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Effects of rice straw and nitrogen fertilization on greenhouse gas emissions and carbon storage in tropical flooded soil planted with rice

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ABSTRACT

Effects of four years of inorganic and organic nitrogen (N) management on the emission of three major greenhouse gases (GHGs): methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O), and on soil labile carbon fractions such as water-soluble carbon (C, WSC), microbial biomass carbon (MBC), $KMnO_4$ oxidizable organic carbon ($KMnO_4$-C), carbon management index (CMI) and soil carbon storage were investigated in a flooded rice (*Oryza sativa* L.) field in India. The treatments included an unfertilized control, inorganic nitrogen fertilizer, rice straw + inorganic nitrogen fertilizer and rice straw + green manure. Maximum global warming potential (GWP) (10,188 kg CO_2 equivalent ha^{-1}) was determined for the combined application of rice straw and green manure. Total carbon content and carbon storage in the topsoil were significantly increased for the rice straw + inorganic nitrogen fertilizer treatment. The combined application of rice straw and green manure was more effective in increasing WSC, MBC, $KMnO_4$-C concentrations and CMI than the inorganic fertilizer treatments, although it increased gaseous carbon emission. The combined application of rice straw and an inorganic fertilizer was most effective in sequestrating soil organic carbon (1.39 Mg ha^{-1}), resulting in a higher grain yield. Therefore, it could be the best option for improving productivity and carbon storage in the rice–rice cropping system.

1. Introduction

Rice paddy contributes toward the emissions of the most important greenhouse gases (GHGs) responsible for global warming: carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Soil is one of the important sources and sinks for GHGs that cause global warming and climate change (Janseens et al., 2003). Soil contributes about 20% to the total emission of CO_2 through soil and root respiration, 12% of CH_4 and 60% of anthropogenic N_2O emissions (IPCC, 2007). Global warming may affect the global carbon cycle, thereby distorting the structure and functions of ecosystems. Organic matter concentration, which is quite low (<1.0%) in tropical soils, would become still lower and climatic change may also affect its quality (Smith et al., 2008). Soil biology and microbial populations are expected to change with changes in climatic conditions (Baker, 2004). Currently, biotic carbon sequestration is being considered a viable option for mitigating carbon emissions in the form of CO_2 and CH_4 to the atmosphere. Agricultural activities have profound short- and long-term influence on soil organic carbon (SOC). Mitigation of CO_2 emission from agriculture can be achieved by increasing carbon sequestration in the soil, which implies storage of carbon as soil organic matter (Lal, 2004). Judicious nutrient management is crucial to SOC sequestration in tropical soils (Bhattacharyya et al., 2007). Adequate supply of nutrients in the soil can enhance biomass production and SOC content (Van Kessel and Hartley, 2000). Attainable carbon sequestration is essentially limited by the quantity of carbon input into the soil system. The estimated potential of agricultural intensification on SOC sequestration in Indian soils ranges between 12.7 and 16.5 Tg carbon $year^{-1}$ (Lal, 2003). A recent report by Benbi and Chand (2007) describes increased productivity under improved SOC density (SOC content in the 0–15 cm soil volume) in the 0–15 cm soil layer. There is a strong need to increase SOC density to improve the quality of natural resources for sustainable crop productivity and to mitigate global warming. However, with the rapid economic and social development, tropical paddy soils are subject to degradation as characterized by low organic carbon content and low crop productivity. Therefore, it is necessary to investigate soil carbon dioxide (CO_2) evolution from paddy soils to better understand the mechanisms that regulate carbon storage and loss in extensively cultivated paddy fields. Furthermore, the effects of nitrogen fertilization and rice growth on variation in CO_2 emission under anaerobic conditions from paddy soils needs to be better understood. Many studies reported that the application of

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inorganic fertilizers resulted in significant increases in SOC and its fractions due to the positive effects of the fertilizer on crop growth, and in turn, crop carbon return (Gong et al., 2009; Purakayastha et al., 2008). The application of straw in combination with inorganic fertilizer is an attractive alternative to burning because it can provide essential nutrients for crops (Edmeades, 2003), while reducing carbon release to the atmosphere. To realize the vast SOC sequestration potential for national benefit, adoption of recommended management practices, including the integrated use of organic and inorganic fertilizer, is necessary. The main focus should be on GHG fluxes in relation to fertilizer management practices. The present investigation was conducted: (a) to estimate GHG emissions in the form of CH_4, CO_2, N_2O and global warming potential (GWP) from soils in a rice–rice ecosystem using fertilizer applications, (b) to estimate soil carbon pool, carbon storage and microbial activity in relation to GHG emissions and (c) to identify the most adoptable treatment that offers high SOC storage, increased yield and the lowest GHG emission.

2. Materials and methods

2.1. Experimental site

The study site is situated in 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 basically tropical. The mean annual precipitation is around 1500 mm. The soil is an Aeric Endoaquept with sandy clay loam texture (25.9% clay, 21.6% silt, 52.5% sand), bulk density 1.41 Mg m^{-3}, percolation rate < 10 mm d^{-1}, pH (H_2O) 6.16, cation exchange capacity 15 c mol (p+) kg^{-1}, electrical conductivity 0.5 dS m^{-1}, total carbon 0.49%, total nitrogen 0.049%, Olsen extractable P 18 mg kg^{-1} and available K 286 mg kg^{-1}.

2.2. Crop establishment and treatments

The field experiment was carried out for four years (2008–2011 *kharif* season, July–November in each year) under rice paddy cultivation. The field was ploughed thoroughly and flooded 2–3 days before transplanting for puddling and leveling. *Sesbania aculeata* (Table 1) (green manure) and rice straw (Table 1) were incorporated in the field. The field was plowed by moldboard plough to 20 cm depth, puddled thoroughly and leveled before the starting of the cropping period. Twenty-five days' old rice seedlings (cv. *Gayatri*, crop cycle of 155 days) were transplanted at a spacing of 20 cm × 15 cm with one seedling assigned to each hill. Urea was applied as three split doses: in basal application, in maximum tillering and in the panicle initiation stage of crop development. The treatment plots were laid out in a randomized block design with three replications for each treatment plot. All the field plots remained continuously flooded to a water depth of 6 ± 2 cm during the entire period of crop growth and were drained 12 days before the harvest. The crop was raised as per the locally recommended agronomic practices except for the fertilization, which was done as per the treatments. The treatments in rice included:

T_1 – Control (without any fertilizers or organic amendments);
T_2 – Urea (60 kg nitrogen ha^{-1});
T_3 – Rice straw + urea (1:1 nitrogen basis) (30 kg nitrogen ha^{-1} + 30 kg nitrogen ha^{-1});
T_4 – Rice straw + green manure [*Sesbania aculeata*] (1:1 nitrogen basis) (30 kg nitrogen ha^{-1} + 30 kg nitrogen ha^{-1}).

2.3. Soil sampling and storage

Soil samples were collected at different crop growth stages of the rice crop by a sample probe from depths of 0–15, 15–30, 30–45 and 45–60 cm. Individual soil cores were taken at four different growth stages: maximum tillering (50 days after transplanting), panicle initiation (75 days after transplanting), grain filling (102 days after transplanting) and harvest (126 days after transplanting). Sampling was done with a sample probe from five different places within individual replicated plots (three replicated plots in a randomized block design for each treatment) and these five samples were mixed together to prepare a composite sample for the plot. All the samples were collected from in between the planted rows for better comparison. Immediately after sampling, excess water was allowed to drain off, visible root fragments and stones removed manually and transferred to the laboratory for analyses. Moisture content of individual samples was determined gravimetrically in 10 g portions after drying at 105 °C for 48 h. The fresh soil samples were kept in the refrigerator at 4 °C for biochemical and microbial population analyses. The fresh soil was air-dried for 7 days, sieved through a 2 mm mesh, mixed and stored in sealed plastic jars for analyses of soil carbon and nitrogen fractions.

2.4. Soil carbon fraction analysis

Soil microbial biomass carbon (MBC) was measured by using a modified chloroform fumigation–extraction method with fumigation at atmospheric pressure (Witt et al., 2000a). 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). Oxidizable organic carbon was estimated by dichromate digestion of the soil (Walkley and Black, 1934). Acid hydrolyzable carbohydrate carbon (AHC) was measured by taking the equivalent weight of 2 g of soil extracted with 20 ml of 1.5 M sulfuric acid (H_2SO_4) (Angers and Mehuys, 1989) for 24 h with regular shaking and filtered through glass fiber filters (Whatman GF/C). The carbohydrate content of the extracts was determined by using the anthrone method (Yoshida et al., 1976). Water soluble carbohydrate carbon (WSC) content was estimated by following the procedure of Haynes and Swift (1990). Permanganate oxidizable carbon ($KMnO_4$-C) was determined by following the method described by Blair et al. (1995) with a few modifications. Dry soil (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 2000 rpm. 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 carbon oxidized; assuming that 1 mM $KMnO_4$ is consumed in the oxidation of 0.75 mM or 9 g of carbon. Ammonium-nitrogen (NH_4^+-N) in the soil extract was estimated by Nesslerization (Jackson, 1973) and nitrate-nitrogen (NO_3^--N) by using the 2,4-phenol disulphonic

Table 1
Composition of different organic amendments and initial soil samples before adding treatments (dry weight basis).

Organic amendments	Carbon (g kg^{-1})	Nitrogen (g kg^{-1})	Carbon:nitrogen	Phosphorous (P) (g kg^{-1})	Potassium (K) (g kg^{-1})
Green manure (*Sesbania aculeata*)	370	25.5	14.5	2.2	23.5
Rice straw	383	6.2	61.7	1.4	19.4
Initial soil	4.9	0.49	10.1	0.018	0.286

acid method (Bremner, 1965). Ninhydrin-reactive nitrogen (NRN) in 20-g soil samples was extracted with 0.5 M potassium sulfate (K_2SO_4) and estimated colorimetrically after mixing the soil extracts with ninhydrin (Badalucco et al., 1992). Total carbon (TC) and total nitrogen contents were estimated by dry combustion in a carbon–hydrogen–nitrogen analyzer (CHN analyzer) (FLASH 2000 Organic Elemental analyzer, M/S Thermo Scientific).

2.5. Soil carbon storage, carbon management index (CMI) analysis

Carbon storage was calculated in terms of the increase in carbon stock in soil. Data on initial and final organic carbon concentrations in the urea, rice straw + urea, rice straw + green manure and unfertilized (control) treatments were collected. The mass of SOC in the soil was calculated as described by Pathak et al. (2011) MSOC = SOC × BD × T; where MSOC is the mass of total carbon (Mg ha^{-1}), SOC is the change in percentage of total carbon concentration in soil in a given period of time, BD is bulk density (Mg m^{-3}) and T is the thickness of the soil layer (cm). Carbon management index (CMI) was calculated for each treatment by using a reference sample value according to the method of Blair et al. (1995). The mean of the three control samples (unfertilized control plot) was used as the reference in the experiment (Gong et al., 2009). Based on changes in the total carbon content between the reference and sample sites, a carbon pool index (CPI) was calculated as follows as described by Lou et al. (2011): CPI = [sample TC/TC of reference soil]; based on the changes in the C lability (L) = [($KMnO_4$-C/(TC − $KMnO_4$-C)] the lability index was determined as: LI = [sample L/reference L] and CMI = [CPI × LI × 100].

2.6. Soil enzymatic activities and microbial populations

Dehydrogenase activity was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) (Casida et al., 1964). Fluorescein diacetate (FDA) hydrolysis activity measurements were made by following the method of Adam and Duncan (2001). β-Glucosidase activity was assayed by following the procedure of Eivazi and Tabatabai (1988). Urease activity was measured by following the method of Tabatabai and Bremner (1972). Five grams of soil were incubated with 5 ml of 0.05 M tris (hydroxymethyl) aminomethane (THAM) buffer (pH 9.0) and 1 ml of 0.2% of urea solution at 37 °C for 2 h. Excess urea was extracted with a potassium chloride-phenyl mercuric acetate (KCl–PMA) solution and estimated colorimetrically at 527 nm. Heterotrophic microbial populations were cultured by using the media of Rand et al. (1975). The methanogenic bacterial population was enumerated by using the anaerobic culture tube technique (Kasper and Tiedje, 1982). Culturable NH_4^+ and NO_2^--oxidizing autotrophs were enumerated by following the most probable number (MPN) method (Schmidt and Belser, 1982). Populations of denitrifying bacteria were estimated by method of Abd-el-Malek et al. (1974).

2.7. Methane and nitrous oxide flux measurements

Methane and nitrous oxide flux from the rice field plots was monitored by using the manual closed chamber method (Datta et al., 2009). 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. The gas samplings were done soon after the transplanting of the rice crop at closed intervals throughout the cropping season. The samplings were done at 3, 7, 17, 21, 26, 33, 38, 42, 47, 54, 58, 62, 69, 76, 81, 88, 95, 102, 107, 112, 118, 123 and 128 days after transplanting (DATs) of the rice crop. The sampling days encompassed the greenhouse gas fluxes from flooded rice paddy starting from initial growth phase of the crop to its vegetative, reproductive phase till its physiological maturity during the cropping season. For measuring CH_4 and N_2O emissions, six rice hills were covered with a locally 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) and connected to polyethylene tubing was used to mix the air inside the chamber and draw the air samples into Tedlar gas-sampling bags (M/s Aerovironment Inc.) at fixed intervals of 0, 15 and 30 min. Gas samples from the sampling bags were analyzed for CH_4 by gas chromatography. Methane concentrations in the air samples collected from the crop canopy 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 inch 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 (nitrogen) flow was maintained at 15 ml min^{-1}. The gas chromatograph was calibrated before and after each set of measurements by using 5.38, 9.03 and 10.8 μL CH_4 L^{-1} in N_2 (Scotty II analyzed gases, M/s Altech associates Inc., USA) as the primary standard and 1.95 μL L^{-1} in air as the secondary standard to provide a standard curve that was linear over the concentration range used in this study. N_2O concentrations 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 inch 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 (nitrogen) flow was maintained at 15 ml min^{-1}. The gas chromatograph was calibrated before and after each set of measurements by using 100 parts per billion (ppb) N_2O in N_2 (Scotty II analyzed gases, M/s Altech Associates Inc., USA) as the primary standard and 316 ppb N_2O in N_2 (National Physical Laboratory, New Delhi) 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 was expressed as kg ha^{-1}.

2.8. Carbon dioxide flux measurement

CO_2 flux was measured 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 high, 10 cm in diameter, and had the 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 in each plot until CO_2 flux measurements were recorded in the data logger. The CO_2 flux was recorded in the inter-row positions of the rice plants. All measurements were taken between 09:00 and 12:00 h and between 15:00 and 17:00 h. The average of the morning and evening flux was considered as the daily flux.

2.9. Global warming potential (GWP) measurements

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 situation. 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 by using the Intergovernmental Panel on Climate Change (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 treatment conditions (IPCC, 2007). Carbon equivalent emissions (CEE) and carbon efficiency ratios (CERs) of the treatments were calculated by using the following equations (Bhatia et al., 2005):

CEE = [GWP × 12]/44 and CER = [grain yield (in terms of carbon) of the rice/CEE]; the carbon concentration in the grain was measured and found to be 43%.

2.10. Statistical analysis

Individual character datasets were subjected to analysis of variance and the means were separated by Duncan's Multiple Range Test (DMRT) at the 0.05 level of probability by using the statistical software, SPSS (Statistical Package for Social Sciences), version 7.5. Pearson correlation (r) was applied to analyze the simple correlations between CH_4, CO_2-C, N_2O flux, select soil parameters, enzymatic activities and the microbial populations.

3. Results

3.1. Methane (CH_4) emission

Fertilizer application had significant ($p < 0.05$) effects on methane emission. Methane flux varied significantly between 1.9 and 5.7 mg m^{-2} h^{-1} [Fig. 1(a)] during the entire cropping period. The highest flux was observed in 76 days after transplanting (76 DAT) irrespective of the treatment and a statistically significant ($p < 0.05$) maximum value of 5.7 mg m^{-2} h^{-1} [Fig. 1(a)] was observed in the case of the combined treatment with rice straw and urea. Methane emission followed almost similar patterns in control as well as fertilizer-treated plots although the magnitude of emissions varied. On a seasonal basis, cumulative emission of methane was lowest (69.7 kg ha^{-1}) in the control treatment and was the highest (122.7 kg ha^{-1}) after the combined

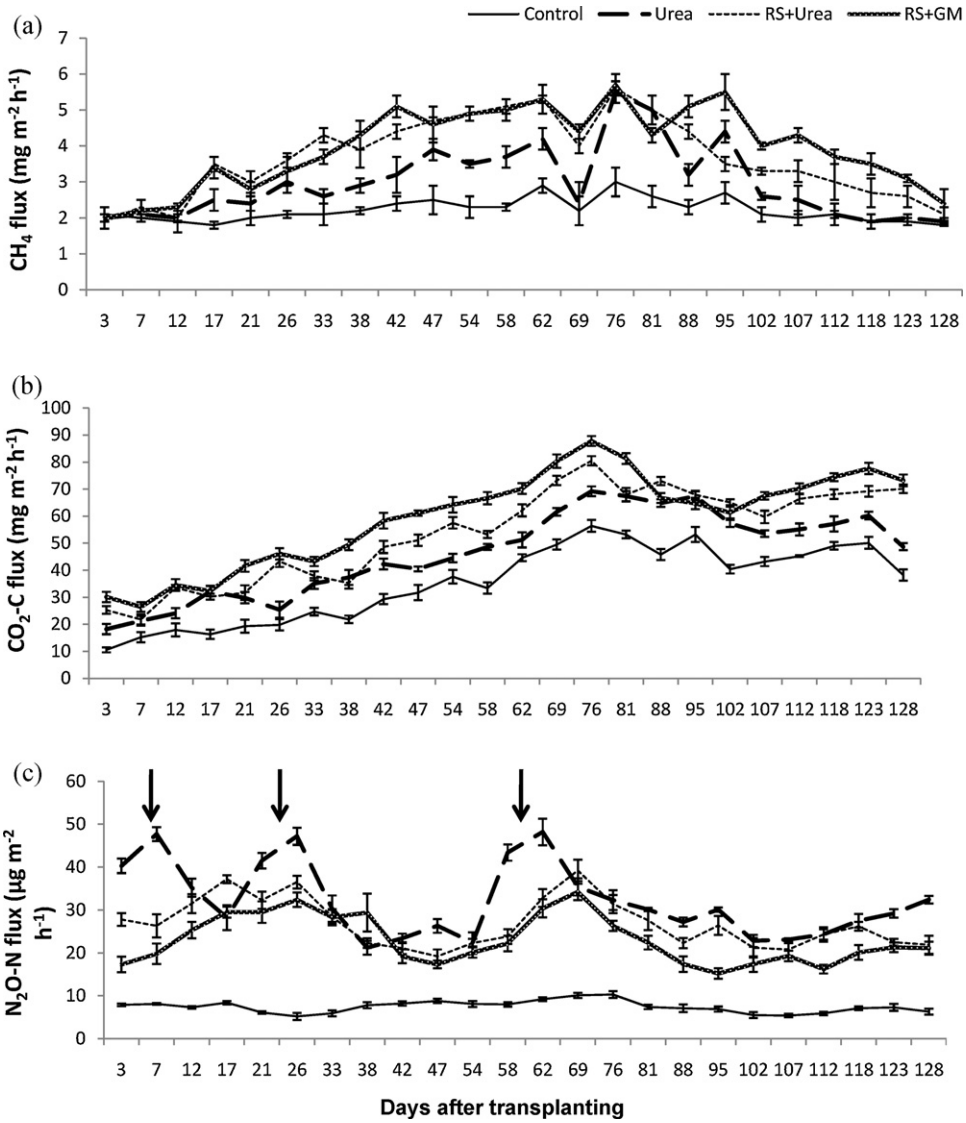

Fig. 1. Methane (CH_4) (a), carbon dioxide (CO_2-C) (b) and nitrous oxide (N_2O-N) (c) flux from soils during different days after transplantation of rice crop after 4 years of cultivation under the various treatments: unfertilized control, urea, rice straw + urea and rice straw + green manure (GM). Error bars are the standard deviations of three replicate observations. Arrow indicates time of fertilizer applications.

Table 2
Green house gas emissions on seasonal basis and related parameters after the application of inorganic fertilizers and organic manure to flooded soil planted with rice (*cv. Gayatri*).

Treatment	CH_4 emission (kg ha^{-1})	CO_2-C emission (kg ha^{-1})	N_2O-N emission (kg ha^{-1})	GWP of rice system (kg CO_2 ha^{-1})	Carbon equivalent emission (CEE) (kg C ha^{-1})	Yield (Mg ha^{-1})	Carbon efficiency ratio (CER)
Control	69.7a	1100.3a	0.23a	5862a	1599a	3.53a	0.95b
Urea	92.6b	1447.7b	1.00d	8084b	2205b	5.13b	1.00b
Rice straw + urea	115.4c	1680.6c	0.84c	9418c	2568c	5.57b	0.93b
Rice straw + green manure	122.7c	1858.5d	0.72b	10188d	2779d	5.30b	0.82a

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

application of rice straw and green manure (Table 2). However, methane emission after application of rice straw and green manure was at par with the combined application of rice straw + urea (115.4 kg ha^{-1}) on a seasonal basis (Table 2). Cumulative methane emissions were in the order of rice straw + green manure (122.7 kg ha^{-1}) = rice straw + urea (115.4 kg ha^{-1}) > urea (92.6 kg ha^{-1}) > control (69.7 kg ha^{-1}) (Table 2). Application of rice straw + urea and rice straw + green manure increased the methane flux significantly ($p < 0.05$) by 66 and 75% over the control, respectively, on a seasonal basis.

3.2. Carbon dioxide-carbon (CO_2-C) emission

Soil carbon dioxide flux significantly ($p < 0.05$) increased soon after the transplantation of the rice crop and reached the maximum at 76 days after transplanting (76 DAT) irrespective of the treatment [Fig. 1(b)]. The CO_2 flux after the treatments varied between 10.5 and 87.8 mg CO_2-C m^{-2} h^{-1} [Fig. 1(b)]. The highest CO_2 flux (87.8 mg CO_2-C m^{-2} h^{-1}) was observed in the case of the combined treatment of rice straw and green manure [Fig. 1(b)]. Cumulative seasonal emissions of CO_2-C ranged from 1100.3 kg ha^{-1} in the control treatment to 1858.5 kg ha^{-1} in the rice straw + green manure treatment (Table 2). Cumulative CO_2-C emissions were in the order of rice straw + green manure (1858.5 kg ha^{-1}) > rice straw + urea (1680.6 kg ha^{-1}) > urea (1447.7 kg ha^{-1}) > control (1100.3 kg ha^{-1}) (Table 2). Application of rice straw + urea and rice straw + green manure increased the methane flux significantly ($p < 0.05$) by 53 and 69% over the control, respectively, on a seasonal basis.

3.3. Nitrous oxide emission (N_2O) emission

Significantly lower N_2O emissions were recorded following the application of rice straw + green manure when compared to the other treatments. In contrast, distinctly higher N_2O emissions were recorded when urea either in a single dose or combined with rice straw was applied to the field [Fig. 1(c)]. A peak of N_2O was initially recorded immediately after urea application in the urea treatment [Fig. 1(c)]. On the other hand, the initial peak of N_2O was delayed in the rice straw + urea and rice straw + green manure treatments [Fig. 1(c)]. Cumulative N_2O-N emissions were in the order of urea (1.0 kg ha^{-1}) > rice straw + urea (0.84 kg ha^{-1}) > rice straw + green manure (0.72 kg ha^{-1}) > control (0.23 kg ha^{-1}) (Table 2).

3.4. Global warming potential (GWP) and carbon equivalent emission (CEE)

Global warming potential in terms of gaseous carbon emission varied significantly ($p < 0.05$) with various treatments between 5862 kg CO_2 equivalent ha^{-1} in the control to 10,163 kg CO_2 equivalent ha^{-1} after the composite application of rice straw and green manure treatment (Table 2). GWP on CO_2 equivalent basis was in the order of rice straw + green manure (10,188 kg CO_2 equivalent ha^{-1}) > rice straw + urea (9418 kg CO_2 equivalent ha^{-1}) > urea (8084 kg CO_2 equivalent ha^{-1}) > control (5862 kg CO_2 equivalent ha^{-1}) (Table 2). CEE was the lowest (1599 kg carbon ha^{-1}) in the control (Table 2). Among the treatments, the highest CEE (2779 kg carbon ha^{-1}) was observed in the rice straw + green manure treatment (Table 2).

3.5. Yield of rice and carbon efficiency ratio (CER)

Yields of rice ranged from 3.53 to 5.57 Mg ha^{-1} (Table 2). The yields after treatment with rice straw + urea (5.57 Mg ha^{-1}), rice straw + green manure (5.30 Mg ha^{-1}) and urea alone (5.13 Mg ha^{-1}) were similar but significantly higher than the control (3.53 Mg ha^{-1}, Table 2). During the previous three years of study, the grain yield of rice ranged from 3.50 to 3.87 (SD ± 0.19), 5.10 to 5.23 (SD ± 0.07), 5.47 to 5.54 (SD ± 0.04) and 5.28 to 5.34 (SD ± 0.03) Mg ha^{-1} under the control, urea, rice straw + urea and rice straw + green manure treatment, respectively. The CER, *i.e.*, the carbon fixed in grain by rice per unit of carbon emitted, was the highest (1.0) in the urea-treated plots, followed by the control (0.95), and then, finally the rice straw + urea treatment (0.93) (Table 2). The lowest CER (0.82) was observed in the rice straw + green manure treatment (Table 2).

3.6. Soil carbon pools

3.6.1. Soil carbon fractions

Microbial biomass carbon (MBC) ranged from 192.7 to 416.9 mg kg^{-1} and accounted for 3.9–5.7% of the total carbon in the soils under study (Table 3). The application of rice straw + green manure resulted in a significantly ($p < 0.05$) higher (416.9 mg kg^{-1}) accumulation of microbial biomass carbon (Table 3) than the other treatments. The application of urea and rice straw + urea showed significant increase in MBC by 65 and 75%, respectively, when compared to the control (Table 3). The combined application of rice straw + urea significantly increased the total carbon content (7.49 g kg^{-1}) compared to the other treatments (Table 3). The ratio of MBC to organic carbon was significantly ($p < 0.05$) higher in the rice straw + green manure (9.50) treatment than in the other treatments (Table 3). The readily mineralizable carbon (RMC) content was highest (188.8 mg kg^{-1}) in plots receiving the rice straw + green manure and the lowest in unamended control plots (37.8 mg kg^{-1}, Table 3). The water-soluble carbohydrate carbon (WSC) and acid-hydrolyzable carbohydrate carbon (AHC) contents significantly ($p < 0.05$) varied among the different treatments from 11.1 to 48.3 mg carbon kg^{-1} and 396.5 to 604 mg carbon kg^{-1}, respectively, and the highest value was obtained for the combined treatment of rice straw and green manure (Table 3). WSC and AHC accounted for 0.29–0.88% and 10.3–11.1% of organic carbon, respectively. Permanganate-oxidizable carbon ($KMnO_4$-C) varied from 315.2 to 472.7 mg carbon kg^{-1} for the different treatments and the highest value (472.7 mg carbon kg^{-1}) was recorded for the combined treatment of rice straw and green manure (Table 3). The soil carbon management index (CMI) showed a significant decline in the order

Table 3
Soil carbon fractions and carbon management index in different inorganic and organic manure-treated soil [0–15 cm] planted with rice (*cv. Gayatri*) under flooded conditions.

Treatment	MBC (mg kg^{-1})	RMC (mg kg^{-1})	WSC (mg kg^{-1})	AHC (mg kg^{-1})	TC (g kg^{-1})	MBC/OC (%)	$KMnO_4$-C (mg kg^{-1})	CMI
Control	192.7a	37.8a	11.1a	396.5a	4.94a	5.05a	315.2a	100a
Urea	317.0b	86.8b	29.5b	487.3b	5.81b	7.60b	367.1b	116.4b
Rice straw + urea	337.8b	147.2c	34.5c	542.0c	7.49d	7.64b	434.9c	137.1c
Rice straw + green manure	416.9c	188.8d	48.3d	604.0d	7.26c	9.50c	472.7d	150.2d

Mean values from 4 years of experimentation presented in table.
MBC: microbial biomass carbon; RMC: readily mineralizable carbon; WSC: water-soluble carbohydrate carbon; AHC: acid-hydrolyzable carbohydrate carbon; TC: total carbon in soil; MBC/OC: ratio of soil microbial biomass carbon to soil organic carbon; $KMnO_4$-C: permanganate oxidizable carbon; CMI: soil carbon management index.
Note: In each column, the mean values followed by common letters are not significantly different ($p < 0.05$) between treatments according to Duncan's multiple range test (DMRT).

Table 4
Soil nitrogen fractions and total carbon to total nitrogen ratio in different inorganic and organic manure-treated soil [0–15 cm] planted with rice (*cv. Gayatri*) under flooded conditions.

Treatment	TN (g kg^{-1})	TC:TN	NH_4^+-N (μg g^{-1} soil)	NO_3^--N (μg g^{-1} soil)	NRN (μg g^{-1} soil)
Control	0.49a	10.1a	11.1a	5.6a	3.3a
Urea	0.53b	11.0b	38.6d	22.7c	10.5d
Rice straw + urea	0.64d	11.7c	28.8c	19.8c	8.8c
Rice straw + green manure	0.59c	12.3d	22.8b	15.1b	6.9b

Mean values of 4 years of experimentation presented in table.
TN: total nitrogen; TC:TN: total carbon to total nitrogen ratio; NH_4^+-N: ammonical nitrogen; NO_3^--N: nitrate nitrogen; NRN: ninhydrin-reactive nitrogen.
Note: In each column, the mean values followed by common letters are not significantly different ($p < 0.05$) between treatments according to Duncan's multiple range test (DMRT).

of rice straw + green manure (150.2) > rice straw + urea (137.1) > urea (116.4) > control (100.0) (Table 3). There were no significant differences in the carbon fractions from the lower soil depths (data not shown). Different applications of fertilizers had significantly affected total nitrogen, carbon: nitrogen ratio and nitrogen fractions such as ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and ninhydrin-reactive nitrogen (NRN) contents (Table 4). Total nitrogen content was found to be significantly higher (0.64 g kg^{-1}) in the combined treatment of rice straw + urea treatment compared to the other treatments under study (Table 4). The carbon:nitrogen ratio was found to be significantly higher (12.3) in the combined treatment of rice straw + green manure (Table 4) than in any of the other treatments. The ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were significantly increased in soil treated with urea (Table 4). Like ammonium nitrogen and nitrate nitrogen contents in the soil, significantly higher (10.5 μg g^{-1} soil) ninhydrin-reactive nitrogen (NRN) content was recorded in the soils from plots treated with urea followed by rice straw + urea (8.8 μg g^{-1} soil), rice straw + green manure (6.9 μg g^{-1} soil) and control (3.3 μg g^{-1} soil, Table 4).

3.6.2. Soil organic carbon, bulk density and carbon storage

Soil organic carbon (SOC) was found to be significantly ($p < 0.05$) higher (4.42 g kg^{-1}) in rice straw + urea treatment than in the control (3.82 g kg^{-1}) (Table 5). There was significant decrease in the carbon content in the subsurface soil (depths of 15–30, 30–45 and 45–60 cm) (Table 5). There were significant differences in the bulk density at different depths of the soil, the highest (1.72 Mg m^{-3}) one being obtained from a soil depth of 45–60 cm after the combined treatment of rice straw and green

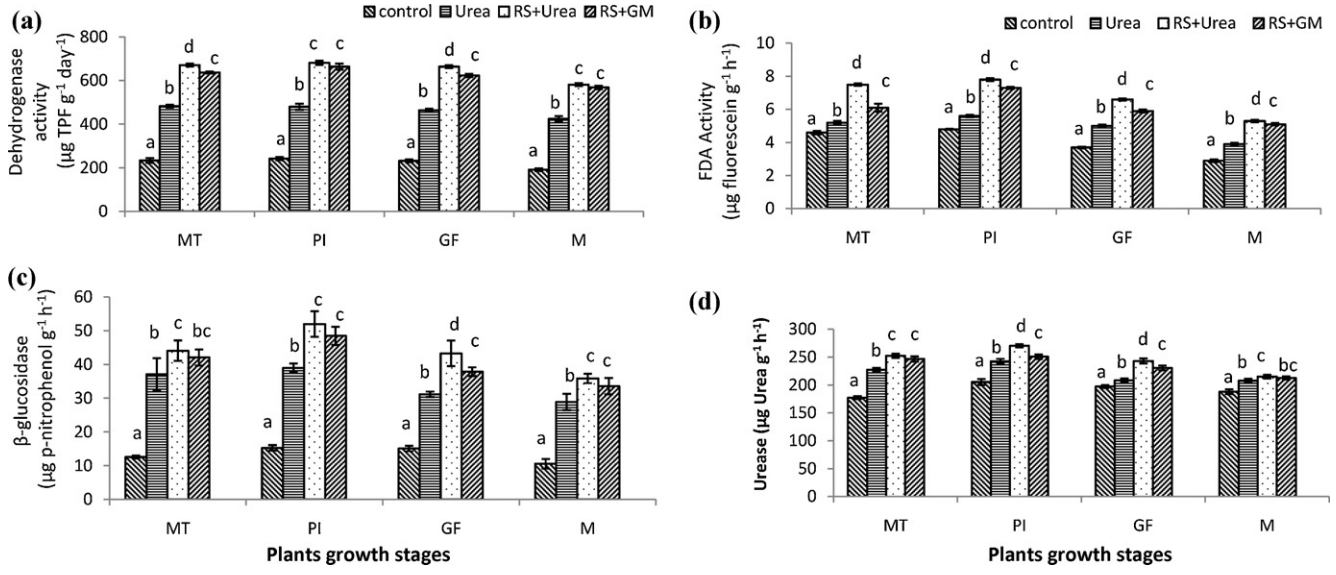

Fig. 2. Dehydrogenase (a), fluorescein diacetate (FDA) (b), β-glucosidase (c) and urease (d) activity of the rhizosphere soils (0–15 cm soil depth) during various stages of crop growth for different treatments: unfertilized control, urea, rice straw (RS) + urea and rice straw (RS) + green manure (GM). 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 stage.

manure (Table 5). Total SOC storage in the 60-cm soil profile in the rice–rice cropping system was significantly higher (1.39 Mg ha^{-1}) after the combined application of rice straw and urea, followed by rice straw + green manure (0.88 Mg ha^{-1}), and then urea (0.29 Mg ha^{-1}) (Table 5). Interestingly, there was no build-up of carbon in the control plots (Table 5). Carbon storage rate was also found to be significantly higher (0.35 Mg carbon ha^{-1} yr^{-1}) in the combined application of rice straw + urea (Table 5).

3.7. Soil enzyme activities and microbial populations

Soil enzyme activities and microbial populations were strongly affected by the application of inorganic or combined doses of inorganic and organic fertilizers. Application of manure increased dehydrogenase activity, which is an index of microbial activity of soil. Soil dehydrogenase activity was enhanced significantly ($p < 0.05$) on the application of rice straw + urea when compared to the other treatments throughout all crop growth phases [Fig. 2(a)]. Dehydrogenase activity ranged from 191.1 to 681.4 μg TPF g^{-1} d^{-1} [Fig. 2(a)] and maximal activity was obtained in the panicle initiation stage of crop development. The highest FDA activity was found after the combined application of rice straw and urea (7.8 μg fluorescein g^{-1} h^{-1}), followed by rice straw and green manure (7.0 μg fluorescein g^{-1} h^{-1}) [Fig. 2(b)]. The β-glucosidase activity ranged from 10.6 to 52 μg *p*-nitrophenol g^{-1} h^{-1} throughout different crop development stages, the maximal activity (52 μg p-nitrophenol g^{-1} h^{-1}) being observed in the plots receiving both rice straw and urea in the panicle initiation stage [Fig. 2(c)]. The activity of urease followed the same trend as those seen for the other three enzymes under study. Interestingly, a single application of urea in the recommended dose inhibited urease activity. On an average, urease activity ranged from 177.4 to 251 μg urea g^{-1} h^{-1} [Fig. 2(d)], with the maximal activity (251 μg urea g^{-1} h^{-1}) of urease being found after the combined treatment of rice straw and urea [Fig. 2(d)]. The abundance of heterotrophs was significantly ($p < 0.05$) enhanced by different fertilizations in comparison with the control condition [Fig. 3(a)]. The highest log colony forming units (cfu) (9.29) value was observed in the rice straw + green manure treatment in the panicle initiation stage [Fig. 3(a)]. Methanogenic populations were in the range of 3.07–3.95 log MPN, and the largest population was observed in the combined treatment of rice straw and green manure in the panicle initiation stage [Fig. 3(b)A]. Ammonifier, nitrifier and denitrifier populations increased steadily up to the panicle initiation stage and decreased moderately thereafter [Fig. 3(b) B, C and D]. The log MPN values of ammonifiers, nitrifiers and denitrifiers were found to be maximal (3.64, 3.45 and 5.12, respectively) in the urea-treated plots compared to the other treatments [Fig. 3(b) B, C and D], which gave rise to high rates of N_2O-N flux.

3.8. Correlation analysis

Significant and positive correlations were found between CH_4, CO_2-C as well as N_2O flux and different soil carbon pools (MBC, RMC, TC), global warming potential, carbon storage and the carbon management index. Soil enzyme activities (dehydrogenase, FDA, β-glucosidase and urease) of the microbial populations showed significant positive correlations with soil organic carbon, indicating that soil functional and microbial population dynamics (with respect to GHG emission) depends upon the soil carbon concentrations. The CH_4 and CO_2-C flux positively correlated with MBC ($r = 0.92^{**}$, 0.92^{**}), RMC ($r = 0.97^{**}$, 0.96^{**}), TC ($r = 0.96^{**}$, 0.93^{**}), GWP ($r = 0.98^{**}$, 1.00^{**}), carbon storage ($r = 0.93^{**}$, 0.91^{**}) and CMI ($r = 0.97^{**}$, 0.97^{**}) (Table 6). In this study, the organic carbon content of soil correlated positively with activities of dehydrogenase ($r = 0.99^{**}$), FDA ($r = 0.95^{**}$), β-glucosidase

Table 5
Soil organic carbon, bulk density and carbon storage in different inorganic and organic manure-treated soil planted with rice (*cv. Gayatri*) under flooded conditions.

Treatments	Initial organic carbon (4 years before) (g kg^{-1})				Present organic carbon (after 4 years) (g kg^{-1})				Bulk density (Mg m^{-3})				Carbon storage (Mg C ha^{-1})	Carbon storage (Mg C ha^{-1} yr^{-1})
	0–15 cm	15–30 cm	30–45 cm	45–60 cm	0–15 cm	15–30 cm	30–45 cm	45–60 cm	0–15 cm	15–30 cm	30–45 cm	45–60 cm		
Control	4.14a	2.12a	1.66a	1.60a	3.82a	1.77a	1.64a	1.59a	1.41a	1.62a	1.66a	1.713ab	−1.63a	−0.41a
Urea	4.14a	2.12a	1.66a	1.60a	4.17b	2.14b	1.71b	1.62a	1.42a	1.63a	1.67a	1.716ab	0.29b	0.07b
Rice straw + urea	4.14a	2.12a	1.66a	1.60a	4.42c	2.30c	1.76c	1.64a	1.42a	1.63a	1.67a	1.70a	1.39c	0.35c
Rice straw + green manure	4.14a	2.12a	1.66a	1.60a	4.39c	2.17b	1.73b	1.62a	1.42a	1.63a	1.67a	1.72b	0.88c	0.22c

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

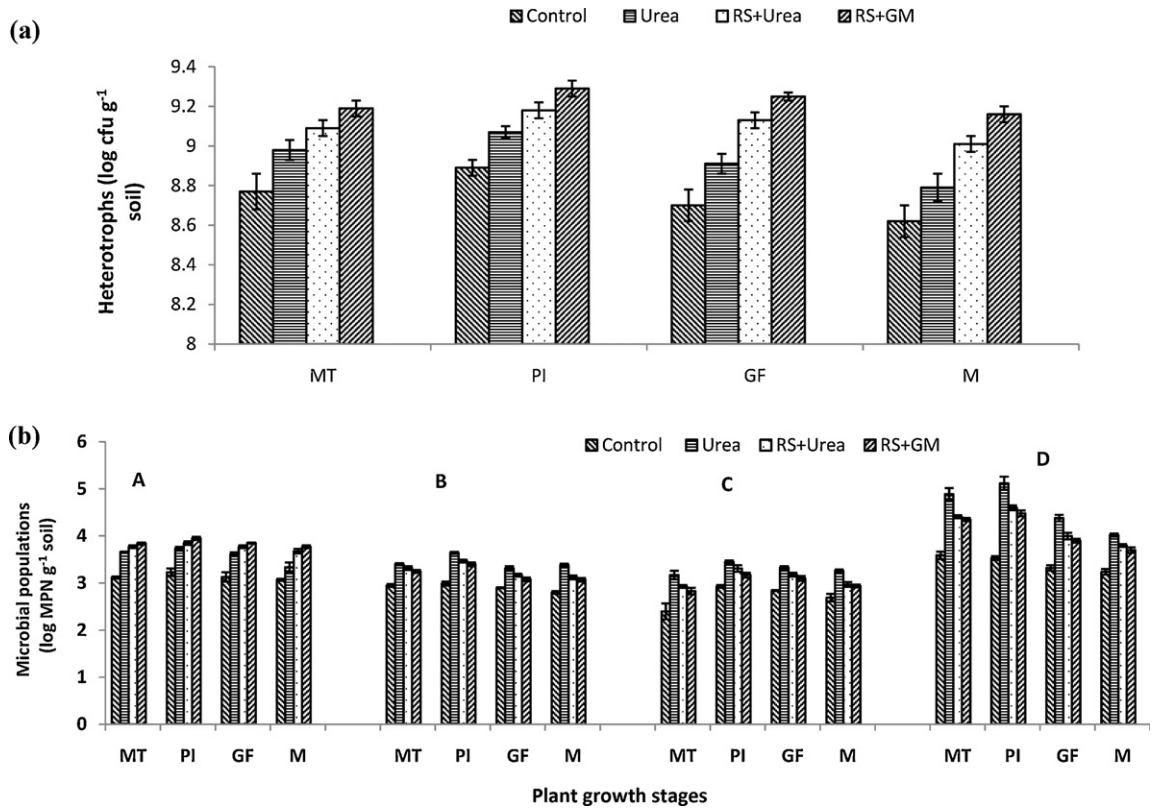

Fig. 3. Effects of inorganic fertilizers and organic amendments after 4 years of cultivation on (a) heterotrophic microbes and (b) methanogens (A), ammonia oxidizers (B), nitrite oxidizers (C), denitrifier (D) population dynamics of soil planted with rice. Treatment included unfertilized control, urea, rice straw (RS) + urea and rice straw (RS) + green manure (GM). Here, MT = maximum tillering stage; PI = panicle initiation stage; GF = grain filling stage; M = maturity stage.

($r = 0.98^{**}$), urease ($r = 0.97^{**}$), the heterotrophs ($r = 0.94^{**}$), methanogens ($r = 0.98^{**}$), ammonifiers ($r = 0.65^{*}$), nitrifiers ($r = 0.61^{*}$) and denitrifiers ($r = 0.65^{*}$) in the soil (Table 7).

4. Discussion

4.1. Effects of fertilization on methane (CH_4) emission

Rice cultivation is an important anthropogenic source of atmospheric CH_4. In the present study, the application of rice straw, nitrogen fertilizer and organic manures resulted in an increase in CH_4 emission. Under submerged conditions, emission of CH_4 resulted from carbon mineralization (Kimura et al., 2004), which was enhanced by fertilization. Microbial biomass and enzyme activity increased due to SOC mineralization but the predominant pathway of CH_4 formation was generally considered to be unchanged after different fertilizer treatments. The capacity of CH_4 production was likely to depend on the available carbon resources for microbes. Application of different fertilizers, especially in combination with manure, has been shown to enhance the

Table 6
Correlation matrix among methane, carbon dioxide emission, nitrous oxide emission, soil carbon and nitrogen fractions, global warming potential and yield in different inorganic fertilizer- and organic manure-treated soil planted with rice (*cv. Gayatri*) under flooded conditions.

	CO_2	CMI	CST	GWP	MBC	CH_4	AN	NN	NRN	N_2O	RMC	TC	Y
CO_2	1.00												
CMI	0.97^{**}	1.00											
CST	0.91^{**}	0.87^{**}	1.00										
GWP	1.00^{**}	0.97^{**}	0.94^{**}	1.00									
MBC	0.92^{**}	0.92^{**}	0.86^{**}	0.93^{**}	1.00								
CH_4	0.98^{**}	0.97^{**}	0.93^{**}	0.98^{**}	0.92^{**}	1.00							
AN	0.42^{ns}	0.28^{ns}	0.65^{*}	0.48^{ns}	0.47^{ns}	0.39^{ns}	1.00						
NN	0.58^{*}	0.44^{ns}	0.77^{**}	0.63^{*}	0.56^{ns}	0.54^{ns}	0.97^{**}	1.00					
NRN	0.51^{ns}	0.39^{ns}	0.75^{**}	0.57^{ns}	0.55^{ns}	0.50^{ns}	0.98^{**}	0.98^{**}	1.00				
N_2O	0.62^{*}	0.50^{ns}	0.81^{**}	0.68^{*}	0.64^{*}	0.60^{*}	0.96^{**}	0.98^{**}	0.99^{**}	1.00			
RMC	0.96^{**}	0.97^{**}	0.86^{**}	0.96^{**}	0.92^{**}	0.97^{**}	0.26^{ns}	0.42^{ns}	0.38^{ns}	0.49^{ns}	1.00		
TC	0.93^{**}	0.94^{**}	0.93^{**}	0.94^{**}	0.83^{**}	0.96^{**}	0.34^{ns}	0.53^{ns}	0.47^{ns}	0.55^{ns}	0.93^{**}	1.00	
Y	0.86^{**}	0.78^{**}	0.96^{**}	0.89^{**}	0.81^{**}	0.85^{**}	0.74^{**}	0.84^{**}	0.81^{**}	0.85^{**}	0.77^{**}	0.84^{**}	1.00

CO_2: seasonal carbon released from carbon dioxide (CO_2-C) emission; CMI: soil carbon management index; CST: soil carbon storage; GWP: global warming potential; MBC: microbial biomass carbon; CH_4: seasonal methane emission flux; AN: ammonium nitrogen content in soil; NN: nitrate nitrogen content in soil; NRN: ninhydrin-reactive nitrogen content in soil; N_2O: seasonal nitrogen released from nitrous oxide (N_2O-N) emission; RMC: readily mineralizable carbon; TC: total carbon content in soil; Yield: rice yield after harvest.
ns = non-significant at 1% and 5% levels of significance.
* Correlation is significant at $p < 0.05$.
** Correlation is significant at $p < 0.01$.

Table 7
Correlation matrix among soil organic carbon, select soil labile fractions soil enzyme activities and microbial populations in different inorganic and organic manure-treated soil planted with rice (*cv. Gayatri*) under flooded conditions.

	AHC	AMO	BGL	DEN	DHA	FDA	HET	POC	METH	NIT	OC	URS	WSC
AHC	1.00												
AMO	0.51^{ns}	1.00											
BGL	0.89**	0.74**	1.00										
DEN	0.53^{ns}	0.99**	0.74**	1.00									
DHA	0.92**	0.64*	0.98**	0.64*	1.00								
FDA	0.85**	0.49^{ns}	0.93**	0.48^{ns}	0.97**	1.00							
HET	0.99**	0.49^{ns}	0.91**	0.51^{ns}	0.94**	0.89**	1.00						
POC	0.97**	0.43^{ns}	0.88**	0.43^{ns}	0.94**	0.90**	0.98**	1.00					
METH	0.96**	0.68*	0.97**	0.69*	0.98**	0.90**	0.96**	0.94**	1.00				
NIT	0.46^{ns}	0.99**	0.70*	0.99**	0.58*	0.43^{ns}	0.43^{ns}	0.35^{ns}	0.63*	1.00			
OC	0.93**	0.65*	0.98**	0.65*	0.99**	0.95**	0.94**	0.93**	0.98**	0.61*	1.00		
URS	0.86**	0.66*	0.98**	0.66*	0.98**	0.97**	0.90**	0.88**	0.95**	0.62*	0.97**	1.00	
WSC	0.98**	0.56^{ns}	0.88**	0.58*	0.91**	0.80**	0.97**	0.96**	0.96**	0.50^{ns}	0.90**	0.84**	1.00

AHC: acid-hydrolyzable carbohydrate carbon; AMO: ammonifier population; BGL: β-glucosidase activity; DEN: denitrifying bacterial population; DHA: soil dehydrogenase activity; FDA: fluorescein diacetate activity; HET: heterotrophic microbial population; POC: permanganate oxidizable carbon; METH: methanogen population; NIT: nitrifying bacterial population; OC: organic carbon; URS: soil urease activity; WSC: water-soluble carbohydrate carbon.
ns = non-significant at 1% and 5% levels of significance.
* Correlation is significant at $p < 0.05$.
** Correlation is significant at $p < 0.01$.

bioavailable pool of organic carbon and, in turn, promote the CH_4 production by the utilization of readily bioavailable organic carbon by methanogenic microbes (Zheng et al., 2007). Diurnal variation of methane 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 (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). Extrapolation of daily flux data measured at closed frequency throughout the cropping period to seasonal flux is well established as reported by Bhatia et al. (2005), Das et al. (2011), Datta et al. (2009). The peaks of the methane flux were observed in 76 days after transplanting (76 DAT) of the rice crop irrespective of the treatments. The 76 DAT corresponds just after the panicle initiation stage of crop development. The higher methane 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 (for example methanogens, heterotrophs) were the highest during panicle initiation stage of crop development. The application of green manure and crop residues enhanced emission of methane by providing additional carbon substrates in comparison to the unfertilized conditions (Lu et al., 2000). The significantly ($p < 0.05$) different fluxes of CH_4 in the various treatments can be explained by the soil biomass carbon/organic carbon (MBC/OC) ratio. It has been suggested that the biomass carbon/organic carbon ratio reflects the potential for mineralization of soil organic matter after the addition of organic materials; the higher the ratio, the higher is the tendency of the organic matter to mineralize (Garcla-orenes et al., 2010). Based on this concept, soil treated with both rice straw and green manure would be characterized by the high mineralization potential of its organic matter, which in turn, corresponds to high gaseous carbon flux from the soil. In this study, all labile pools such as RMC, WSC and AHC in the soil show significantly higher values in the rice straw + green manure treatment, which promoted the growth of methanogens and produced higher methane emissions. Substrates such as green manure or rice straw decompose to produce acetate, which is the key component for the growth of the methanogens.

4.2. Effects of fertilization on carbon dioxide (CO_2) emission

The supply of nutrients via organic products would be expected to affect CO_2 flux in the soil by increasing carbon input from enhanced plant productivity and crop residue returned to soil. The application of organic amendments can affect mineralization rates of soil organic matter and contribute to increases in soil organic matter content by increasing residue input with increased crop production (Iqbal et al., 2009). Few datasets are currently available to characterize the effects of the application of inorganic fertilizer, inorganic fertilizer with organic manure or organic manure alone on soil CO_2 flux from the flooded rice paddy. In this study, the highest CO_2 flux was observed in the rice straw + green manure treatment, probably due to the efficient use of carbon for microbial growth in response to the application of the fertilizers (Fisk and Fahey, 2001). The peaks of the CO_2 flux were observed in 76 days after transplanting (76 DAT), i.e. just after panicle initiation stage. The higher flux was due to the availability of the C substrates in that 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). Extrapolation of daily CO_2 flux data measured at closed intervals throughout the cropping period to seasonal CO_2 flux is well known (Bhatia et al., 2005). Microbial biomass tends to be dynamic in soil with limited nutrient availability (Fontaine et al., 2004). Under such conditions, net soil carbon loss increases due to the enhancement in soil organic carbon mineralization. Rice straw with a high carbon/nitrogen ratio tends to decompose slowly due to limited nitrogen availability caused by net immobilization (Khalil et al., 2001). Rice straw in combination with urea resulted in a lower emission rate than rice straw in combination with green manure, possibly due to low turnover rates of the more stable microbial biomass. The rice straw when becomes associated with green manure, the labile carbon source 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).

4.3. Effects of fertilization on nitrous oxide (N_2O) emission

Urea application, either singly or in combination with rice straw, significantly increased the N_2O flux. Nitrification and denitrification are the two major microbial processes responsible for N_2O emission from flooded rice soils. Denitrification also acts as the sink for N_2O, especially under the submerged conditions of flooded rice soil. 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). Extrapolation of daily N_2O-N flux data measured at closed intervals throughout the cropping period to seasonal N_2O-N flux is also well studied (Bhatia et al., 2010; Das et al., 2011; Datta et al., 2009; Malla et al., 2005). Correlation analysis between ammoniacal nitrogen, nitrate nitrogen and N_2O-N showed a highly significant positive relationship ($r = 0.96^{**}$, 0.98^{**}), indicating that N_2O emission is a microbial process that depends on available carbon and nitrogen sources available to microorganisms. Ninhydrin-reactive nitrogen (NRN) content, an index of labile nitrogen available from microbial biomass in the rhizosphere soil (Nayak et al., 2007), was seen to increase significantly in soils treated with urea. Furthermore, NRN contents of the soils showed significant correlation with N_2O emission ($r = 0.99^{**}$), demonstrating the utilization of the readily available nitrogen source by microorganisms in response to the application of urea to produce N_2O. In this study, the lower emissions in the rice straw + green manure treatment were due to the slow decomposition of nitrogen due to the higher carbon:nitrogen ratio associated with this combination when compared to the other fertilizers (Bhatia et al., 2005).

4.4. Global warming potential (GWP) and carbon equivalent emission (CEE)

It is notably important that fertilization through the incorporation of organic manures or inorganic fertilizers significantly ($p < 0.05$) increased the total GWP of paddy soil. Correlation analysis showed that GWP of different manurial treatments were significantly ($p < 0.01$) correlated with the CH_4 flux ($r = 0.98^{**}$), CO_2-C flux ($r = 1.00^{**}$) and N_2O-N flux ($r = 0.68^{*}$). This indicates that the increase or decrease in GWP and CEE values would follow the same trend followed by CH_4, CO_2 and N_2O fluxes under the different treatment conditions.

4.5. Yields of rice and carbon efficiency ratio (CER)

In this study, yields of rice were similar for the different treated plots, although a comparatively higher yield was obtained in the rice straw + urea treatment, possibly due to the availability of nutrients. In agreement with the results reported by Goyal et al. (1997), our results also showed a higher crop yield with the addition of *Sesbania* in the form of green manure. The carbon efficiency ratio (CER) was the largest in the urea treatment due to lower gaseous carbon emission but similar to the control and rice straw + urea treatment, and was the smallest in the rice straw + green manure treatment due to the high gaseous carbon emission.

4.6. Effects of fertilization on soil carbon pools

4.6.1. Effects of fertilization on soil carbon fractions

The application of inorganic fertilizers, by themselves or in combination with organic manures, has been reported to significantly affect SOC and its fractions due to the significant increase in carbon input after manure application (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. Green manuring provides a potent source of labile carbon content. Hence, the application of green manure in combination with other manures, such as rice straw, leads to a high degree of gaseous carbon flux, a greater labile carbon pool and a low reserve of total carbon. Total nitrogen content was the highest in the rice straw + urea treatment because of the slow release of organically bound N (Bhatia et al., 2005), leading to accumulation of total nitrogen in the soil. The soluble carbon 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 carbon fractions, such as RMC, WSC, AHC and $KMnO_4$-C, all increased after the addition of green manure in combination with rice straw. There was a significant correlation between RMC and total carbon content ($r = 0.93^{**}$). 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 a single dose or in combination may contribute more labile carbon that can act as a source of energy and nutrients (Manna et al., 2007). Whitbread et al. (1998) suggested that the soil carbon management index (CMI) be used to describe soil fertility as it is a more sensitive indicator of the rate of change in SOC in response to soil management changes, than single measures such as the total SOC. In our study, CMI was more significantly enhanced by the organic treatments than by the nitrogen treatment. This was probably due to the increase in annual carbon input and the variations in organic matter quality, thus modifying the liability of carbon to $KMnO_4$ oxidation. These results are similar to those reported by Blair et al. (2006) who reported that manure alone and manure with inorganic fertilizer significantly increased CMI in comparison to any other chemical fertilizer treatment.

4.6.2. Effects of fertilization on organic carbon and carbon storage

Soil organic carbon (SOC) generally increases with carbon input before the soil becomes carbon saturated. Inorganic fertilizer, alone or in combination with organic manures, has been widely shown to increase SOC content (Blair et al., 2006; Purakayastha et al., 2008). This reflects the considerable carbon supplementation to soil with the applied fertilizers. Balanced fertilization is expected to increase SOC because of greater carbon input associated with enhanced primary production and crop residues returned to the soil. High rate of carbon sequestration in the rice system is due to the soil being under a unique flooded moisture regime for 3–4 months under the rice crop, and secondly, due to high biomass production in rice. Inadequate amounts of oxygen under submerged conditions lead to even modest oxygen demand for microbial activity not being met if large pores are filled with water, resulting in decreased rates of decomposition (Kukal et al., 2009). Sahrawat (2004) described preferential accumulation of organic matter in submerged rice soils as compared to aerobic soils due to the incomplete decomposition of organic materials and decreased humification of organic matter under flooded conditions. Consequently, the overall organic matter decomposition rates are slower in submerged soils than in aerobic soils. This results in a net accumulation of organic matter in soils that remain flooded for several years. Witt et al. (2000) also reported 11–12% greater carbon sequestration in soils that were continuously cropped with rice for two years than in maize–rice rotation systems with higher amounts sequestered in nitrogen fertilizer treatments. In this study, application of rice straw along with urea

as inorganic nitrogen fertilizer led to higher accumulation of carbon in the soil. The high lignin content in rice straw has been shown to lead to slow decomposition (Bhatia et al., 2005), which probably results in the accumulation of total carbon content observed in the present study.

4.7. Effects of fertilization on soil enzymes and microbial populations

The activities of assayed enzymes were generally well correlated with the organic carbon content because all these parameters were increased substantially by increasing returns of organic residues. Indeed, in general, there is a significant correlation between the activity of soil enzymes and organic carbon content (Graham and Haynes, 2005). In this study also, there were positive correlations between organic carbon content in soil and dehydrogenase ($r = 0.99^{**}$), FDA ($r = 0.95^{**}$), β-glucosidase ($r = 0.98^{**}$) and urease ($r = 0.97^{**}$) activity in the soil. 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). Dehydrogenase activity basically depends on the metabolic state of the soil biota. A significant increase in dehydrogenase activity occurred in the organically treated plots, especially the ones treated with urea. Total microbial activity, in terms of fluorescein diacetate hydrolysis, 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. β-Glucosidase is widely abundant, and is synthesized by soil microorganisms in response to the presence of suitable substrates. The highest β-glucosidase activity was recorded in the plot treated with urea + rice straw, probably due to the enrichment in fresh plant materials of a cellulolitic nature, which acted as substrates for the β-glucosidase enzyme. Urease activity decreased with the increasing application of NH_3 based-nitrogen fertilizers, as seen in the urea-treated plots, probably due to the presence of the end product of the enzymatic reaction (NH_4^+) that suppressed the synthesis of urease (Dick et al., 1988). Urease activity was found to be the highest for the combined treatment of rice straw and urea. Heterotrophic microbial populations were larger in the combined treatment of rice straw and green manure due to the bioavailability of growth-promoting substances, and showed positively correlation with soil labile carbon pools. Similarly, significant ($p < 0.05$) correlations were observed between methanogen populations and labile carbon pools such as WSC ($r = 0.96^{**}$), AHC ($r = 0.96^{**}$) and $KMnO_4$-C ($r = 0.94^{**}$). Nitrification and denitrification are the two major microbial processes responsible for N_2O emission from flooded rice soils. Ammonifiers and nitrifiers are involved in the nitrification process whereas denitrifiers 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.

5. Conclusions

The application of inorganic fertilizers in combination with organic manures for four years to a rice–rice tropical agroecosystem resulted in soil carbon build up and increase in crop productivity. However, carbon storage and carbon sequestration capacity were influenced by both the recalcitrant and labile nature of the inputs, varied significantly among the different treatments. The combined application of urea or green manure with rice straw resulted in higher GHG emissions, and also helped to build up carbon in the soil. Thus, the combination of an inorganic fertilizer, such as urea, with rice straw on a 1:1 nitrogen basis that resulted in a significant build-up of soil carbon, enhancement of crop yield and lower GHG emission when compared to rice straw and green manure, could be a viable option to mitigate global warming and maintain soil health.

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