



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 (CH₄), nitrous oxide (N₂O) and carbon dioxide (CO₂-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.

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1. Introduction

The increasing trend of greenhouse gases (GHGs) content in the atmosphere, such as those of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), 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 CO₂ 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 fractions, such as water soluble organic carbon (WSC), microbial biomass carbon (MBC), readily mineralizable carbon (RMC) and KMnO₄ 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 greenhouse 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 $15.2 \text{ cmol (p+) kg}^{-1}$, 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 $20 \text{ cm} \times 15 \text{ cm}$ 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 $7 \pm 3 \text{ cm}$ 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.

T₁ – control (without any fertilizers or organic manures)

T₂ – nitrogen (60 kg N ha^{-1} in wet season; 80 kg N ha^{-1} in dry season)

T₃ – FYM (5 Mg ha^{-1}) only in wet season

T₄ – FYM + nitrogen ($5 \text{ Mg ha}^{-1} + 60 \text{ kg N ha}^{-1}$ in wet season; 80 kg N ha^{-1} in dry season)

T₅ – NPK (60:30:30 kg ha^{-1} in wet season; $80:40:40 \text{ kg ha}^{-1}$ in dry season)

T₆ – FYM + NPK ($5 \text{ Mg ha}^{-1} + 60:30:30 \text{ kg ha}^{-1}$ in wet season; $80:40:40 \text{ kg ha}^{-1}$ 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 $0.5 \text{ M K}_2\text{SO}_4$ (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 $3400 \times g$. 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 $0.5 \text{ M K}_2\text{SO}_4$ 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

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