversity as influenced by 39 years long-term fertilizer management in tropical rice

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ABSTRACT

Impacts of 39-years of fertilizer and manure application on greenhouse gas (GHG) emissions viz. methane, carbon dioxide and nitrous oxide, soil labile carbon (C) and nitrogen (N) pools, functional microbial diversity were investigated in a tropical flooded rice (*Oryza sativa* L.). The treatments included non-fertilized control, N, farmyard manure (FYM), FYM + N, NPK and FYM + NPK. Annual cumulative GHGs emissions after 39 years of intensive rice–rice cultivation were significantly higher in FYM + NPK treatments than other treatments. The global warming potential (GWP) in 100 years time scale and carbon equivalent emission (CEE) were increased significantly under the combined application of FYM + NPK by 88.4% over control. The carbon efficiency ratio (CER) was significantly higher ($p \leq 0.05$) in NPK as compared to others. The annual emissions of methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2-C) in FYM + NPK were 177.6, 1.28, 1407 kg ha^{-1}, respectively, in tropical rice–rice system (wet season rice-fallow–dry season rice-fallow) which were significantly higher ($p \leq 0.05$) than other treatments. Although the GHGs emissions were more under FYM + NPK treatment, it helps to maintain soil fertility and supported sustainable rice yield. The soil labile C, N pools, soil enzymatic activities and microbial populations were significantly higher under this treatment which is the indicators of improved soil fertility. Stepwise regression analysis of GHGs emission with related soil parameters was performed to predict seasonal fluxes from tropical rice.

1. Introduction

The increasing trend of greenhouse gases (GHGs) content in the atmosphere, such as those of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), are expected to contribute to global warming and reduction in the content of these gases have become a major issue (Balota et al., 2004; Lal, 2004a). Agriculture has been one of the important source and sink of these gases. Controlling and regulating the release of these gases in agricultural soils through judicious land-use and appropriate management practices can mitigate the process of climate change (Lal, 2004a; Wright et al., 2004). Soil organic carbon (SOC) pool is the largest among the terrestrial carbon (C) pools (Lal, 2004b). The management and enhancement of SOC is important for sustainable agriculture. SOC is also the source and sink of atmospheric CO_2 and plays a key role in global C cycling. Soil total organic C (TOC) content can be easily measured by conventional methods. The changes in TOC due to management practices are difficult to detect since these changes occur slowly and are relatively small compared to the vast background of SOC, which vary both spatially and temporally (Purakayastha et al., 2008). The identification of some more sensitive labile SOC factions, such as water soluble organic carbon (WSC), microbial biomass carbon (MBC), readily mineralizable carbon (RMC) and $KMnO_4$ oxidized organic C (POC), contributes to elucidate changes in TOC at early stages of changes in management practices (Gong et al., 2009; Purakayastha et al., 2008). Soil C and nitrogen (N) contents and storage are influenced by soil-forming and anthropogenic factors. Human activities such as fertilizer practices and cropping systems play a key role in the regulation of C and N contents in agricultural soils and emissions of green house gases (Gal et al., 2007; Jagadamma et al., 2007). The dynamics of organic C and N and the factors which influence them have been studied widely using laboratory simulation, long-term field experiments, and regional investigations (Dou et al., 2007; Zanatta et al., 2007). The labile C and N contents in agroecosystems can be

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increased by long-term fertilizer application, particularly by application of organic manure and chemical fertilizer (Zhang et al., 2009) and hence contributing to more GHGs in the atmosphere. Soil C and N cycles are often coupled and this coupling is one of the important mechanisms for the response of terrestrial ecosystem to climate change. Inputs of N to ecosystems can affect C accumulation and distribution within soil–plant systems (Coulter et al., 2009). Hyvonen et al. (2008) found that an increase in N use decreased SOC mineralization under long term fertilizer experiment and support C sequestration in soils (Hungate et al., 2003; Lal, 2004a). Long-term experiments with contrasting fertilization and organic matter inputs may offer a unique potential for quantifying changes in soil C and N content of soil (Schjonning et al., 1994). The dynamics of soil C and N often vary with varying rate chemical and organic fertilization, hence, the change of soil labile C and N contents in relation to GHGs emissions would be worth investigating in long term fertilizer treatments. Therefore a study was conducted in a 39 years old long term fertilizer experiment in rice–rice system at Central Rice Research Institute Cuttack, a tropical region of India to assess the (i) annual GHGs emission and global warming potential (GWP) from flooded rice paddy under different nutrient management and (ii) changes in soil labile C, N and soil functional microbial diversities and coupling their relationships with the GHG emission.

2. Materials and methods

2.1. Experimental site

The study site is situated at the experimental farm of the Central Rice Research Institute, Cuttack (20°25′N, 85°55′E; 24 m above mean sea level) in the eastern part of India. The climate is tropical monsoon with mean annual precipitation is around 1500 mm most of which is received during June to September. The soil is an Aeric Endoaquept with sandy clay loam texture (30.9% clay, 16.6% silt, 52.5% sand), bulk density 1.40 Mg m^{-3}, percolation rate 10 mm d^{-1}, pH (using 1:2.5, soil:water suspension) 6.6, cation exchange capacity 15.2 cmol (p+) kg^{-1}, electrical conductivity (EC) 0.5 dS m^{-1}, total C 0.78%, total N 0.08%.

2.2. Crop establishment and treatments

The field experiment was carried out for 39 years starting from 1969 under rice–rice cultivation with ten treatments which were replicated thrice on a randomized block design. The field was ploughed thoroughly and flooded 2–3 days before transplanting for puddling and leveling. Twenty five (25) days old rice seedlings were transplanted at a spacing of 20 cm × 15 cm with two to three seedlings per hill in both wet (July–December) and dry season (January–April). Farmyard manure was applied in the field once a year during the wet season at the rate of 5 Mg ha^{-1}. Nitrogen was applied in the form of urea 50% as basal and the rest in two equal split after transplanting as top dressing. Top dressing of N fertilizer (urea) was done at 23 and 76 days after transplanting during wet season and 22 and 56 days after transplanting during dry season. Full dose of P and K was applied as basal just before transplanting in the form of single super phosphate and muriate of potash (KCl). All the field plots remained continuously flooded to a water depth of 7 ± 3 cm during the entire period of crop growth and were drained 10 days before the harvest. The crop was raised as per the local recommended agronomic practices except for the fertilization, which was done as per the treatments. Out of ten treatments, following six treatments were selected for the present study.

T_1 – control (without any fertilizers or organic manures)

T_2 – nitrogen (60 kg N ha^{-1} in wet season; 80 kg N ha^{-1} in dry season)

T_3 – FYM (5 Mg ha^{-1}) only in wet season

T_4 – FYM + nitrogen (5 Mg ha^{-1} + 60 kg N ha^{-1} in wet season; 80 kg N ha^{-1} in dry season)

T_5 – NPK (60:30:30 kg ha^{-1} in wet season; 80:40:40 kg ha^{-1} in dry season)

T_6 – FYM + NPK (5 Mg ha^{-1} + 60:30:30 kg ha^{-1} in wet season; 80:40:40 kg ha^{-1} in dry season)

2.3. Soil sampling and storage

Soil samples were collected at three locations randomly in each plot by a sample probe (at the depth of 0–15 cm) at different crop growth stages, viz., maximum tillering, panicle initiation, grain filling and at the harvest both in wet and dry season during 2009–2010, thoroughly mixed and composite samples were prepared. One part of fresh soil samples was kept in refrigerator at 4 °C for biochemical and microbial population analysis. Other part was air dried for 7 days and processed, using 2 mm sieve, stored in sealed plastic jars for analyses of soil carbon, nitrogen fractions.

2.4. Soil carbon and nitrogen fractions analysis

Soil microbial biomass-C (MBC) was measured by modified chloroform fumigation–extraction method with fumigation at atmospheric pressure (Witt et al., 2000). Readily mineralizable carbon (RMC) content of the soil samples was estimated after extraction with 0.5 M K_2SO_4 (Inubushi et al., 1991) followed by wet digestion of the soil extract with dichromate (Vance et al., 1987). The water soluble carbohydrate carbon (WSC) was estimated followed by the procedure of Haynes and Swift (1990). Permanganate oxidizable carbon (POC) was determined following the method described by Blair et al. (1995) with little modifications. Dry soil of 3 g was weighed into 50 ml centrifuge tubes and 30 ml of 20 mM $KMnO_4$ was added. The centrifuge tubes were shaken for 15 min and centrifuged for 5 min at 3400 × *g*. The absorbance of the supernatant and standards was read at 565 nm. The change in the concentration of $KMnO_4$ was used to estimate the amount of C oxidized; assuming that 1 mM $KMnO_4$ is consumed in the oxidation of 0.75 mM or 9 g of C. Ammonium-N (AMON) in the soil extract was estimated by nesslerization (Jackson, 1973) and Nitrate-N (NITRN) nitrogen by 2,4-phenol disulfonic acid method (Bremner, 1965). Ninhydrin reactive nitrogen (NRN), in 20 g soil samples was extracted with 0.5 M K_2SO_4 and was estimated colorimetrically after mixing the soil extracts with ninhydrin (Badalucco et al., 1992). Microbial biomass nitrogen (MBN) was determined using the fumigation–extraction method (Brookes et al., 1985). Ferrous iron (Fe^{2+}) content in 10 g subsamples was extracted with 50 ml of 1 N sodium acetate in HCl (pH 2.8) and assayed by reacting with orthophenanthroline (Murti et al., 1966). The soil pH and Eh was measured at 3–7 days interval throughout the year and expressed in mV by using a portable pH/ORP (oxidation–reduction potential) meter using platinum-calomel electrode as reference immersed into the reduced zone (about 1–2 cm below the oxidized zone).

2.5. Soil enzymatic activities and microbial populations

Dehydrogenase (DHA) activity was determined by reduction of 2,3,5-triphenyltetrazolium chloride (TTC) (Casida et al., 1964). Fluorescein diacetate (FDA) hydrolysis activity measurements were made following the method of Adam and Duncan (2001). The β-glucosidase (BGLU) activity was assayed following the procedure of Eivazi and Tabatabai (1988). Urease (UREASE) activity was

measured following the method of Tabatabai and Bremner (1972). The heterotrophic (HET) microbial populations were enumerated by using the media of Rand et al. (1975) while methanogenic (METH) bacterial population was enumerated by anaerobic culture tube technique (Kasper and Tiedje, 1982). Culturable NH_4^+ and NO_2^- oxidizing atutotrophs (AMOOX and NITROX, respectively) were enumerated by the MPN method (Schmidt and Belser, 1982). Populations of denitrifying bacteria (DENITRI) were estimated by method of Abd-el-Malek et al. (1974).

2.6. Methane and nitrous oxide flux measurement

Methane and N_2O flux from the rice field plots were monitored throughout the year by using the manual closed chamber method. Sampling for CH_4 and N_2O flux measurements was done from all the replicated plots in the morning (09:00–09:30 h) and afternoon (15:00–15:30 h), and the average of the morning and afternoon fluxes was used as the flux for the day (Bhattacharyya et al., 2012; Datta et al., 2009). The gas samplings were done soon after the transplanting of the rice crop at 3–7 days intervals throughout the year including wet season (2009), dry season (2010) and two fallow periods each after the wet and dry season. For measuring CH_4 and N_2O emissions, six rice hills were covered with a fabricated Perspex chamber (53 cm × 37 cm × 51 cm, length × width × height from seedling to tillering, and 53 cm × 37 cm × 71 cm, length × width × height from maximum tillering to maturity stages). A battery-operated air circulation pump with an air displacement of 1.5 L min^{-1} (M/s Aerovironment Inc., Monrovia, CA, USA) was connected to polyethylene tubing to mix the air inside the chamber and draw the air samples into Tedlar gas-sampling bags (M/s Aerovironment Inc.) at 0, 15 and 30 min intervals. Methane concentrations in the air samples collected were analyzed in a Chemito 2000 gas chromatograph (M/s Thermo Scientific) equipped with a flame ionization detector (FID) and Porapak Q column (6 feet long, 1/8 in. outer diameter, 80/100 mesh size, stainless steel column). The temperature of the injector, column and detector was maintained at 150, 50 and 230 °C, respectively. The carrier gas (N_2) flow was maintained at 15 ml min^{-1}. The gas chromatograph was calibrated before and after each set of measurements by using 1.2 and 1.8 μl CH_4 L^{-1} in N_2 (Chemtron Science Laboratories, India) as the primary standard to provide a standard curve that was linear over the concentration range used in this study. Nitrous oxide concentration in the air samples collected in the Tedlar sampling bags were analyzed in a Chemito 2000 gas chromatograph (M/s Thermo Scientific) equipped with an electron capture detector (ECD) and a Porapak Q column (6 feet long, 1/8 in. outer diameter, 80/100 mesh, stainless steel column). The injector, column and detector were maintained at 200, 60 and 340 °C, respectively, and the carrier gas (N_2) flow was maintained at 15 ml min^{-1}. The gas chromatograph was calibrated before and after each set of measurements by using 110 parts per billion (ppb) N_2O in N_2 (Chemtron Science Laboratories, India) as the primary standard and 310 and 398 ppb N_2O in N_2 (Chemtron Science Laboratories, India) as the secondary standard. Fluxes of CH_4 and N_2O were calculated by successive linear interpolation of the average emissions on the sampling days, assuming that the emissions followed a linear trend during the periods when no sampling was done (Datta et al., 2009). Cumulative CH_4 and N_2O emissions for the entire cropping period were computed by plotting the flux values against the days of sampling calculating and were expressed as kg ha^{-1}.

2.7. Carbon dioxide flux measurement

The CO_2 flux was measured at 2–7 days intervals with an environmental gas monitor chamber attached to a data logger (model EGM-4, PP system, Haverhill, MA). A flag was placed as a marker in the plot where CO_2 flux was measured throughout the study period. The chamber was 15 cm tall, 10 cm in diameter and had capacity to measure CO_2 flux from 0 to 9.99 g CO_2-C m^{-2} h^{-1}. The chamber was placed at the soil surface for 2 min for each plot until CO_2 flux measurement was recorded in the data logger. The CO_2 flux was recorded in the inter-row position of the rice plants. All measurements were taken between 9:00 A.M. to 12:00 noon in the morning and between 3:00 P.M. to 5:00 P.M. in the afternoon (Bhattacharyya et al., 2012; Sainju et al., 2008; Iqbal et al., 2009). The average between the morning and evening flux was considered as the daily flux and cumulative emission was computed by the method followed in case of CH_4 and N_2O estimation.

2.8. Global warming potential measurement

Global warming potential (GWP) is an index defined as the cumulative radiative forcing between the present and some chosen later time horizon caused by a unit mass of gas emitted during the present condition (Bhattacharyya et al., 2012). It is used to compare the effectiveness of each GHG to trap heat in the atmosphere relative to some standard gas, by convention CO_2. Integrated evaluation of GHG emissions expressed as global warming potential (GWP) was computed using the IPCC factors for calculating the combined GWPs for 100 years [GWP = 24.5 × CH_4 + CO_2 + 320 × N_2O kg CO_2 equivalent ha^{-1}] from CH_4, N_2O and CO_2 efflux values under different treatments (IPCC, 2007). The CEE and CER of the treatments were calculated using the following equations (Bhatia et al., 2005):

$$\text{CEE} = \frac{\text{GWP} \times 12}{44} \quad \text{and} \quad \text{CER} = \frac{\text{grain yield (in terms of C) of the rice}}{\text{CEE}};$$

the C concentration in the grain was measured as 43%.

2.9. Statistical analysis

Individual character datasets were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT) at the 0.05 level of probability. The correlations among the different soil parameters and stepwise regression of seasonal GHGs emission with related soil parameters were done using SPSS version 20.0.

3. Results

3.1. Soil redox potential (Eh), pH and Fe^{2+} content

Soil Eh decreased after transplantation under flooding condition up to panicle initiation stage (76 and 230th days of year in the wet and dry season, respectively) and increased thereafter in all the treatments and the Eh values were positive in the fallow periods [Fig. 1(a)–(f)]. The lowest Eh value was recorded under FYM + NPK treatment (Fig. 1) which was significantly lower than other treatments. While the ferrous iron (Fe^{2+}) content was highest (1911.1 μg g^{-1} and 1665.4 μg g^{-1} in the wet and dry season, respectively) in FYM + NPK treatment at the panicle initiation stage of crop development (Table 1). The soil pH during the entire experimental period ranged between 5.75 and 6.01 [Fig. 1(a)–(f)].

3.2. Soil carbon and nitrogen fractions

The MBC ranged from 122.5 to 358.5 μg g^{-1} during the wet and dry season and its contents were higher during the wet season than that during dry season [Fig. 2(a)]. The application of FYM + NPK showed highest (358.5 μg g^{-1}) accumulation of MBC during the

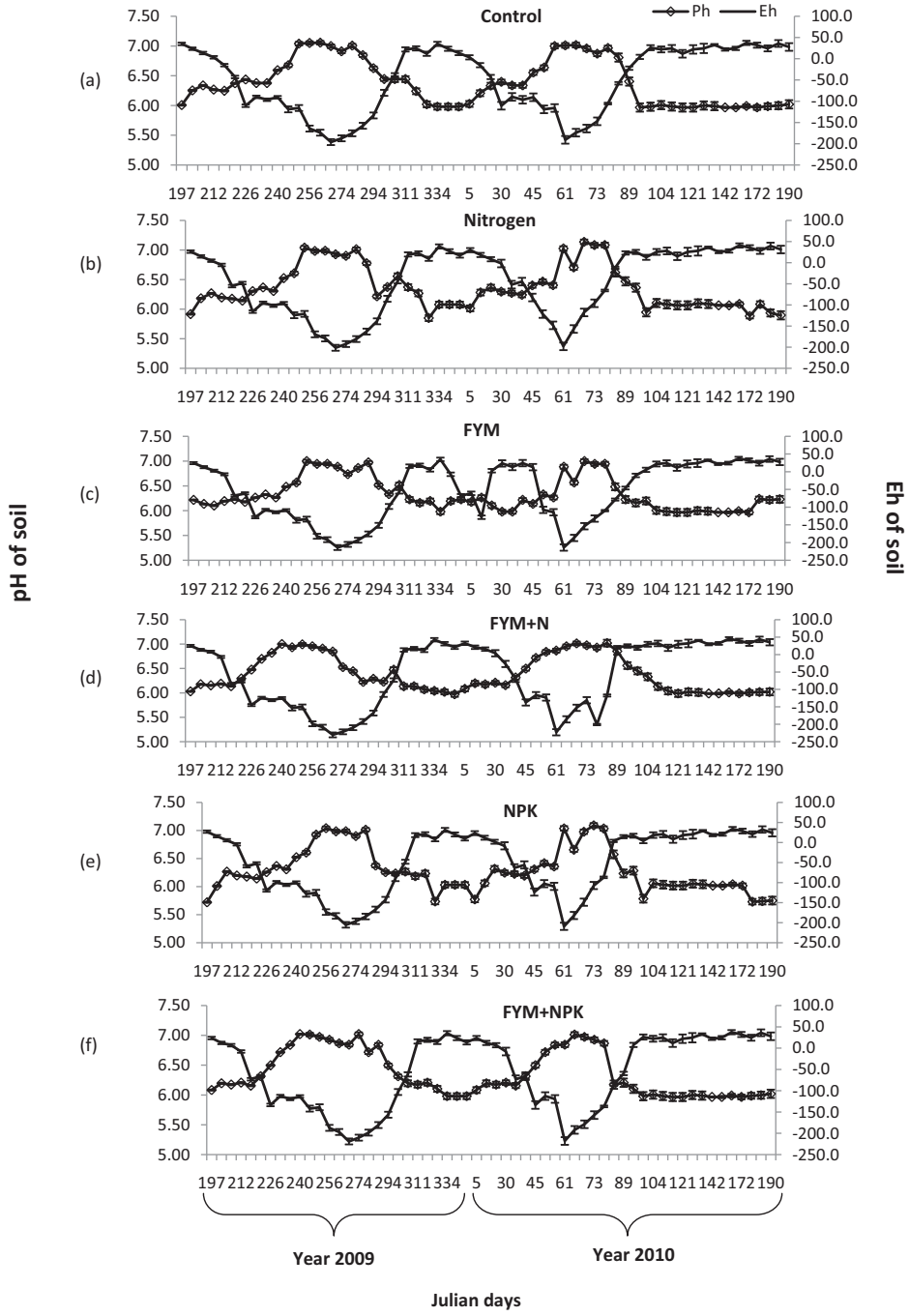

Fig. 1. Effect of 39 years of rice cultivation under the various treatments of long term fertilizer experiment under flooding condition on pH and Eh of an alluvial soil planted to rice. Means of three replicate values plotted, bars/half-bars indicate the standard deviations.

Table 1
Ferric iron (Fe^{2+}) content ($\mu g\,g^{-1}$) in soil at different plant growth stages of flooded rice after 39 years of fertilizer and manure application.

Treatment	Wet season				Dry season			
	Maximum tillering	Panicle initiation	Grain filling	Maturity	Maximum tillering	Panicle initiation	Grain filling	Maturity
Control	1151.1[a]	1805.1[a]	1115.4[a]	583.0[a]	1006.1[a]	1577.6[a]	974.8[a]	509.5[a]
Nitrogen	1177.6[ab]	1831.6[ab]	1136.8[ab]	606.5[ab]	1026.2[ab]	1596.1[ab]	990.6[ab]	528.5[ab]
FYM	1224.8[bc]	1878.8[bc]	1164.2[ab]	619.8[ab]	1067.3[bc]	1637.2[bc]	1014.5[ab]	540.1[ab]
FYM + nitrogen	1230.7[bc]	1894.6[bc]	1180.1[ab]	634.9[ab]	1072.4[bc]	1651.0[bc]	1028.4[ab]	553.3[ab]
NPK	1191.0[abc]	1841.9[ab]	1140.1[ab]	613.8[ab]	1037.8[abc]	1605.1[ab]	993.5[ab]	534.9[ab]
FYM + NPK	1243.8[c]	1911.1[c]	1188.2[b]	648.1[b]	1083.9[c]	1665.4[c]	1035.4[b]	564.7[b]

Note: In each column the mean values followed by common letters are not significantly different ($p < 0.05$) between treatments by Duncan's multiple range test (DMRT).

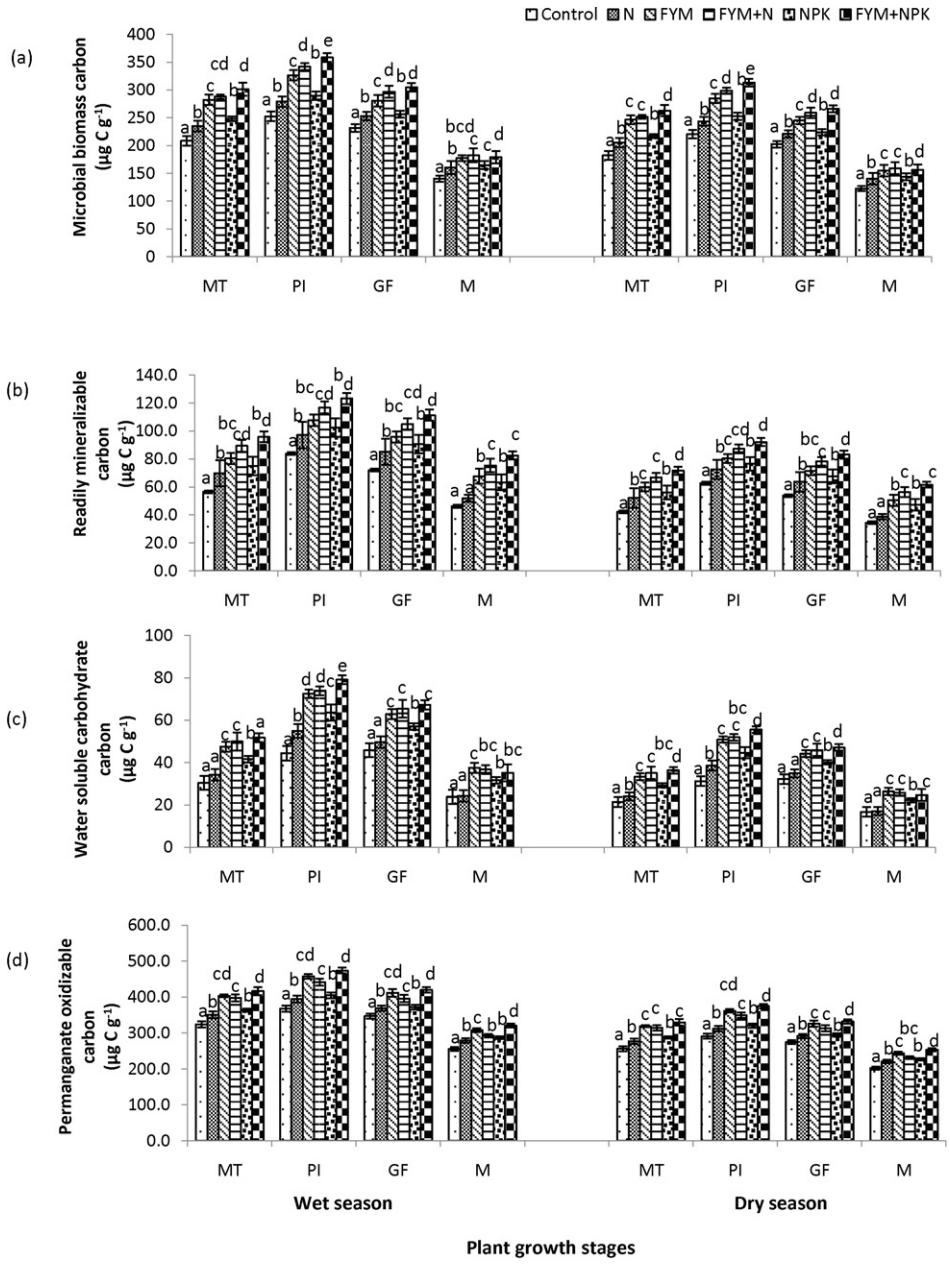

Fig. 2. Microbial biomass carbon (a), readily mineralizable carbon (b), water soluble carbohydrate carbon (c) and permanganate oxidizable carbon (d) of the rhizosphere soils (0–15 cm soil depth) during various stages of crop growth after 39 years of rice cultivation under the various treatments of long term fertilizer experiment under flooding condition. Columns with error bars followed by the common letters are not significantly different ($p < 0.05$) between treatments at particular crop growth stage by Duncan's multiple range test (DMRT). Here, MT = maximum tillering, PI = panicle initiation, GF = grain filling and M = maturity.

wet season [Fig. 2(a)]. Similarly, the RMC content was highest (123.2 $\mu g\ g^{-1}$) in plots receiving both FYM and NPK and the lowest in unamended control plots (46.1 $\mu g\ g^{-1}$) during the wet season [Fig. 2(b)]. The WSC varied from 16.7 to 79.1 $\mu g\ C\ g^{-1}$ in both the seasons and the highest value was obtained in case of the combined application of FYM and NPK [Fig. 2(c)] during wet season. The POC also ranged from 202.2 to 473.8 $\mu g\ C\ g^{-1}$ in the different treatments during the wet and dry season and the highest value (473.8 $\mu g\ C\ g^{-1}$) was recorded in FYM + NPK treatment during the wet season [Fig. 2(d)]. The fertilization practices had significantly affected nitrogen fractions viz., AMON, NRN, MBN, and NITRN [Fig. 3(a)–(d)]. Of all the treatments, the AMON, NRN, MBN, NITRN values were highest in FYM + NPK treatment at the panicle initiation stage of crop development [Fig. 3(a)–(d)]. Most of the N fractions showed comparatively higher values in dry season as compared to that of the wet season [Fig. 3(a)–(d)].

3.3. Soil enzyme activities and microbial population

Soil enzyme activities and microbial populations were strongly influenced by the application of FYM. In the present study, the highest DHA (596.5, 569.4 $\mu g\ TPF\ g^{-1}\ d^{-1}$), FDA (8.14, 5.68 μg fluorescein $g^{-1}\ h^{-1}$), BGLU (42.1, 31.8 μg p-nitrophenol $g^{-1}\ h^{-1}$) and UREASE (632.3, 738.1 μg urea $g^{-1}\ h^{-1}$) activity was observed in the plots receiving FYM + NPK at panicle initiation stage in the wet and dry season, respectively [Fig. 4(a)–(d)]. The highest HET (9.23 log CFU) and METH (4.02 log MPN) microbial population was observed in the FYM + NPK treatment in

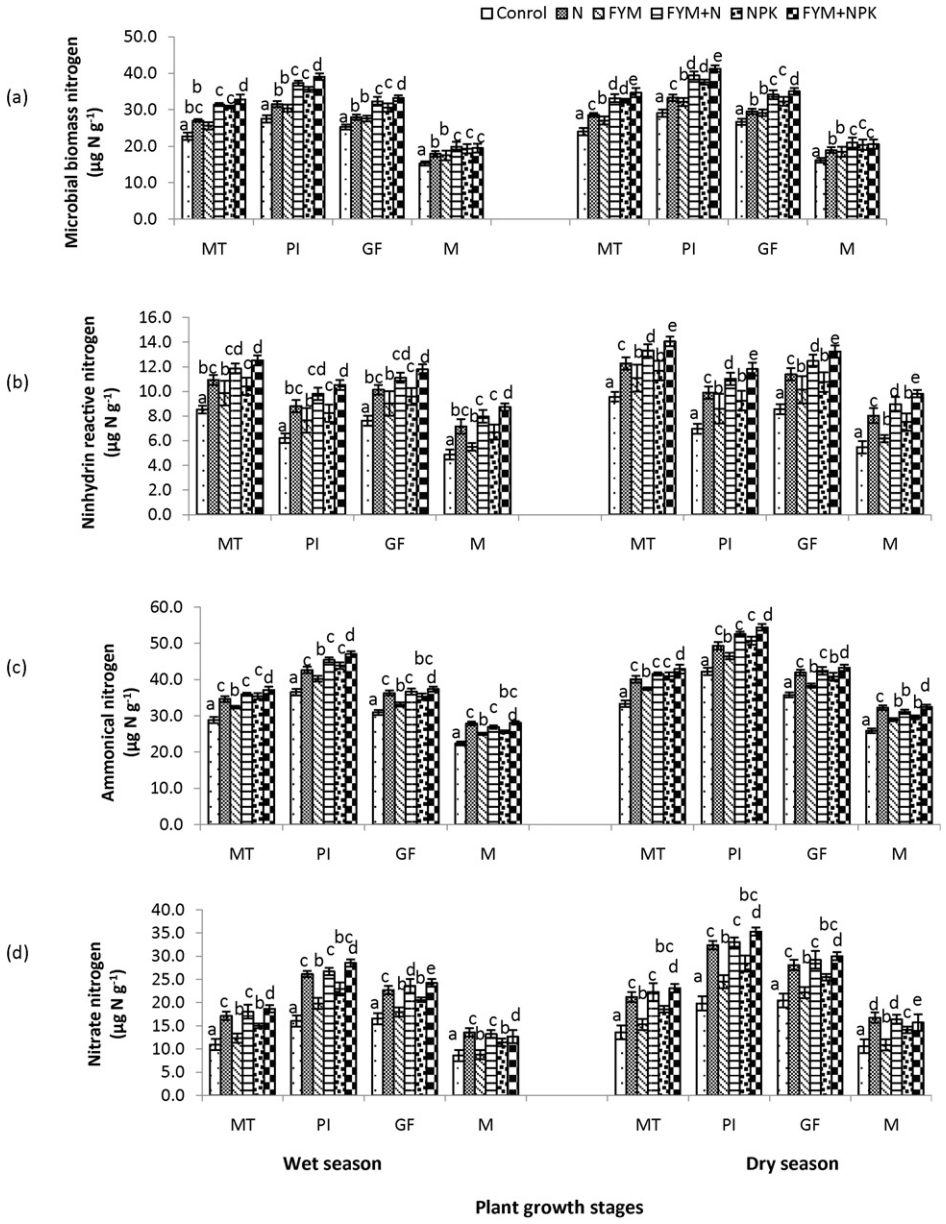

Fig. 3. Microbial biomass nitrogen (a), ninhydrin reactive nitrogen (b), ammoniacal nitrogen (c) and nitrate nitrogen (d) of the rhizosphere soils (0–15 cm soil depth) during various stages of crop growth after 39 years of rice cultivation under the various treatments of long term fertilizer experiment under flooding condition. Columns with error bars followed by the common letters are not significantly different ($p < 0.05$) between treatments at particular crop growth stage by Duncan's multiple range test (DMRT). Here, MT = maximum tillering, PI = panicle initiation, GF = grain filling and M = maturity.

the panicle initiation stage in the wet season [Fig. 5(a) and (b)], where in highest AMOOX, NITROX and DENTRI populations were also recorded [Fig. 5(c), (d), and (e)].

3.4. Methane emission

Fertilizer application had significant ($p \leq 0.05$) influence on CH_4 emission. The fluxes of CH_4 varied from 0.23 to 5.06 mg m^{-2} h^{-1} [Fig. 6(a)] throughout the year. In the wet season the highest flux (5.06 mg m^{-2} h^{-1}) was observed in the panicle initiation stage of crop development at 270th Julian day (76 DAT), 2009 where as in dry season the highest flux (4.26 mg m^{-2} h^{-1}) was observed at 65th Julian day (62 DAT), 2010 under FYM + NPK treatment [Fig. 6(a)]. Similar pattern with varying magnitude of temporal CH_4 emission was observed in control as well as fertilizer treated plots. Cumulative CH_4 emission was significantly ($p \leq 0.05$) higher during the wet season (53.3–101.1 kg ha^{-1}) as compared to that of the dry season (34.8–68.8 kg ha^{-1}) (Table 2). Annual cumulative CH_4 emission was lowest (93.3 kg ha^{-1}) in the control and the highest (177.6 kg ha^{-1}) in FYM + NPK (Table 2); and the order of emissions were: FYM + NPK (177.6 kg ha^{-1}) > FYM + N (160.2 kg ha^{-1}) > FYM (124.1 kg ha^{-1}) = NPK (128.1 kg ha^{-1}) > nitrogen (160.3 kg ha^{-1}) > control (93.3 kg ha^{-1}). The CH_4 flux over the fallow period ranged from 5.2 to 7.7 kg ha^{-1} (Table 2).

3.5. Carbon dioxide-C flux

Soil CO_2-C flux increased soon after the transplanting of the rice crop and reached at its peak at the panicle initiation stage of crop development falling between 256 and 270 Julian days (2009) in wet season and at 63 Julian days (2010) in dry season

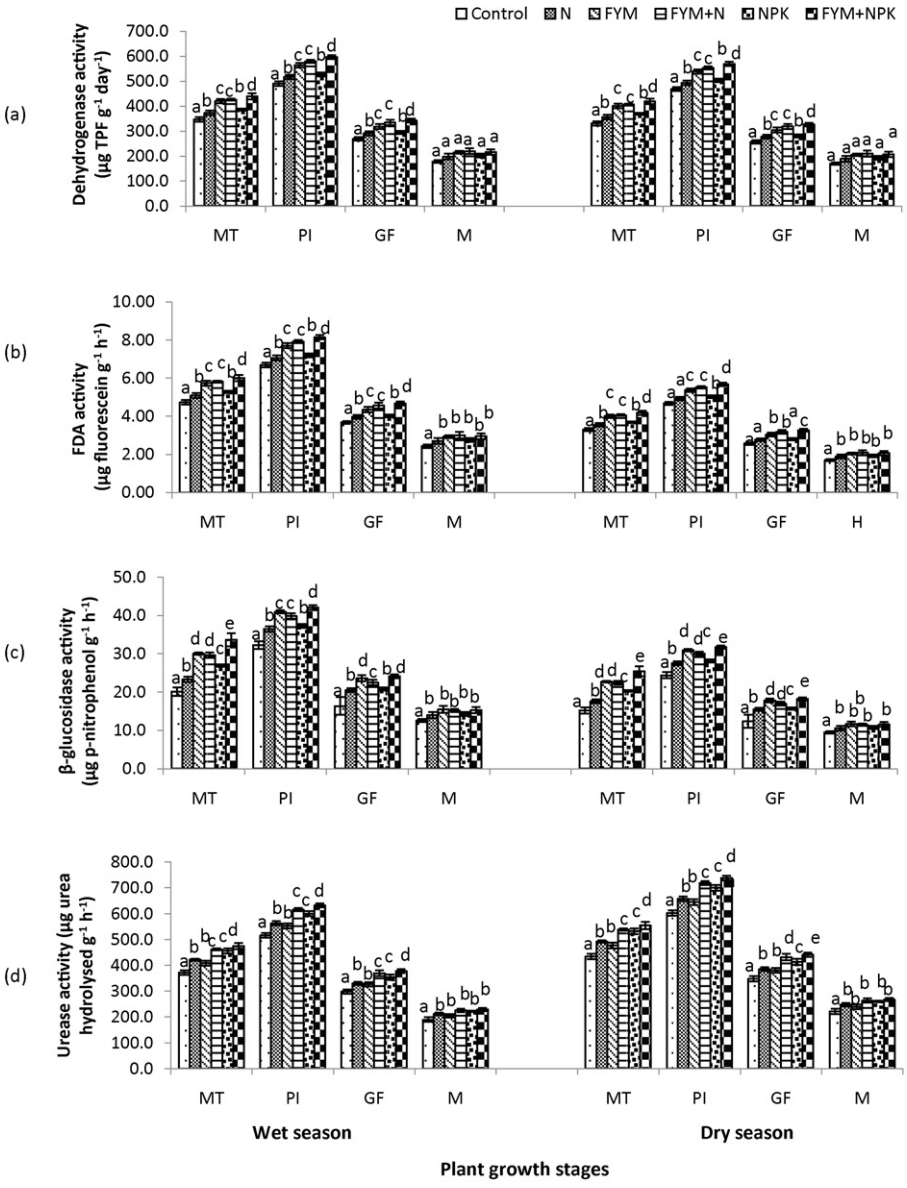

Fig. 4. Dehydrogenase (a), fluorescein diacetate (FDA) (b), β-glucosidase (c) and urease (d) activity of the rhizosphere soils (0–15 cm soil depth) during various stages of crop growth after 39 years of rice cultivation under the various treatments of long term fertilizer experiment under flooding condition. Columns with error bars followed by the common letters are not significantly different ($p < 0.05$) between treatments at particular crop growth stage by Duncan's multiple range test (DMRT). Here, MT = maximum tillering, PI = panicle initiation, GF = grain filling and M = maturity.

irrespective of the treatments [Fig. 6(b)]. Treatment wise, the CO_2-C flux varied from 4.6 to 36.9 mg CO_2-C m^{-2} h^{-1} [Fig. 6(b)] and higher fluxes were observed during the dry season than that during the wet season [Fig. 6(b)]; cumulative emission being 383.5–624.4 kg ha^{-1} and 337.3–605.8 kg ha^{-1} in the dry and wet season, respectively (Table 2). Cumulative CO_2-C emissions on annual basis were in the order of FYM + NPK (1407.0 kg ha^{-1}) > FYM + N (1271.6 kg ha^{-1}) > NPK (1210.1 kg ha^{-1}) > FYM (1106.3 kg ha^{-1}) > nitrogen (1034.0 kg ha^{-1}) > control (833.0 kg ha^{-1}) (Table 2). No significant treatment differences were observed during the fallow period and the cumulative being 112.5–176.8 kg ha^{-1} (Table 2).

3.6. Nitrous oxide emission

Three peaks of N_2O emissions were observed both in wet and dry season [Fig. 6(c)] immediately following the application of urea fertilizer. Unlike CH_4 fluxes, the N_2O-N fluxes were significantly ($p < 0.05$) higher during the dry season (0.24–0.83 kg ha^{-1}) than that during the wet season (0.21–0.76 kg ha^{-1}) 1) (Table 2). Cumulative annual N_2O-N emissions were in the order of FYM + NPK (1.82 kg ha^{-1}) > FYM + N (1.51 kg ha^{-1}) > NPK (1.46 kg ha^{-1}) = nitrogen (1.42 kg ha^{-1}) > FYM (1.15 kg ha^{-1}) > control (0.82 kg ha^{-1}) (Table 2). The N_2O-N flux over the fallow period ranged from 0.13 to 0.23 kg ha^{-1} and was observed highest in the plots treated with FYM + NPK (Table 2).

3.7. Global warming potential and CEE

The GWP under various treatments varied significantly ($p \leq 0.05$) from 2649 to 5084 kg CO_2 equivalent ha^{-1} in wet season and from 2381 to 4395 kg CO_2 equivalent ha^{-1} in dry season in control and FYM + NPK treatment, respectively (Table 3). The highest CEE of 1386 kg C ha^{-1} in wet and 1199 kg C ha^{-1} in dry was recorded in FYM + NPK treatment, while lowest of 723 kg C ha^{-1} in wet and 649 kg C ha^{-1} in dry season was noticed in control (Table 3).

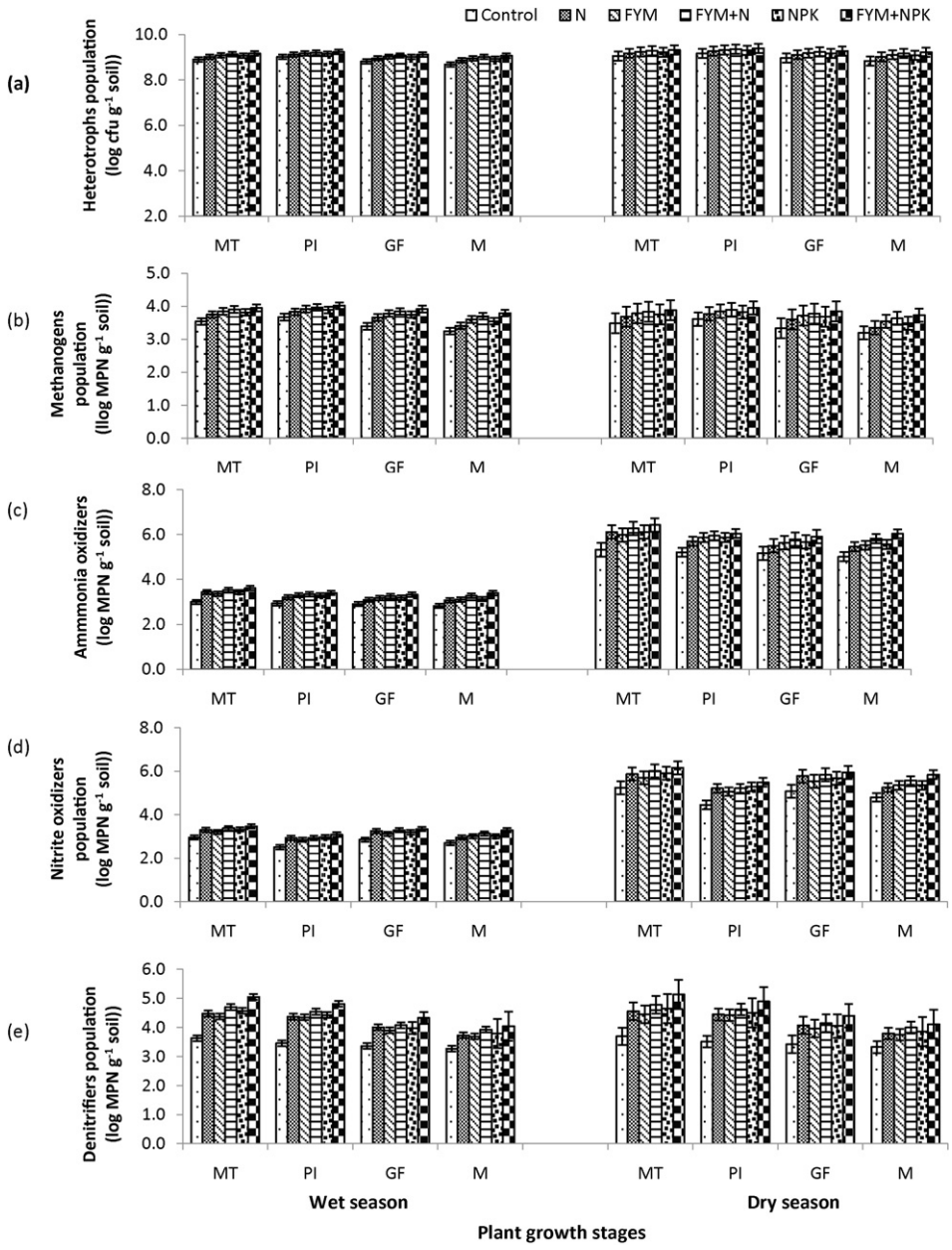

Fig. 5. Heterotrophs (a), methanogens (b), ammonia oxidizers (c) nitrite oxidizers (d) and denitrifiers (e) population in the rhizosphere soils (0–15 cm soil depth) during various stages of crop growth after 39 years of rice cultivation under the various treatments of long term fertilizer experiment under flooding condition. Columns with error bars followed by the common letters are not significantly different ($p < 0.05$) between treatments at particular crop growth stage by Duncan's multiple range test (DMRT). Here, MT = maximum tillering, PI = panicle initiation, GF = grain filling and M = maturity.

3.8. Yield of rice and CER

The grain yields of rice in wet season (average of the year 2005–2009) ranged from 3.54 to 5.99 Mg ha^{-1} and 2.64–5.36 Mg ha^{-1} in the dry season (average of the year 2005–06 to 2009–10) (Table 3). The yields under FYM + NPK (5.99, 5.36 Mg ha^{-1}), FYM + N (5.27, 4.22 Mg ha^{-1}), NPK (5.29, 4.66 Mg ha^{-1}) and in the FYM treatment (4.50, 3.48 Mg ha^{-1}) were significantly higher compared to control (3.54, 2.64 Mg ha^{-1}) in the wet and dry season, respectively (Table 3). The CER, i.e., C fixed in rice grain per unit of C emitted, was the highest (2.13 and 2.06 in the wet and dry season, respectively) in the NPK treated plots (Table 3).

3.9. Stepwise regression analysis

Pearson correlation analysis of different soil C and N pools, enzymatic activities and microbial population were done to see the dependency of soil parameters. It was found that most of the parameters were highly related ($p < 0.01$) to each other except ammonium oxidizer. After that, to predict GHGs emission on seasonal basis stepwise regression analysis was performed by using all related soil parameters in each crop growth stages, taking seasonal mean of CH_4, N_2O and CO_2 emission as dependent variable. Based on regression coefficient values seasonal GHGs emission could be better predicted by taking the soil parameters at PI stage (Table 4).

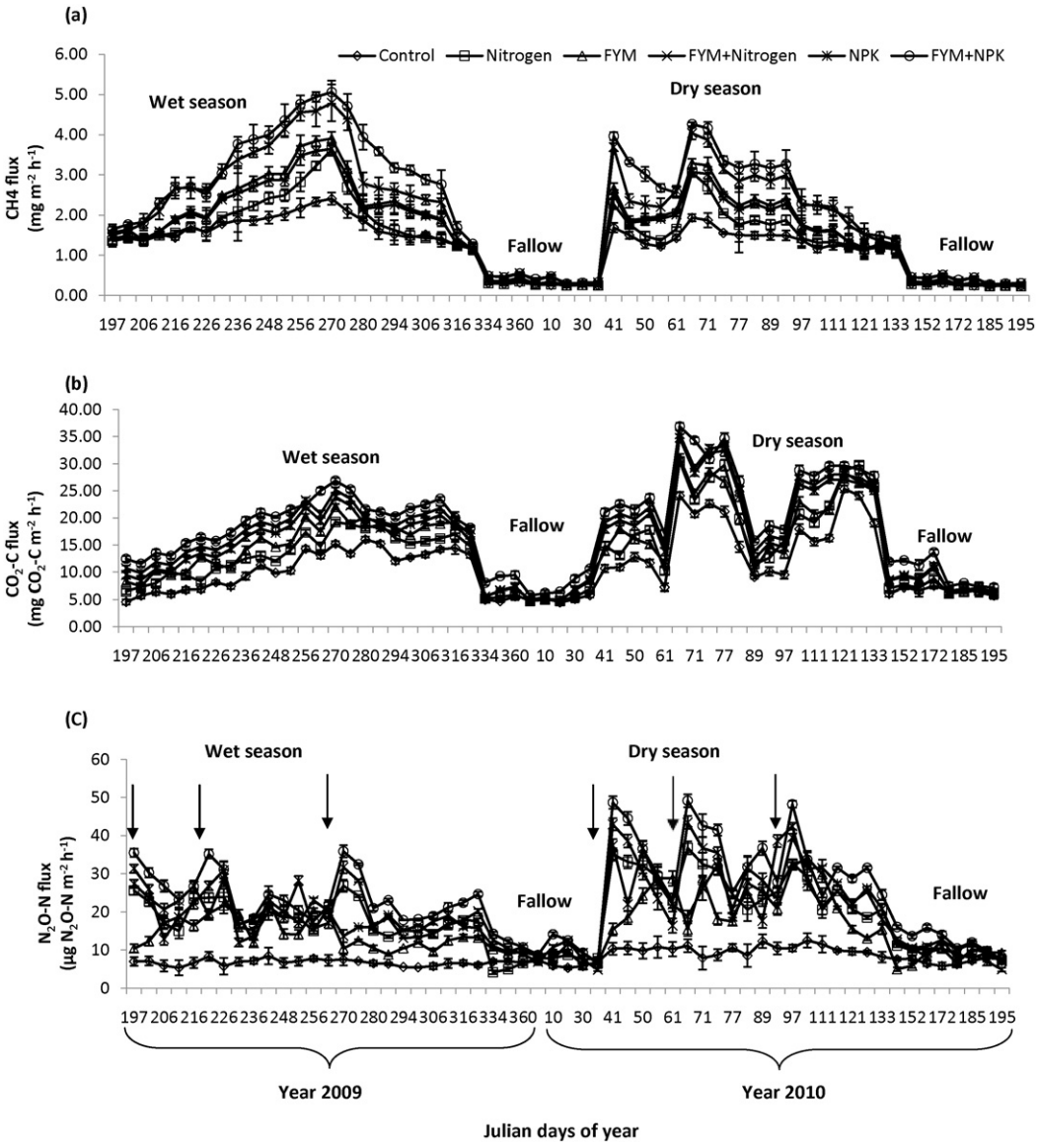

Fig. 6. Methane (CH_4) (a), carbon dioxide (CO_2-C) (b) and nitrous oxide (N_2O-N) (c) flux from soils during different days after transplantation of rice crop after 39 years of cultivation under the various treatments of long term fertilizer experiment under flooding condition. Error bars are the standard deviations of three replicate observations. Upper arrow indicates the time of urea application in the field in the corresponding treatments.

4. Discussion

4.1. Effects of fertilization on soil carbon pools

The application of inorganic fertilizers, by themselves or in combination has been reported to significantly affect SOC and its fractions due to the significant increase in carbon input (Ma et al., 2011). Soil MBC regulates soil organic matter decomposition and nutrient cycling, and thus plays a key role in maintaining function and sustainability of terrestrial ecosystems. MBC has been included in current soil monitoring concepts due to its rapid response and high sensitivity to management practices and environmental changes. FYM provides a potent source of labile C content, as it is rich in nutrients that suit the growth of microbial

Table 2
Annual green house gas emission from flooded rice fields after 39 years of fertilizer and manure application.

Treatment	Methane emission (kg ha^{-1})				CO_2-C emission (kg ha^{-1})				N_2O-N emission (kg ha^{-1})			
	Wet season	Dry season	Fallow	Annual	Wet season	Dry season	Fallow	Annual	Wet season	Dry season	Fallow	Annual
Control	53.3^{a}	34.8^{a}	5.2^{a}	93.3^{a}	337.3^{a}	383.5^{a}	112.5^{a}	833.3^{a}	0.21^{a}	0.24^{a}	0.13^{a}	0.58^{a}
Nitrogen	60.1^{b}	42.0^{b}	5.2^{a}	107.3^{b}	422.6^{b}	492.3^{b}	119.1^{a}	1034.0^{b}	0.60^{c}	0.65^{c}	0.17^{b}	1.42^{c}
FYM	72.5^{c}	50.3^{c}	6.0^{b}	128.8^{c}	476.9^{c}	492.2^{b}	137.2^{b}	1106.3^{c}	0.46^{b}	0.54^{b}	0.15^{ab}	1.15^{b}
FYM + nitrogen	89.7^{d}	63.0^{d}	7.5^{c}	160.2^{d}	551.9^{e}	582.9^{d}	136.8^{b}	1271.6^{d}	0.63^{c}	0.70^{d}	0.18^{b}	1.51^{d}
NPK	70.0^{c}	48.3^{c}	5.8^{ab}	124.1^{c}	517.4^{d}	557.5^{c}	135.2^{b}	1210.1^{cd}	0.61^{c}	0.66^{c}	0.19^{b}	1.46^{c}
FYM + NPK	101.1^{e}	68.8^{d}	7.7^{c}	177.6^{e}	605.8^{f}	624.4^{e}	176.8^{c}	1407.0^{e}	0.76^{d}	0.83^{e}	0.23^{c}	1.82^{e}

Note: In each column the mean values followed by common letters are not significantly different ($p < 0.05$) between treatments by Duncan's multiple range test (DMRT).

Table 3
GWP, CEE, grain yield and CER from flooded rice fields after 39 years of fertilizer and manure application.

Treatment	GWP (kg CO_2 equivalent ha^{-1})		CEE (kg ha^{-1})		Yield (Mg ha^{-1})		CER	
	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season
Control	2649[a]	2381[a]	723[a]	649[a]	3.54[a]	2.64[a]	2.11[c]	1.75[b]
Nitrogen	3323[b]	3163[b]	906[b]	863[b]	4.43[b]	3.45[b]	2.10[c]	1.72[b]
FYM	3757[c]	3312[c]	1025[c]	903[c]	4.50[c]	3.48[bc]	1.89[b]	1.66[a]
FYM + nitrogen	4540[e]	4034[e]	1238[e]	1100[e]	5.27[d]	4.22[c]	1.83[a]	1.65[a]
NPK	3919[d]	3563[d]	1069[d]	972[d]	5.29[d]	4.66[d]	2.13[c]	2.06[d]
FYM + NPK	5084[f]	4395[f]	1386[f]	1199[f]	5.99[e]	5.36[e]	1.86[ab]	1.92[c]

GWP: global warming potential; CEE: carbon equivalent emission; Yield: grain yield of rice; CER: carbon efficiency ratio.
Note: In each column the mean values followed by common letters are not significantly different ($p < 0.05$) between treatments by Duncan's multiple range test (DMRT).

populations. Hence, the application of FYM + NPK builds up higher labile C. The soluble C fraction is an important pool with respect to soil organic matter turnover in agricultural soils, as it acts as a readily decomposable substrate for soil microorganisms and as a short-term reservoir of plant nutrients (Garcla-orenes et al., 2010). Labile C fractions, such as RMC, WSC and POC, all increased after the addition of FYM + NPK. WSC consists of an array of molecules that generally reflect the composition of total SOC due to the equilibrium between the soluble and solid phases of SOC, and is regarded as an indicator of soil quality and functioning. Application of inorganic fertilizer in combination with organic manure contributes more labile carbon that can act as a source of energy and nutrients (Manna et al., 2007).

4.2. Effects of fertilization on soil enzymes and microbial populations

The activities of assayed enzymes were highly correlated with the organic C and N content because there activities were increased substantially by increasing returns of labile C and N source (Graham and Haynes, 2005). Soil organic matter is the substrate for many soil enzymes and protects them through the formation of enzyme complexes with clay and humus (Tabatabai, 1994). DHA basically depends on the metabolic state of the soil biota, highest activities of which occurred under FYM + NPK recording highest heterotrophs. Total microbial activity, in terms of FDA, has been used to determine amounts of active microflora producing extracellular enzymes (Adam and Duncan, 2001). These enzymes can persist in soil as parts of inorganic complexes or in association with organic colloids. BGLU is widely abundant, and is synthesized by soil microorganisms in response to the presence of suitable substrates. Higher UREASE activity under FYM + NPK treatment is due to the presence of the end product of the enzymatic reaction (NH_4^+) (Dick et al., 1988). Heterotrophic microbial populations were higher in the treatment of FYM + NPK due to the bioavailability of growth-promoting substances, and showed positive correlation with soil labile C pools. Nitrification and denitrification are the two major microbial processes responsible for N_2O emission from flooded rice soils. AMOOX and NITROX are involved in the nitrification process whereas DENITRI are involved in the denitrification process. Highly significant positive correlations existed among these organisms, which suggested that these two processes occurred simultaneously in the rice field to give rise to N_2O flux.

4.3. Effects of fertilization on CH_4 emission

Rice cultivation is an important anthropogenic source of atmospheric methane. In a long term rice–rice system, the application of FYM + NPK resulted in an increase in CH_4 emission over the other treatments. Under submerged condition, emission of CH_4 resulted from carbon mineralization (Kimura et al., 2004), which was enhanced by application of chemical fertilizer and manure. Microbial biomass and enzyme activity increased due to SOC mineralization and enhanced substrate supply leading to increased CH_4 production although the predominant pathway of CH_4 formation remained unchanged. Application of different fertilizers, especially in combination with FYM, has been shown to enhance the bioavailable pool of organic C and, in turn, promote the CH_4 production by the utilization of readily bioavailable organic C by methanogenic microbes (Zheng et al., 2007). Diurnal variation of CH_4 flux under field conditions showed that the emission was maximum at mid day or early afternoon 12:00–15:00 h and minimum at midnight (24:00 h) at tillering, panicle initiation and maturity stage of rice crop (Satpathy et al., 1997). It was standardized and well established that under this agro-ecological region the gas samplings at 9:00–9:30 h and 15:00–15:30 h were the most representable one for daily flux measurement and nullified the diurnal variation of flux (Bhattacharyya et al., 2012; Das et al., 2011; Datta et al., 2009; Nayak et al., 2006). Estimation of seasonal CH_4 emissions during the crop season was done by successive linear interpolation of average emission on the sampling days assuming that emission followed a linear trend during the periods when no sample was taken (Bhatia et al., 2011). The highest flux of the CH_4 was observed at 270 Julian days in 2009 and 65 Julian days in 2010 (76 DAT of the wet season crop and 62 DAT of the dry season crop, respectively) of the rice crop

Table 4
Regression relationships of seasonal GHGs emission with related soil parameters.

Dependable variable	Regression equation	R^2 ($p < 0.05$)
Kharif season		
Methane	$CH_4 = 20.3$ (FDA) + 1.5 (MBC) + 0.82 (RMC) + 1.06 (METH) − 128.2 (constant)	$R^2 = 0.92$
Nitrous oxide	$N_2O = 0.3$ (NITROX) + 0.03 (AMON) + 1.3 (DENITRI) − 1.8 (constant)	$R^2 = 0.96$
Carbon dioxide	$CO_2 = 297.2$ (HET) + 6.3 (BGLU) + 0.93 (RMC) − 2680.1 (constant)	$R^2 = 0.96$
Rabi season		
Methane	$CH_4 = 20.8$ (FDA) + 1.03 (MBC) + 0.86 (RMC) + 1.14 (METH) − 94.03 (constant)	$R^2 = 0.94$
Nitrous oxide	$N_2O = 0.01$ (NITRN) + 1.15 (UREASE) + 0.3 (DENITRI) − 0.98 (constant)	$R^2 = 0.95$
Carbon dioxide	$CO_2 = 353.4$ (HET) + 1.3 (UREASE) + 0.96 (RMC) − 3275.1 (constant)	$R^2 = 0.96$

Note: NITRN: nitrate nitrogen; FDA: fluorescein diacetate; BGLU: β-glucosidase; UREASE: urease activity; HET: heterotrophic microbes; METH: methanogens; NITROX: nitrite oxidizers; DENITRI: denitrifiers; AMON: ammoniacal nitrogen; MBC: microbial biomass carbon; RMC: readily mineralizable carbon.

irrespective of the treatments which corresponds just after the panicle initiation stage of crop development. The higher CH_4 flux in the panicle initiation stage was also reported by Gogoi et al. (2005). It was evident from our study that the microbial activity both in terms of extracellular enzyme activity and populations (methanogens, heterotrophs) were the highest during panicle initiation stage of crop development. The application of FYM + NPK and FYM + N enhanced emission of CH_4 by providing additional C substrates in comparison to the unfertilized conditions (Lu et al., 2000). Methane production is largely favored by a soil Eh value lower than −150 mV, a pH between 6 and 8 and supply of low molecular fatty acids derived from easily degradable organic matter (Neue et al., 2000). The main electron donor in flooded rice soils is readily decomposable organic matter and there were much faster rate of reduction due to the incorporation of easily decomposable organic matter soon after flooding (Xu et al., 2000) resulting lowest soil Eh under FYM + NPK treatment. In mineral phase, under low pH and Eh condition, the reduction of Fe^{3+} increases resulting increased water soluble Fe^{2+} concentration (Ponnamperuma, 1972). In the present study, lower redox potential, favorable pH and increased water soluble Fe^{2+} under FYM + NPK treatment resulted higher CH_4 emission.

4.4. Effects of fertilization on CO_2 emission

The application of fertilizers on long term basis can affect mineralization rates of soil organic matter and contribute to increase in soil organic matter content by increasing residue input with increased crop production (Iqbal et al., 2009). Rudrappa et al. (2006) reported that balanced fertilization in the form of FYM + NPK increased the carbon concentration in 0–15 cm layer of soil in comparison to FYM and NPK alone. There are limited datasets currently available to characterize the effects of the application of fertilizers on long term basis on soil CO_2 flux from the flooded rice paddy. In the present study, the highest CO_2 flux was observed in the FYM + NPK treatment, due to the efficient use of carbon for microbial growth in response to the application of the fertilizers (Fisk and Fahey, 2001). The highest peak of the CO_2 flux was observed just after panicle initiation stage. The higher flux was due to the availability of the C substrates during this period and higher microbial activity as reported by Iqbal et al. (2009) and Campbell et al. (2001). All the measurements were taken between 09:00 and 12:00 h and between 15:00 and 17:00 h of the day to reduce variability in CO_2 flux due to diurnal changes in temperature and considered as the representative time as reported by Sainju et al. (2008) and Iqbal et al. (2009). The balanced fertilization in the form of FYM + NPK provided the labile source of carbon that supports the growth of microbial biomass, which is dynamic and promotes the priming effect of soil organic matter resulting into higher CO_2-C flux (Singh et al., 2009). In this study while estimating CO_2 emissions from the rice field, C-fixation through photosynthesis has not been taken into account.

4.5. Effects of fertilization on N_2O emission

Urea application, either alone or in combination with FYM or in the form of NPK, significantly increased the N_2O flux. Nitrification and denitrification are the two major microbial processes responsible for N_2O emission from flooded rice soils. Although nitrification is an aerobic process and denitrification is an anaerobic process, both processes have been known to occur in tandem in flooded rice soils. Like nitrification, denitrification is a major source of N_2O emission, especially under anaerobic conditions with higher carbon availability (Kyaw and Toyota, 2007). Correlation analysis between microbial biomass nitrogen, ninhydrin reactive nitrogen and N_2O-N showed a highly significant positive relationship, indicating that N_2O emission is a microbial process that depends on available carbon and nitrogen sources available to microorganisms. NRN content increased significantly in soils treated with FYM + NPK, FYM + N and nitrogen alone, which is an index of labile nitrogen available from microbial biomass in the rhizosphere soil (Nayak et al., 2007) leading to higher N_2O emission.

4.6. Global warming potential and CEE

Fertilization through the incorporation of FYM + NPK, FYM + N, FYM and NPK significantly ($p \leq 0.05$) increased the total GWP of paddy soil in comparison to that of the non fertilized and non manured soil. Correlation analysis showed that GWP of different manurial treatments were significantly ($p \leq 0.05$) correlated with the GHGs flux.

4.7. Yields of rice and CER

The yield of rice was significantly higher under FYM + NPK treatment compared to other treatments possibly due to the better availability of nutrients. The CER was the highest under the NPK treatment due to lower gaseous C emission and relatively higher yield. Though highest yield was observed under NPK + FYM, because of highest emission, the CER value was lesser than NPK applied plots. If the emission of GHG for industrial production of NPK which is already present in FYM taken in to account, there would be further improvement of CER value for NPK + FYM treatment.

4.8. Relationship of GHGs emission and soil parameters

Active METH population in the soil rich with RMC in the tropical paddy field enhanced CH_4 production, whose flux can be predicted from FDA, RMC, MBC and METH population. Similarly HET population, BGLU and RMC value can suitably predict the CO_2-C flux from tropical paddy field in kharif season. Whereas, AMON concentration, NITROX, NITRN and DENITRI population in the soil are important parameters that can govern the emission of N_2O-N from soil.

5. Conclusion

In rice–rice cropping systems, application of FYM with NPK as balanced fertilization resulted in higher labile C and N pools, soil enzymatic activities, soil microbial populations after a period of 39 years and sustain grain yield. The annual emissions of CH_4, CO_2-C and N_2O-N from tropical rice ranged from 93.3 to 177.6 kg ha^{-1}, 833.3 to 1407 kg ha^{-1} and 0.58 to 1.82 kg ha^{-1}, respectively. Though the GHGs emissions and GWP were higher under the combined application of FYM + NPK but emissions per unit grain yield were moderate under this treatment. Therefore, the combined application of FYM + NPK is a viable option in managing soil fertility, moderating GHG emissions and sustaining rice yield in tropical flooded soils. For estimation of net C balance, in addition to gaseous C emissions, the sequestration potential of tropical rice under long term fertilization need to be considered.

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