



## Original Articles

## Long-term management of rice agroecosystem towards climate change mitigation

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## ABSTRACT

The Intergovernmental Panel on Climate Change recognize to agriculture the responsibility for about 15 % of global anthropogenic greenhouse gas (GHG) emissions, contributing to global warming. The increasing nutrient inputs in industrial agriculture affect the GHG concentration in the atmosphere and varies substantially due to rate and type of fertilizers applied to the crops, making the management more or less sustainable. In this perspective, this study has investigated at small scale the effect of different adjusted agricultural management practices, based on different nutrient dosage, to optimize the effect of rice cropping systems on carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) emissions. Farmyard manure (FYM), rice stubbles and *Azolla* integrated with chemical fertilizers have been correlated with microbial and enzymatic activities, and with different carbon and nitrogen fractions in acid Inceptisol. Results have revealed that the integrated nutrient management used in rice-rice agroecosystem yielded a peak for CO<sub>2</sub> and CH<sub>4</sub> emissions, whereas two peaks for N<sub>2</sub>O emission. This study has shown an increase in greenhouse gas emission intensity (GHGI) and grain yield of rice in the following order: rice stubbles > FYM > *Azolla* and it has confirmed CH<sub>4</sub> emission as the dominant contributor to GHGI from the rice-rice agroecosystem. When analyzed together GHGs emission and soil properties, a positive correlation was found with biological properties as well as with the different carbon and nitrogen fractions in soil. The highest GHGI has been highlighted in the treatment where recommended dose of chemical fertilizers has been combined with rice stubbles, primarily due to the increase in CH<sub>4</sub> emissions. In contrast, the lowest GHGI has been exhibited in *Azolla* treated plot, probably due to the cumulative effects of the photosynthetic rate of *Azolla*, the release of oxygen from the *Azolla* roots, and the physical protection capacity of the *Azolla* cover, which partially avoid the CH<sub>4</sub> diffusion from the standing water. The seasonality did not affect the estimated rates of GHGI that have been lower both in case of winter and autumn rice compared to previous studies, probably for dissimilarities in management practices. Further research is required in other cropping sequences for addressing the ecological contribution of smallholder agriculture to help reducing GHG emissions, thus, mitigating global warming with actions at local scale.

### 1. Introduction

Soil plays a double role since it is the reservoir of plant nutrients, but it can also be considered as an indirect sink of greenhouse gases (GHGs) as it can hold 190 Pg nitrogen (Mackenzie, 1998) and 1,500–2,400 Pg carbon (Lal, 2004; Powlson, 2005). One of the main drivers impacting

negatively on soil is agriculture, essential for ensuring livelihood security but, at the same time, it is one of the most significant contributors in enhancing the greenhouse effect (Pathak, 2015) accounting for 10–12 % of worldwide GHG emissions (IPCC, 2007; Sheila et al., 2020; Magazzino et al., 2023). Soil emissions have a high share in total GHGs emissions and, in particular: 35 % of carbon dioxide, 47 % of methane,

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53 % of nitrous oxide, and 21 % of nitric oxide (IPCC, 2007). The main source of CO<sub>2</sub> emission is agriculture with effects on global warming, contributing to around 25 % GHG effects to global anthropogenic GHG (Carlson et al., 2017).

Today, with the increasing inputs requirements in agriculture for nutrient management, the GHG concentration in the atmosphere is increasing (Dalal et al., 2003; Malagó et al., 2019). Depending on the crop type and management choices, GHG emission varies substantially due to rate and type of N fertilizers applied to the crops (Schwenke and Haigh, 2016). The upland dry rice (*Oryza sativa* L.) systems mostly emit CO<sub>2</sub> and N<sub>2</sub>O; however, flooded rice emits both CH<sub>4</sub> and N<sub>2</sub>O. Consequently, GHGs emissions from rice soils are nowadays a main issue in climate change research. In this context, the retention and management of rice residues is the basic part of farmyard manure (FYM) and green manure in rice crops, and they have an increasingly importance for an eco-friendly sustainable agricultural system (Timsina and Connor, 2001; Rinasoa et al., 2023). Crop residues act as an integral component of integrated nutrient management (INM) in rice cultivation, since it contains nitrogen (N) (3.0–8.2 kg), phosphorus (P) (0.2–0.6 kg), potassium (K) (7.2–23.3 kg) per ton of dry matter (Rengel et al., 2007), and represent a crucial organic source for improving the soil health and quality (Kumar, 2003). Farmyard manure (FYM) is one of the major sources of organic inputs in paddy fields (Baishya et al., 2015). In this perspective, *Azolla* is commonly used as a biofertilizers, since the N fixed by the symbiotic cyanobacterium *Anabaena azollae* becomes available for crop uptake after its decomposition (Mian and Stewart, 1985).

In addition, the results of past long-term experiments have indicated that in the crop yields, the qualitative and quantitative organic inputs to the soil affect its C fractions and C sequestration capacity (Nayak et al., 2012; Tong et al., 2015; Gogoi et al., 2021a). Applications of organic inputs enhance the nutrient availability to crops and increase microbial diversity and their activities in soil (Marschner et al., 2003; Gogoi et al., 2010a). Soil management of inorganic and organic nutrients enhances soil physico-chemical properties (Baishya et al., 2017) strongly affecting soil ecosystem services provisions, such as soil biodiversity, primary productivity, and nutrient recycling in soil bio-geo-chemical cycles (Gogoi et al., 2021b).

Several research highlighted increased N<sub>2</sub>O emissions due to FYM application (De Wever et al., 2002; Velthof et al., 2003; Rochette et al., 2008; Vangelis et al., 2022), with the exception of Meng et al. (2005) and Jager et al. (2011). Previous studies have shown that the addition of straw in rice crops could reduce (Zhang et al., 2013) or enhance (Xu et al., 2000; Yuan et al., 2014) CH<sub>4</sub> emissions. Similarly, decreases (Bharati et al., 2000; Ali et al., 2015; Mujiyo et al., 2016; Kimani et al., 2018) or increases (Chen et al., 1997; Ying et al., 2000) in CH<sub>4</sub> emissions were reported following the application of *Azolla*. Hence, these contrasting findings together with the lacking in analyzing all the GHGs emissions jointly would suggest that soil and management practices could strongly affect GHGs (*viz.*, CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O) emissions in rice systems, thus further studies are necessary to better clarify and fully understand this process. The novelty of this research is that it considers the effects of different management practices on all GHGs emissions, to identify which agricultural practice could represent the most sustainable strategy to mitigate climate change.

In this perspective, the aim of this research is to analyze the data derived from a permanent plot experiment under a rice-rice system that was setup in 1987–1988 in the Assam state of north-east (NE) India adopting a full integrated nutrient management (INM) system. This is the first study on GHGs emissions comparing, in terms of effects, the consequence of three decades of organic inputs application (FYM, rice residues and *Azolla*) with chemical fertilizers in double-rice cropping systems. Furthermore, the correlation of different nutrient management with microbial and enzymatic activities and with different C and N fractions in acid Inceptisol are analysed under the prevailing climatic condition of the study area. These estimates of GHGs emissions from Indian rice fields assume a special importance since India contributes

with 29.4 % (44.3 million ha) to the global world rice area, where only 16.4 million ha are irrigated area (Kumar and Gupta, 2017). In addition, to meet global food needs by the end of 2030, a 40 % increase of rice production is required (FAO, 2009), with a consequent increase of GHGs emissions. In the past, many studies have been carried out in India for estimating GHGs coming from the rice fields (Bharati et al., 2000; Bhatia et al., 2013; Pathak, 2015; Kumar and Gupta, 2017). In this sense, previous research has shown that CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions occur simultaneously from the rice fields depending on the cultivation method, nutrient management, and properties (mostly soil pH, redox potential, microbial activity, organic C, soil texture and moisture status of soil) (Akiyama et al., 2006; Snyder et al., 2009; Nishimura et al., 2011; Liu et al., 2023). Even if relevant research on CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions from the paddy ecosystem are available since 1980 s, most studies did not focus on these gases mutually, rather they have studied them in an isolated manner (Zheng et al., 2000; Ghosh et al., 2003; Datta et al., 2009; Kimani et al., 2018; Gangopadhyay et al., 2022). Hence, further studies are required because of the uncertainties caused by the gaps in the knowledge on rice agroecosystems, the impact of different crop management, soil types, and the scarcity of direct field data on emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, probably depending on environmental conditions (soil typology and climate), and on the sustainable management of the organic inputs to the soil.

## 2. Materials and methods

### 2.1. Study area and long-term treatment details

The study area is represented by a farm managed by the Assam Agricultural University (AAU) in India (latitude 26°48'N, longitude 95°50'E, and altitude of 86.6 m above mean sea level) (Fig. 1), characterized by a subtropical (humid) climate with the average yearly rainfall of 1900 mm, showing the minimum average temperature (18.5 °C) in January and the maximum average temperature (28.5 °C) in August.

The long-term study has begun in 1987–1988 and it is based on 8 treatment combinations (with individual plot size of 10 m × 10 m), replicating thrice in a randomized block design (total of 24 plots) (Table 1). The farm has been the study area of several research activities (Baishya et al., 2015; Gogoi et al., 2021a; Gogoi et al., 2021b); therefore, the details of the crop management are reported in synthesis in Appendix 1 (Supplementary materials).

### 2.2. Soil sampling and analysis

At the beginning of the long-term study in 1987, soil samples were collected from a depth of 0–0.30 m and were classified as Aeric Endoaquept (Inceptisol) following the USDA (United State Department of Agriculture) Soil Taxonomy. Given the dominant kaolinitic minerals, the soil texture was classified as to be a sandy clay loam (Piper, 1966) with acidic in reaction (pH 5.0). The initial values of the different soil properties are accessible in Appendix 2 (Supplementary materials). Soil properties during the field experiment (2019 to 2022) were measured after the harvest of the winter rice and autumn rice continuously for three years. Following colorimetric methods, NH<sub>4</sub>-N and NO<sub>3</sub>-N fractions in soil were determined as outlined by Baruah and Borthakur (1997). The different carbon (C) fractions in soil were determined following Chan et al. (2001) and Jha et al. (2013). The method of Jenkinson and Powelson (1976) was used for estimation of microbial biomass C in soil. Determination of total organic C (TOC) and total C (TC) were carried out as described by Tiessen and Moir (1993) and Jha et al. (2013), respectively. Later, the soil total inorganic C (TIC) was estimated subtracting TOC from TC. Through serial dilution technique, the microbial counting (bacteria and fungi count) was performed (Martin 1950). Determination of enzymatic activities such as urease activity, fluorescein diacetate (FDA) hydrolysis, dehydrogenase (DHD) and phosphomonoesterase (PMEase) were performed following the



Fig. 1. Study area.

**Table 1**  
Treatment combinations used in the rice-rice agroecosystem.

ID	Treatment Winter rice(cv. <i>Ranjit</i> )	Autumn rice(cv. <i>Disang</i> )	Symbol
T <sub>1</sub>	No fertilizer, No manure (control)	Control	Unfertilized-control
T <sub>2</sub>	Recommended dose of chemical fertilizers (RDF)	RDF	RDF
T <sub>3</sub>	50 %RDF + Farmyard manure (FYM) 2.5 t ha <sup>-1</sup>	RDF	RDF + FYM
T <sub>4</sub>	75 % RDF + FYM 1.25 t ha <sup>-1</sup>	75 % RDF	<sup>3</sup> / <sub>4</sub> RDF + FYM
T <sub>5</sub>	50 %RDF + Rice stubbles (RS) 3.0 t ha <sup>-1</sup>	RDF	RDF + RS
T <sub>6</sub>	75 % RDF + RS 1.5 t ha <sup>-1</sup>	75 % RDF	<sup>3</sup> / <sub>4</sub> RDF + RS
T <sub>7</sub>	50 % RDF + <i>Azolla</i> 0.5 t ha <sup>-1</sup>	RDF	RDF + <i>Azolla</i>
T <sub>8</sub>	75 % RDF + <i>Azolla</i> 0.25 t ha <sup>-1</sup>	75 % RDF	<sup>3</sup> / <sub>4</sub> RDF + <i>Azolla</i>

RDF of 45 kg N, 30 kg P and 40 kg K ha<sup>-1</sup>

methods of Fawcett and Scott (1960), Adam and Duncan (2001), Casida et al. (1964) and Tabatabai and Bremner (1969), respectively.

### 2.3. GHG sampling and analysis

For greenhouse gas (GHG) flux measurement, the gas samples from the rice field were collected continuously for three years (2019–2022) following the technique of Hutchinson and Mosier (1981). Close chambers (0.50 m length × 0.30 m width × 1.20 m height) and made of white colored transparent acrylic sheets (6 mm thickness) fitted with rubber septa for sampling have been used in the six plants of the rice crop for the sampling. In particular, the chamber was accommodated on

a rectangular aluminium frame (0.50 m × 0.30 m × 0.10 m) having a rectangular channel (filled with water), while the air inside was mixed by a fan fitted inside the chamber. The inside temperature was recorded using a thermometer within the chamber. Air samples were collected inserting the needle through a self-sealing rubber septum at a pre-set interval of 15 min (at 09:00–09:30 and 15:00–15:30 h of the day). Air samplings were done at 7 days interval starting from the first transplanting date to about 15 days before harvest of the crops.

The air samples collected were analyzed simultaneously (within 24 h) in the laboratory for the three gases using a Gas Chromatography (GC) system (Thermo Scientific™ TRACE™ 1110 GC). The GC system was equipped with a valve oven and two detectors, an ECD (electron capture detector) and a FID (flame ionization detector) with methanizer. The temperatures of the oven, FID, ECD, and the methanizer were kept constant at 80, 300, 250, and 350 °C, respectively. The FID was used for determining CO<sub>2</sub> and CH<sub>4</sub>, whereas ECD was used for determining trace levels of N<sub>2</sub>O (Dhole and Kadam, 2013). Following Ali et al. (2008), GHG fluxes were calculated using the equation (1):

$$F = \frac{dc}{dt} \times \frac{M \times V \times 273}{A \times 22.4 \times (273 + T)} \quad (1)$$

Here,  $F$ : N<sub>2</sub>O or CH<sub>4</sub> or CO<sub>2</sub> flux (where, N<sub>2</sub>O is expressed as μg m<sup>-2</sup>h<sup>-1</sup>, and CH<sub>4</sub> and CO<sub>2</sub> in mg m<sup>-2</sup>h<sup>-1</sup>),  $\frac{dc}{dt}$  is their concentration change with time (μmol mol<sup>-1</sup>),  $M$ : molar mass of N<sub>2</sub>O or CH<sub>4</sub> or CO<sub>2</sub> (44 for N<sub>2</sub>O, 16 for CH<sub>4</sub>, 44 for CO<sub>2</sub>),  $V$ : chamber volume (m<sup>3</sup>),  $A$ : chamber area (m<sup>2</sup>),  $T$ : inside air temperature of the chamber (°C), 273: temperature coefficient, and 22.4: standard molar volume (L mol<sup>-1</sup>) of N<sub>2</sub>O or CH<sub>4</sub> or CO<sub>2</sub> (under 0 °C, 100 kPa).

Total GHG emission during the cropping season was calculated from

the measured fluxes directly, and days with no measurements were linearly interpolated. Calculations of seasonal total emission were performed as follows (Sun et al., 2020):

$$S = \sum_{i=1}^d \frac{F_{i+1} + F_i}{2} \times (d_{i+1} - d_i) \times 24 \times 10^{-2} \quad (2)$$

Where,  $S$ : seasonal total GHG emission ( $\text{kg hm}^{-2}$ ),  $F$ : GHG flux ( $\text{mg m}^{-2}\text{h}^{-1}$ ),  $i$ : sampling frequency,  $d$ : sampling date (expressed as days after transplanting), and 24: daily hours number.

The greenhouse gas emission intensity (GHGI) was derived from the global warming potentials (GWPs) of the three GHGs and grain yield of rice. The GWPs of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  were estimated taking  $\text{CO}_2$  as the reference gas and considering that GWPs of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  are 28 and 265 times higher, respectively over a 100-year time scale (IPCC, 2014). The GHGI (in  $\text{kg CO}_2\text{-eq t}^{-1}$  grain yield) was calculated as follows:

$$\text{GHGI} = \frac{S_{\text{CO}_2} \times 1 + S_{\text{CH}_4} \times 28 + S_{\text{N}_2\text{O}} \times 265}{\text{Ricegrainyield}} \quad (3)$$

Where,  $S_{\text{CO}_2}$ ,  $S_{\text{CH}_4}$  and  $S_{\text{N}_2\text{O}}$  represents seasonal total emissions of  $\text{CO}_2$  ( $\text{kg CO}_2 \text{ hm}^{-2}$ ),  $\text{CH}_4$  ( $\text{kg CH}_4 \text{ hm}^{-2}$ ) and  $\text{N}_2\text{O}$  ( $\text{kg N}_2\text{O} \text{ hm}^{-2}$ ), respectively; 1, 28 and 265 stands for GWPs of  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  versus  $\text{CO}_2$  over 100 years, respectively while rice grain yield is expressed in  $\text{t hm}^{-2}$ .

## 2.4. Statistical analyses

Following Gomez and Gomez (1984), the ANOVA (analysis of variance) was carried out to assess whether the INM with organic inputs (rice stubbles or FYM or *Azolla*) and reduced doses of chemical fertilizers had a significant influence on GHG emissions from the rice-rice system, soil parameters and rice grain and straw. Least significant difference (LSD) among means, as well as Pearson correlation were performed using the SAS-9.3 version (SAS Institute Inc., 2013).

## 3. Results

### 3.1. Nitrogen and carbon fractions in puddled rice soil

The continuous application of different organic inputs and chemical fertilization in the rice-rice system (32 crop cycles) significantly influenced both the ammoniacal ( $\text{NH}_4\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) fractions in the puddled rice soil (Fig. 2).

The  $\text{NH}_4\text{-N}$  content in soil ranged from 24.1 to 57.6  $\text{kg ha}^{-1}$  after winter rice and from 25.1 to 60.0  $\text{kg ha}^{-1}$  after autumn rice. Similarly, the  $\text{NO}_3\text{-N}$  content in soil varied from 10.4 to 32.9  $\text{kg ha}^{-1}$  at the end of

winter harvest and 10.8 to 35.0  $\text{kg ha}^{-1}$  after autumn harvest. Results clearly indicated that the different INM treatments produced in a net gain in soil N fractions. The different carbon (C) fractions in soil were also significantly affected due to INM treatments (Fig. 3).

The continuous application of RDF + *Azolla* (32-year period) resulted in the highest percent increase in water-soluble carbon (WSC) (+196.6 %) and soil microbial biomass carbon (SMBC) (+218.5 %) in comparison with the unfertilized plot (Fig. 3a). The maximum increase in very labile C (VLC) (+130.6 %) and labile C (LC) (+126.5 %) over the unfertilized-plot were recorded in the plots with RDF + *Azolla* treatment (Fig. 3b). Likewise, significant variations in total organic C and total C content in soil due to long-term INM practices were recorded (Fig. 3c).

### 3.2. Biological properties in puddled rice soil

The long-term INM practices significantly influenced the microbial count in soil (Fig. 4) under the rice-rice cropping sequence.

The bacterial count varied from 5.0 to 8.5  $\log \text{cfu g}^{-1}$  soil after winter harvest (Fig. 4a) and from 5.1 to 9.0  $\log \text{cfu g}^{-1}$  soil after autumn harvest (Fig. 4b). Variations in fungal count ranged from 3.0 to 6.8  $\log \text{cfu g}^{-1}$  soil after winter rice, and from 3.6 to 7.5  $\log \text{cfu g}^{-1}$  soil after autumn rice. The maximum fungal and bacterial counts were recorded in RDF + FYM and RDF + *Azolla*, respectively. Due to INM treatments, significant variations in enzymatic activities in the puddled rice soil were recorded (Table 4). In the present study, dehydrogenase (DH) and urease activities were enhanced up to +73.6 % and +153.3 %, respectively in RDF + *Azolla* treatment after winter rice as compared to the unfertilized plot ( $T_1$ ). Whereas the highest increase in phosphomonoesterase (PMEase) (+69 %) and fluorescein diacetate (FDA) hydrolysis activities (+90 %) were recorded in RDF + FYM after winter rice harvest over the unfertilized-control plot. Similar trends were observed after autumn rice harvest (Table 4). In the present study, the treatment receiving no manures and fertilizers ( $T_1$ ) continuously for 32-years period showed the minimum microbial and enzymatic activities in soil in comparison with the other plots.

For treatment details, refer Table 1. Treatments with similar letters within the identical parameter indicate no significant variation at  $p < 0.05$ .

### 3.3. GHG flux from the rice-rice agroecosystem

The pattern of greenhouse gas (GHG) emissions varied significantly with INM treatments (Appendix 3, 4, 5, 6, 7 and 8, Supplementary materials), whereas variations in the intensity of GHG emissions were

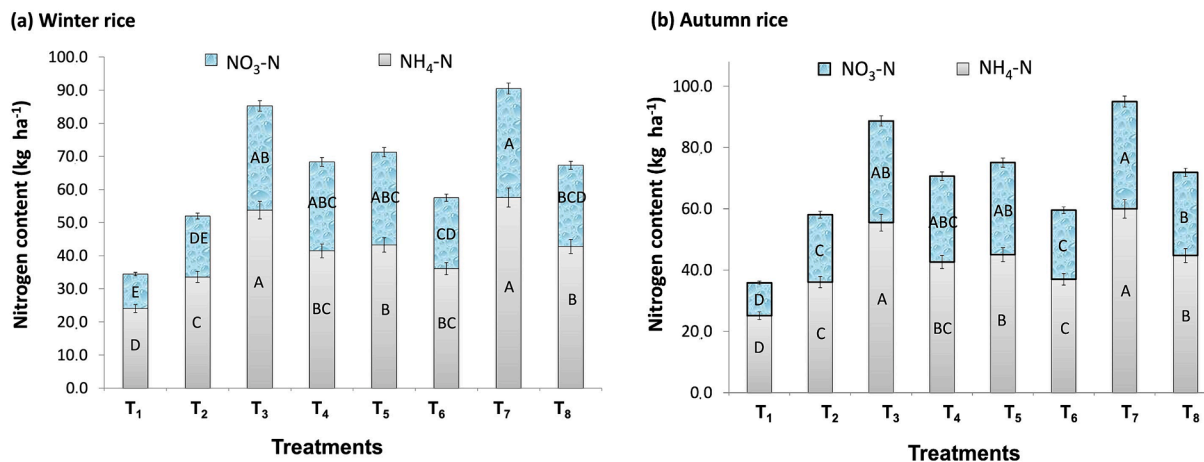
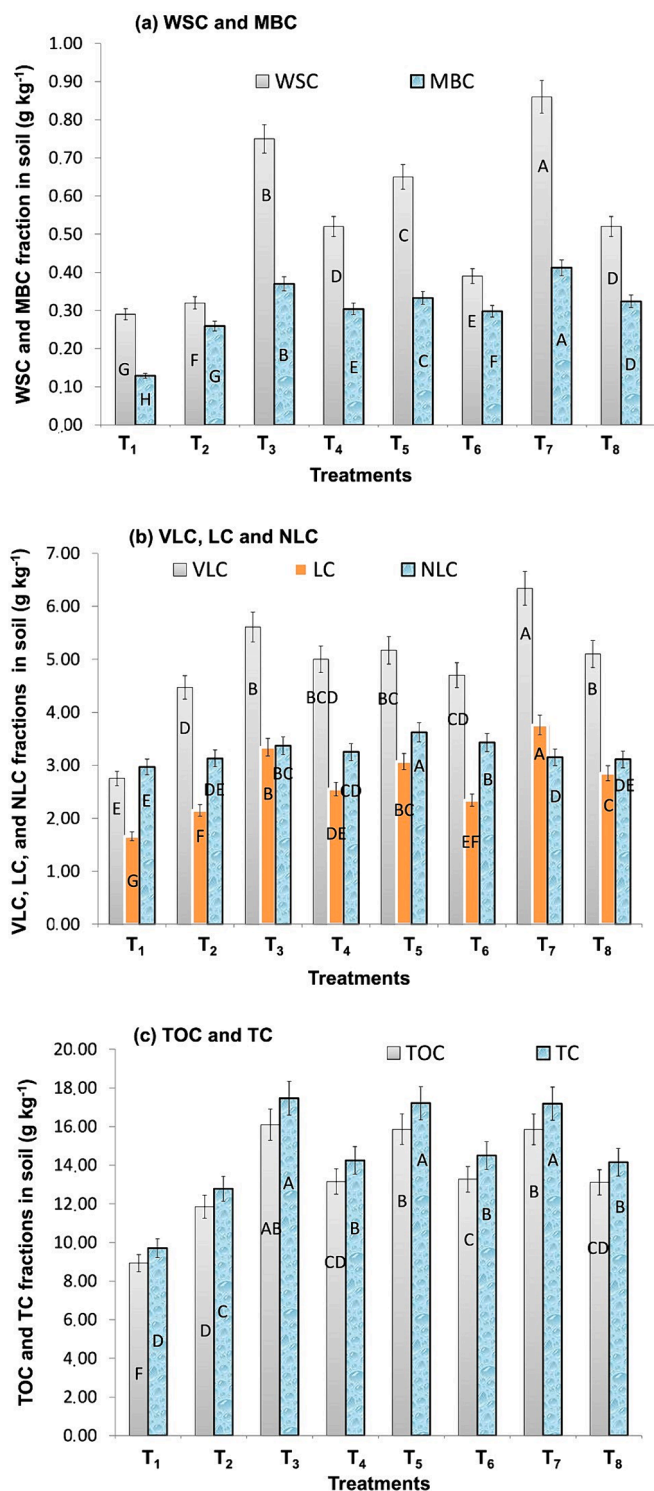


Fig. 2. Effect of long-term INM practices on ammoniacal and nitrate N fractions in soil under rice-rice system (mean value of 3 years). (a) after harvest of winter rice; (b) after harvest of autumn rice. For treatment details, refer Table 1. Treatments with similar letters within the identical parameter indicate no significant variation at  $p < 0.05$ . Vertical bars represent standard error.





**Fig. 3.** The long-term INM effects on carbon fractions in puddled rice soil (mean value of 3 years): (a) shows WSC and MBC, (b) shows VLC, LC, and NLC, and (c) shows TOC and TC. For treatment details, refer Table 1. Treatments with similar letters within the identical parameter indicate no significant variation at  $p < 0.05$ . Vertical bars represent standard error. TC: Total carbon, TOC: Total organic carbon, NLC: Non-labile carbon, LC: Labile carbon, VLC: Very labile carbon, MBC: Microbial biomass carbon, WSC: Water soluble carbon.

observed with changes in growth stages both in winter and autumn rice crops (Fig. 5).

Initially, the emissions of CO<sub>2</sub> and CH<sub>4</sub> increased independently from the INM treatments until the rice crop was fully mature and afterward decreased gradually. Both CO<sub>2</sub> and CH<sub>4</sub> emissions peaked at 60 DAT (days after transplanting) in winter rice and 45 DAT in autumn rice. Among the organic materials, the addition of rice stubbles (i.e., RDF + RS treatment) showed significantly highest emission of CO<sub>2</sub> and CH<sub>4</sub> from the rice-rice system whereas the addition of *Azolla* showed the lowest emission.

A steady raise in N<sub>2</sub>O emission rate was found in all the treatments until the emission peak. We observed two emission peaks for N<sub>2</sub>O emission (Fig. 5) from the winter rice (at 30 and 60 DAT) and autumn rice crop (at 30 and 45 DAT). After the second peak, the emission of N<sub>2</sub>O decreased gradually until the harvesting stage. The highest N<sub>2</sub>O emission from the rice-rice system was from RDF (T<sub>2</sub>), whereas the lowest was from unfertilized treatment (T<sub>1</sub>).

### 3.4. Seasonal total GHG emission

The seasonal total CO<sub>2</sub> emission significantly varied from 116.5 kg CO<sub>2</sub> hm<sup>-2</sup> (T<sub>1</sub>) to 253.9 kg CO<sub>2</sub> hm<sup>-2</sup> (T<sub>5</sub>) during winter rice crop and from 63.2 kg CO<sub>2</sub> hm<sup>-2</sup> (T<sub>1</sub>) to 123.5 kg CO<sub>2</sub> hm<sup>-2</sup> (T<sub>5</sub>) during autumn rice crop (Fig. 6). The total CO<sub>2</sub> emission from the rice-rice system varied from 179.7 kg CO<sub>2</sub> hm<sup>-2</sup> in the unfertilized-control (T<sub>1</sub>) plot to 377.4 kg CO<sub>2</sub> hm<sup>-2</sup> under RDF + RS (T<sub>5</sub>) plot. Similarly, the seasonal total emission of CH<sub>4</sub> varied significantly from 13.9 kg CH<sub>4</sub> hm<sup>-2</sup> (T<sub>1</sub>) to 33.7 kg CH<sub>4</sub> hm<sup>-2</sup> (T<sub>5</sub>) during the winter rice crop, from 8.1 kg CH<sub>4</sub> hm<sup>-2</sup> (T<sub>1</sub>) to 15.8 kg CH<sub>4</sub> hm<sup>-2</sup> (T<sub>5</sub>) during the autumn rice crop, and from 22.0 kg CH<sub>4</sub> hm<sup>-2</sup> (T<sub>1</sub>) to 49.5 kg CH<sub>4</sub> hm<sup>-2</sup> (T<sub>5</sub>) during the entire system of rice-rice sequence (Fig. 6).

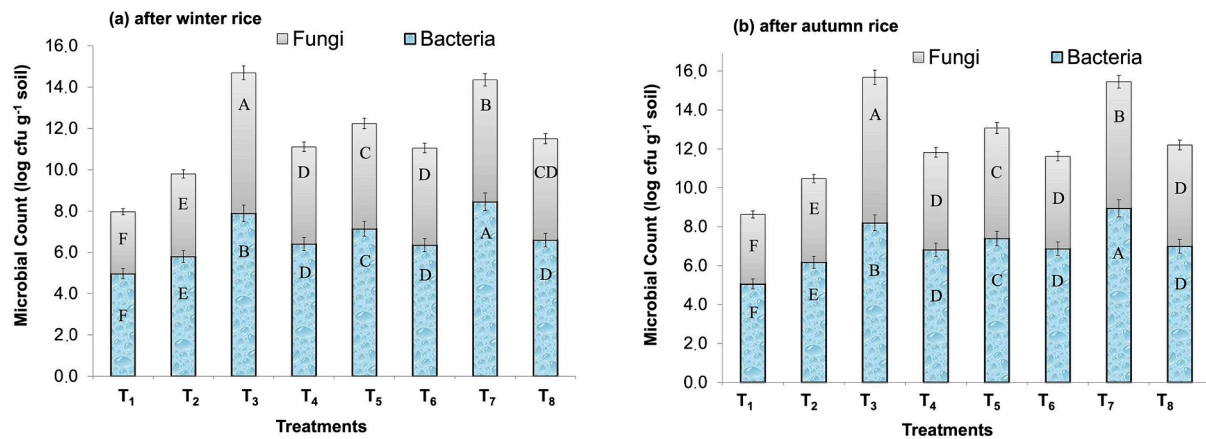
Significant influence of treatment combinations was observed in seasonal total N<sub>2</sub>O emission from the rice-rice system and the highest emission was recorded from the plot receiving RDF (T<sub>2</sub>). The data on seasonal total N<sub>2</sub>O emission during winter rice varied from 0.48 kg N<sub>2</sub>O hm<sup>-2</sup> (T<sub>1</sub>) to 1.07 kg N<sub>2</sub>O hm<sup>-2</sup> (T<sub>2</sub>), and during the autumn rice from 0.22 kg N<sub>2</sub>O hm<sup>-2</sup> (T<sub>1</sub>) to 0.74 kg N<sub>2</sub>O hm<sup>-2</sup> (T<sub>5</sub>) (Fig. 6). This recurrent behavior could be used to analyze the resilience of these systems in terms of capacity to face alternative stable states without losing the capacity to adapt to change (Petrosillo et al., 2021).

### 3.5. Greenhouse gas emission intensity (GHGI)

Greenhouse gas emission intensity (GHGI) is the combined emission of GHGs from the rice ecosystem per unit production of grain yield (Wang et al., 2018). The INM with different organics and mineral fertilizers showed a significant influence on GHGI from the double-cropped rice plots (Fig. 7). The GHGI from winter rice varied from 217.7 kg CO<sub>2</sub>-eq t<sup>-1</sup> grain yield under RDF + *Azolla* (T<sub>7</sub>) to 361.1 kg CO<sub>2</sub>-eq t<sup>-1</sup> grain yield under RDF + RS (T<sub>5</sub>). Likewise, in autumn rice, GHGI was the highest (236.0 kg CO<sub>2</sub>-eq t<sup>-1</sup> grain yield) under RDF + RS (T<sub>5</sub>). Compared to unfertilized-control plot, GHGI under RDF + RS treatment increased by 14.7 % and 15.4 % from winter and autumn rice, respectively. CH<sub>4</sub> emission was the biggest contributor to GHGI from the rice agroecosystem, moreover the increase in GHGI varied with the addition of the different organic inputs (rice stubbles > FYM > *Azolla*).

### 3.6. Yield

Addition of NPK through inorganic and organic sources significantly increased crop yields of the rice-rice agroecosystem compared to the control plot (Fig. 8). Significant variations among the treatments were recorded in total yield of the system. Total grain yields of the system varied between 3.7 and 7.7 Mg ha<sup>-1</sup>, whereas total straw yields of the system varied between 5.6 and 9.8 Mg ha<sup>-1</sup>. The highest system yield was observed in RDF + RS (T<sub>5</sub>) following RDF + FYM (T<sub>3</sub>) as the second-



**Fig. 4.** The long-term INM effects on microbial population in puddled rice soil (mean value of 3 years). (a) Fungal and bacterial count after winter rice; (b) Fungal and bacterial count after autumn rice. For treatment details, refer Table 1. Treatments with similar letters within the identical parameter indicate no significant variation at  $p < 0.05$ . Vertical bars represent standard error.

**Table 4**

The long-term INM effects on enzymatic activities in puddled rice soil (pooled data of 3 years).

Treatments	After winter rice				After autumn rice			
	Dehydrogenase ( $\mu\text{g TTF g}^{-1} \text{soil } 24 \text{ h}^{-1}$ )	Phospho-monoesterase ( $\mu\text{g p-nitrophenol g}^{-1} \text{soil h}^{-1}$ )	Fluorescein di-acetate hydrolysis ( $\mu\text{g fluorescein g}^{-1} \text{soil h}^{-1}$ )	Urease ( $\mu\text{g NH}_4\text{-N g}^{-1} \text{soil } 2 \text{ h}^{-1}$ )	Dehydrogenase ( $\mu\text{g TTF g}^{-1} \text{soil } 24 \text{ h}^{-1}$ )	Phospho-monoesterase ( $\mu\text{g p-nitrophenol g}^{-1} \text{soil h}^{-1}$ )	Fluorescein di-acetate hydrolysis ( $\mu\text{g fluorescein g}^{-1} \text{soil h}^{-1}$ )	Urease ( $\mu\text{g NH}_4\text{-N g}^{-1} \text{soil } 2 \text{ h}^{-1}$ )
T <sub>1</sub>	127.4 <sup>g</sup>	197.2 <sup>f</sup>	5.0 <sup>e</sup>	1.5 <sup>f</sup>	128.1 <sup>e</sup>	203.6 <sup>f</sup>	5.3 <sup>f</sup>	1.7 <sup>e</sup>
T <sub>2</sub>	163.0 <sup>f</sup>	263.0 <sup>e</sup>	6.8 <sup>d</sup>	2.8 <sup>e</sup>	167.1 <sup>d</sup>	265.1 <sup>e</sup>	7.0 <sup>e</sup>	2.9 <sup>d</sup>
T <sub>3</sub>	214.4 <sup>ab</sup>	333.2 <sup>a</sup>	9.5 <sup>a</sup>	3.4 <sup>b</sup>	217.5 <sup>ab</sup>	337.4 <sup>a</sup>	10.0 <sup>a</sup>	3.7 <sup>b</sup>
T <sub>4</sub>	183.6 <sup>de</sup>	303.9 <sup>bcd</sup>	8.5 <sup>c</sup>	3.0 <sup>d</sup>	185.0 <sup>cd</sup>	305.7 <sup>bc</sup>	9.0 <sup>c</sup>	3.3 <sup>c</sup>
T <sub>5</sub>	203.8 <sup>bc</sup>	328.7 <sup>ab</sup>	8.7 <sup>bc</sup>	3.2 <sup>c</sup>	206.1 <sup>abc</sup>	331.6 <sup>a</sup>	9.2 <sup>bc</sup>	3.5 <sup>bc</sup>
T <sub>6</sub>	178.5 <sup>ef</sup>	294.1 <sup>cd</sup>	7.3 <sup>d</sup>	3.1 <sup>cd</sup>	181.9 <sup>cd</sup>	297.5 <sup>cd</sup>	8.2 <sup>d</sup>	3.4 <sup>c</sup>
T <sub>7</sub>	221.1 <sup>a</sup>	314.2 <sup>abc</sup>	9.1 <sup>ab</sup>	3.8 <sup>a</sup>	223.6 <sup>a</sup>	318.0 <sup>ab</sup>	9.5 <sup>b</sup>	4.1 <sup>a</sup>
T <sub>8</sub>	193.1 <sup>cde</sup>	282.9 <sup>d</sup>	8.6 <sup>bc</sup>	3.1 <sup>cd</sup>	195.3 <sup>bc</sup>	284.2 <sup>de</sup>	9.0 <sup>c</sup>	3.3 <sup>c</sup>
SEm( $\pm$ )	5.83	9.00	0.18	0.03	7.94	8.41	0.09	0.06
CV (%)	16.8	13.6	14.0	13.1	13.6	12.7	11.9	9.3

best treatment. Compared to the unfertilized-plot, maximum + 108.1 % increase in grain and + 75.0 % increase in straw yields were observed in the treatment RDF + RS.

### 3.7. Statistical correlations

Pearson correlation analyses allowed evaluating the effect of various soil N and C fractions on the GHG emissions from the rice agro-ecosystem. Emissions of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O were correlated significantly and positively with NH<sub>4</sub>-N and NO<sub>3</sub>-N fractions in soil (Table 5). Similar correlations of CO<sub>2</sub> and CH<sub>4</sub> emissions with carbon fractions in soil were also recorded, except Total Inorganic Carbon (TIC), where the correlations were non-significant. Pearson correlation coefficient (Table 5) revealed significant and positive correlations between the emission of N<sub>2</sub>O and C fractions (MBC, VLC, and TOC). However, the correlations of N<sub>2</sub>O emission were positive but non-significant with WSC, LC, NLC, TIC and TC.

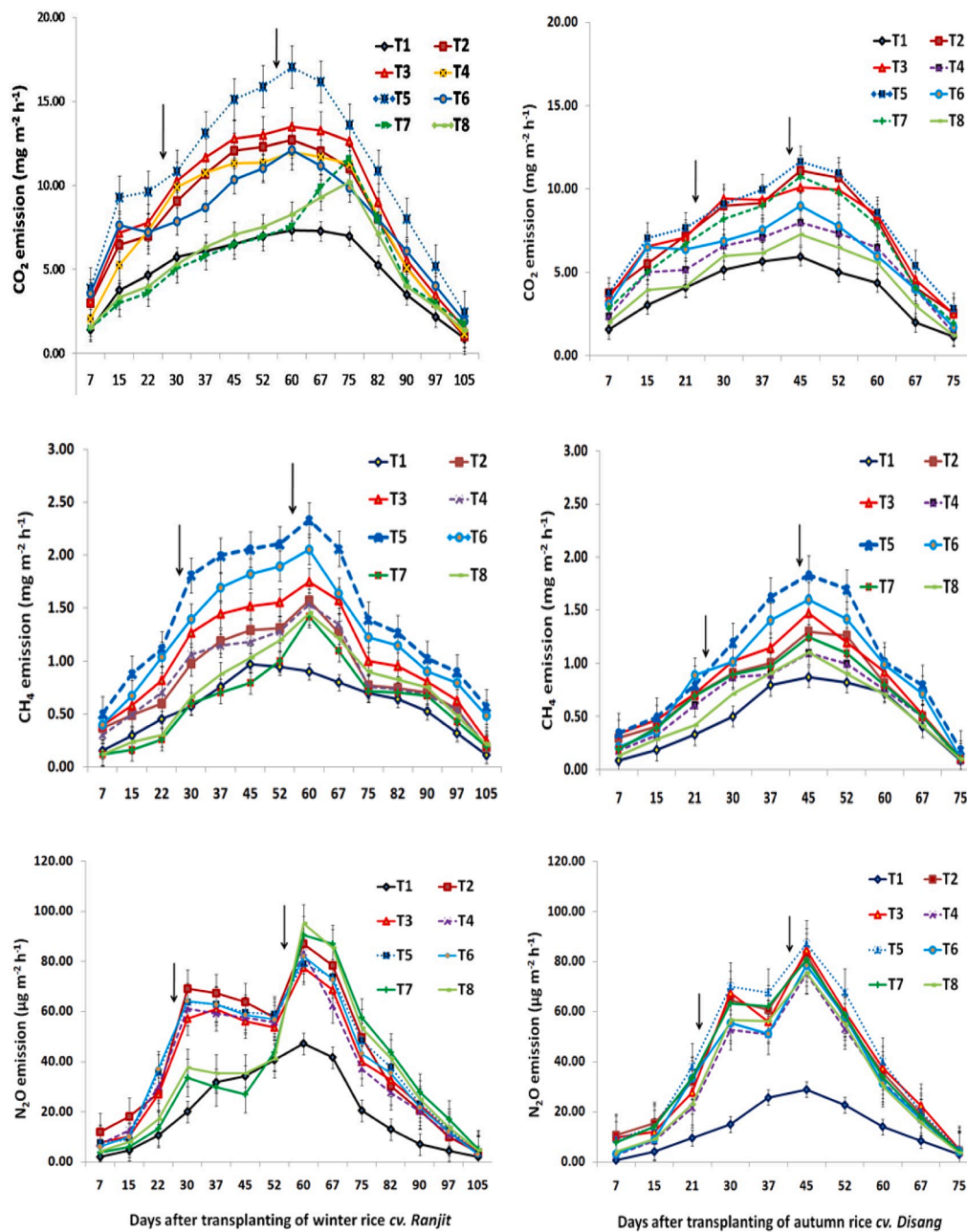
Given the effect that different permanent crops can exert on soil microbial community (Marzaioli et al., 2010), the Pearson correlations among GHG emissions and soil biological properties are shown in Table 6. Microbial abundance such as bacteria and fungi in soil were positively correlated with CO<sub>2</sub> and CH<sub>4</sub> emissions from the rice system. Nevertheless, the correlation with bacteria was significant ( $r = 0.89^{**}$ ;  $r = 0.84^{**}$ ), and with fungi abundance was non-significant ( $r = 0.54$ ;  $r = 0.51$ ). The Pearson correlations of CO<sub>2</sub> emissions with enzymatic activities were found to be significant and positive. Similarly, the relationships of CH<sub>4</sub> emission with fluorescein diacetate ( $r = 0.76^*$ ) and urease activity ( $r = 0.76^*$ ) were found to be significantly positive.

However, phosphomonoesterase activities in soil showed non-significant correlations with CO<sub>2</sub> ( $r = 0.47$ ) and CH<sub>4</sub> emissions ( $r = 0.34$ ). Emission of N<sub>2</sub>O from the rice field was positively correlated with fungal ( $r = 0.73^*$ ) and bacterial abundance ( $r = 0.89^{**}$ ). Similar relationships of N<sub>2</sub>O emission with different enzymatic activities were recorded (Table 6).

## 4. Discussion

The crop yields have increased significantly owing to beneficial effects of integrated application of various sources of nutrients in rice-rice sequence under long-term (Nayak et al., 2012; Mandal et al., 2018). Increase in available soil nutrients resulting from the organic amended treatments has helped in growth and developments of the crop (Gogoi et al., 2021a). Integrated supply of nutrients has led to an increase the microbial activities owing to accumulation of higher soil carbon (Baishya et al., 2015; Bhardwaj et al., 2019) that might have ultimately contributed to higher crop yield. Higher crop yields under better soil health due to INM practices were reported earlier (Gogoi et al., 2010b; Ramdas et al., 2017; Baishya et al., 2017). The lowest yield of crops in the control treatment might be ascribed to poor soil fertility status over the INM treatments. Low C and imbalance nutrient status in unfertilized control plot has depleted soil properties, resulting in low yields of crops. Earlier reports (Nayak et al., 2012; Gogoi et al., 2021a) also showed similar results in unfertilized-control due to depletion of organic C in soil and reduction in available nutrient supply to the crop.

The different nutrient fractions in soil are clearly sensitive to the ecological level of crop management practices (Gogoi et al., 2021a). In



**Fig. 5.** The long-term INM effects on GHG emissions from winter and autumn rice (mean value of 3 years). On the left, during winter rice (*cv. Ranjit*); on the right, during autumn rice (*cv. Disang*). Every point stands for the mean of three replicated values. Vertical bars and arrows indicate standard error and the split application time of urea fertilizer. For treatment details, refer [Table 1](#).

this study, different nitrogen (N) fractions have been significantly influenced by integrated application of chemical fertilizers with organics (FYM/ rice stubbles/ *Azolla*). [Nath et al. \(2012\)](#) and [Mandal et al. \(2018\)](#) recognized an augment in microbial activity under INM treatments that favored N mineralization, ultimately resulting in higher NO<sub>3</sub>-N and NH<sub>4</sub>-N content over initial status in soil. The highest NO<sub>3</sub>-N and NH<sub>4</sub>-N content in soil in RDF + *Azolla* treated plot (T<sub>7</sub>) might be attributed to optimum nutrient input from the addition of *Azolla* as biofertilizer along with the chemical fertilizers. Increase in N content in soil might have resulted from the decomposition of *Azolla* and ammonification of organic N under long run. Hence, the build-up of NO<sub>3</sub>-N and NH<sub>4</sub>-N fractions could be due to the reduction in N losses in organic matter applied plots ([Mandal et al., 2018](#)) as it complexed with clay minerals in soil.

The types and quantity of organic inputs added to soil significantly

influence the different carbon (C) fractions in soil. Among the bio-materials used in this study, the highest portion to the labile C fractions have come from the supplementation of *Azolla* (biofertilizer) followed by FYM and rice stubble along with the chemical fertilizers. The direct addition of C using organics might have increased in labile C fractions under INM plots resulting in higher microbial activity in soil ([Moharana et al., 2012](#); [Bhardwaj et al., 2019](#)). Increase in non-labile C content in the plots receiving rice stubbles and FYM have resulted from the addition of humic acid from the organic inputs ([Singh et al., 2012](#); [Gogoi et al., 2021a](#)) constantly for 32 years in the long run experiment. In the present study, improvement in C status in soil may be related to the additive effect of NPK and organics as well as the interaction between them ([Nayak et al., 2012](#)) due to continuous supply of organic sources *viz.*, FYM or crop residues or *Azolla* to the soil. From the long-term soil fertility management trials, [Gogoi et al. \(2021a\)](#) and [Liang et al. \(2011\)](#)

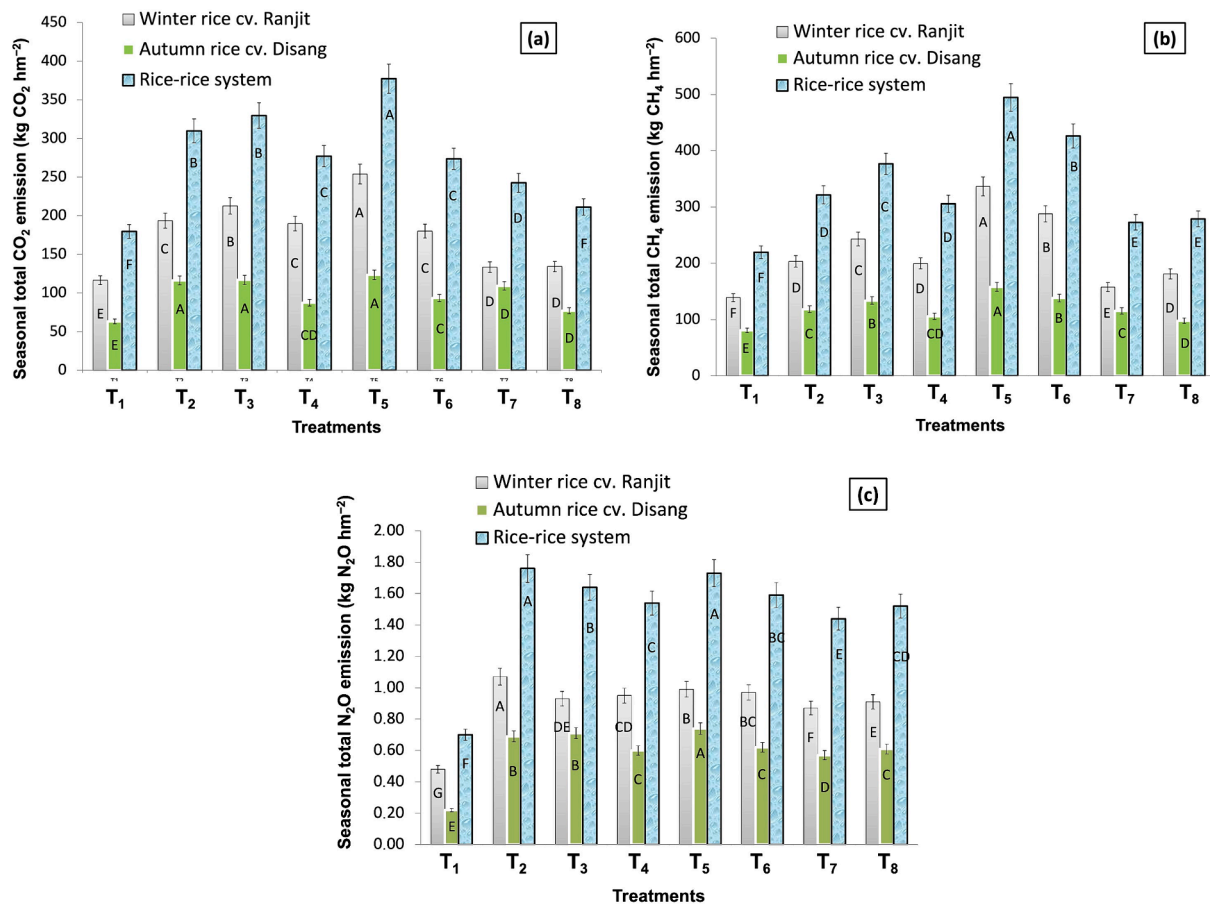


Fig. 6. Effect of long-term INM on seasonal total GHG emission from rice-rice system. For treatment details, refer Table 1. Treatments with similar letters within the identical parameter indicate no significant variation at  $p < 0.05$ . Vertical bars represent standard error.

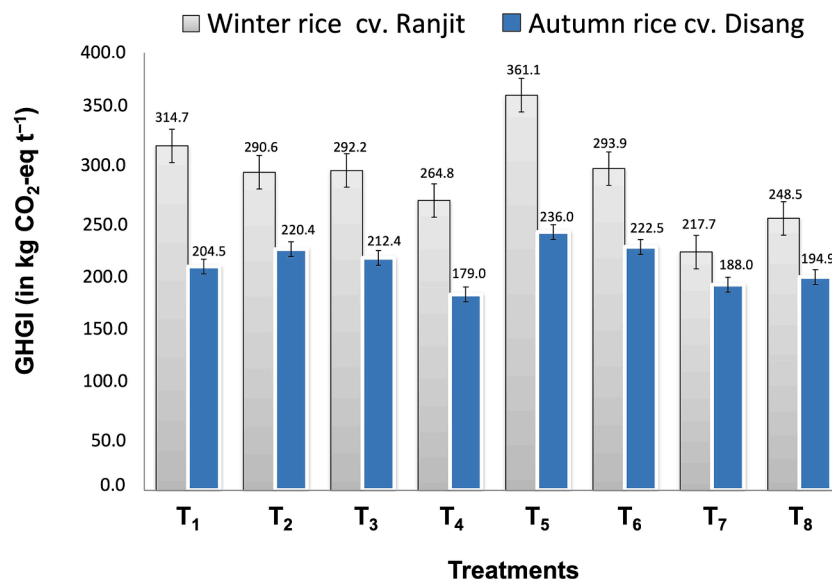


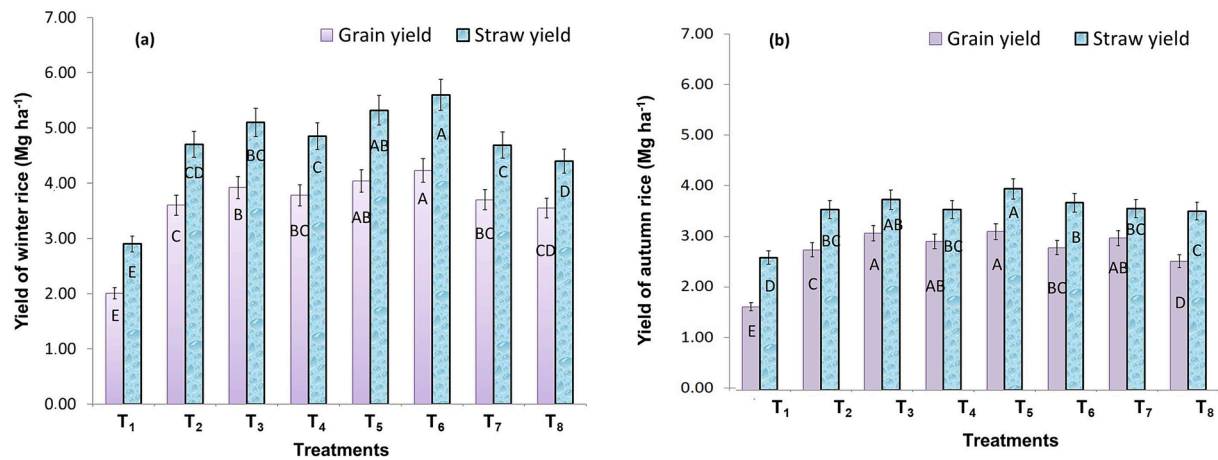
Fig. 7. Effects of long-term INM treatments on the greenhouse gas emission intensity (GHGI) (in kg CO<sub>2</sub>-eq t<sup>-1</sup>) from rice-rice system. Each value above the bars represents the pooled data of 3 years. For treatment details, refer Table 1.

also thoroughly established that inclusion of organic inputs in soil was able to improve soil C over the original levels.

Soil microbial activity is known as greatly associated with the supplementation of manures and organic matter content in soil (Gogoi et al., 2021b). In this study, considerable increase in microbial population due

to addition of organics with chemical fertilizers may be resulted from the incorporation of larger residual biomass and rhizodeposition in soil. Higher level of labile C and N in INM plots might have provided adequate environments for better proliferation of bacteria and fungi in soil (Prakash et al., 2016; Tao et al., 2020). Long-term INM practices





**Fig. 8.** Yield of crops (3 years pooled data) as affected by long-term INM practices. (a) Grain and straw yield of winter rice; (b) Grain and straw yield of autumn rice. Treatments with similar letters within the identical parameter indicate no significant variation at  $p < 0.05$ . For treatment details, refer Table 1. Vertical bars represent standard error.

**Table 5**

Pearson correlation coefficients (r) between the greenhouse gases and the different nitrogen and carbon fractions in puddled rice soil.

Parameters	Soil nitrogen fractions		Soil carbon fractions		VLC	LC	NLC	TOC	TIC	Total C
	NH <sub>4</sub> -N	NO <sub>3</sub> -N	WSC	MBC						
CO <sub>2</sub>	0.74*	0.80*	0.83*	0.87**	0.85**	0.84**	0.73*	0.83**	0.50	0.79*
CH <sub>4</sub>	0.83*	0.81*	0.80*	0.77*	0.73*	0.71*	0.83*	0.84**	0.30	0.73*
N <sub>2</sub> O	0.77*	0.89**	0.67	0.82*	0.79*	0.67	0.50	0.71*	0.47	0.69

\*\*significant at  $p < 0.01$ ; \*significant at  $p < 0.05$ .

Total C: Total carbon; TIC: Total inorganic carbon; TOC: Total organic carbon; NLC: Non-labile carbon; LLC: Less labile carbon; LC: Labile carbon; VLC: Very labile carbon; MBC: Microbial biomass carbon; WSC: Water-soluble carbon

**Table 6**

Pearson correlation coefficients (r) of greenhouse gases with soil microbes and enzymatic activities under the rice-rice system.

Parameters	Soil microbial population		Enzymatic activity Dehydrogenase	Phospho-monoesterase	Fluorescein diacetate hydrolysis	Urease
	Bacteria	Fungi				
CO <sub>2</sub>	0.89**	0.54	0.88**	0.47	0.75*	0.79*
CH <sub>4</sub>	0.84**	0.51	0.32	0.34	0.76*	0.76*
N <sub>2</sub> O	0.89**	0.73*	0.84**	0.76*	0.74*	0.84**

\*\*significant at  $p < 0.01$ ; \*significant at  $p < 0.05$ .

enhance the activities of enzymes in soil viz., dehydrogenase (DH), phospho-monoesterase (PMEase), fluorescein di-acetate (FDA), and urease activity (Nath et al., 2012; Baishya et al., 2017). Normally, soil C and N content controls the enzymatic activities in soil (Mandal et al., 2018). Application of organic amendments with chemical fertilizers augmented the microbial activities in soil that might have released the organically bound phosphorus resulting in higher PMEase activities in soil (Mandal et al., 2007; Gogoi et al., 2021b). The INM practices also increases the lipases, esterases and proteases activities in the soil (Nath et al., 2012), which probably induced the FDA hydrolysis in soil. In our study, urease activities in paddy soil were enhanced by 2.0 – 2.4 times due to long-term INM over control. Due to higher availability of substrates (C and N) resulting from the addition of organic manures (Sheoran et al., 2020) and from the accumulation of rice root exudates, the microbial activity has increased enhancing the soil urease activity (Ramdas et al., 2017).

Carbon dioxide (CO<sub>2</sub>) dynamics around submerged paddy cultivation has been quite different from those of other soil systems. Soil submergence generally has caused significant decline in CO<sub>2</sub> emission (Koizumi, 2001; Nishimura et al., 2011). According to Nishimura et al. (2015), uptake and release of CO<sub>2</sub> (during photosynthesis and respiration, respectively) by the algae and aquatic weeds are among the major factors contributing to the net CO<sub>2</sub> flux from the submerged water

surface. Algae and aquatic weeds assimilated dissolved CO<sub>2</sub> in water which caused significant decline in the dissolved CO<sub>2</sub> concentration in the paddy water. Nevertheless, net CO<sub>2</sub> emission from paddy ecosystem (both by soil and rice plants) has been observed in previous studies (Saito et al., 2005; Minamikawa and Sakai, 2007; Alberto et al., 2012; Ozlu and Kumar, 2018; Yu et al., 2023). In line with this research, this study has shown that the CO<sub>2</sub> emission from the rice agroecosystem is significantly affected by the type and quantity of nutrient sources added into the soil. The effect of organic inputs on CO<sub>2</sub> emission was dependent on the C:N ratio of the organic sources (Flessa and Beese, 1995). Fertility management with the inclusion of organic sources of nutrients have progressively enhanced different C fractions in soil (Fig. 3) affecting CO<sub>2</sub> emission, given the positive correlation found in this research between CO<sub>2</sub> emission and the C fractions in soil (Table 5). An increase in C fractions under INM stimulates the growth and activities of microorganisms (Bhardwaj et al., 2019; Gogoi et al., 2021a) (Table 4, Fig. 4). While decomposing the organic matter, soil microorganisms utilize C as the cellular materials that might also liberate CO<sub>2</sub>. Thus, net emission of CO<sub>2</sub> in this study might be due to cumulative effect of decomposition of added organic inputs (FYM, rice stubbles and Azolla) and rice root respiration. Besides, the air bubbles of submerged paddy soil (1000–1400 mL m<sup>-2</sup>) also contribute considerably to CO<sub>2</sub> emission (Cheng et al., 2008).

The plot with the addition of rice stubbles ( $T_5$ ) has shown the highest emission of  $CO_2$  and this might be owing to the higher C:N ratio (76.4) of rice stubbles (Sarma et al., 2013) as compared to FYM (18.9) and *Azolla* (10.2). Besides, urea fertilizer applied in the fields under stagnant water condition releases  $CO_2$  as urea is converted to ammonium, hydroxide and bicarbonate ions due to action of urease enzyme (Gupta et al., 2021). Here, bicarbonate ions finally breakdown into  $CO_2$  and  $H_2O$ . Previously, Jacinthe et al. (2002) demonstrated that mineral fertilizations along with manure and straw applications (due to increased plant growth) contributed to the soil respiration. Rice stubbles and reduced doses of inorganic fertilizers application might have increased the decomposable C (an energy source for microbes) which was responsible for higher release of  $CO_2$  into the atmosphere (Aulakh et al., 2001). The maximum cumulative emission of  $CO_2$  from the rice residue was also reported by Sarode et al. (2009). Further, the lowest emission of  $CO_2$  with  $1/2$  RDF + *Azolla*-RDF ( $T_7$ ) might be attributed to the photosynthesis process of *Azolla* that consumes  $CO_2$ , as well as to the high content of N in *Azolla* (4.5 %) compared to FYM (0.9 %) and rice stubble (0.74 %). Organic materials with a lower C:N ratio (higher N content) undergoes a faster decomposition compared to materials with higher C:N ratio (lower N content), leading to lower emission of  $CO_2$  to the environment (Datta and Devi, 2001).

Concerning methane, the application of organic materials generally increases  $CH_4$  emission from flooded paddies (Yagi and Minami, 1990; Wassmann et al., 1996; Yu et al., 2023). The higher emission of  $CH_4$  during the winter rice crop under RDF + RS treatment was possibly caused by the increased microbial putrefaction of rice residues (Wassmann et al., 1996). Increased abundance of methanogens due to degradation of rice stubbles was reported (Yuan et al., 2014) leading to enhancement in  $CH_4$  emission. Besides, plant biomass (above-ground and roots) has a strict connection with the  $CH_4$  efflux (Baruah et al., 2010). The lower  $CH_4$  emission observed during the early crop growth stages was probably due to the lower  $CH_4$  transport capability owing to lower leaf area, root and shoot growth during the initial growth stages of rice (Baruah et al., 2010). The emission of  $CH_4$  might be further enhanced during the active growth stages by an increase in stem diameter and effective tiller numbers of rice crop (Liu et al., 2015). At harvesting time, the decline in  $CH_4$  emission was perhaps caused by the presence of dead tissues in rice plant as well as due to a decrease in C availability (Jean and Roger, 2001; Das and Baruah, 2008). In this perspective, this research has shown that the pattern of  $CH_4$  emission from paddy eco-system was significantly influenced by split application of urea fertilizer. The  $CH_4$  emission peak at 60 DAT (days after transplanting) in winter rice might have resulted from the top dressing of urea at 27 and 55 DAT as  $CH_4$  production and emission through rice plants were stimulated by the urea application (Banik et al., 1996) as well as by higher amount of decomposable C deriving from the root exudates and the decaying tissues (Aulakh et al., 2001). Likewise, the two top dressings of urea in autumn rice (22 and 42 DAT) yielded one peak of  $CH_4$  emission at 45 DAT. A single peak of  $CH_4$  emission from rice crops, despite two urea top dressings, might be attributed to the fact that the first top dressing was still effective even during the second top dressing of urea.

In this context, *Azolla*, representing a source of N, has been grown with rice as a dual crop and was thereafter incorporated into the soil in this study. The emission from the plot receiving *Azolla* + chemical fertilizers ( $T_7$  and  $T_8$ ) and from the unfertilized-control ( $T_1$ ) plot was relatively low and comparable during the early growth stages and increased as the winter rice crop matured. The decrease in  $CH_4$  emission at initial stages in the dual culture of *Azolla* with rice crop was reported earlier by other research (Bharati et al., 2000; Liu et al., 2017; Xu et al., 2017; Kimani et al., 2018), but this study showed that the use of rice stubbles ( $T_5$ ) and FYM ( $T_3$ ) in winter rice increased  $CH_4$  emission by 121.6 % and 57.4 %, respectively as compared to *Azolla* ( $T_7$ ). Thus, results indicated that *Azolla* ( $0.5 t ha^{-1}$ ) could reduce the emission of  $CH_4$  by 2.21 and 1.57 times in comparison to rice stubbles ( $3.0 t ha^{-1}$ )

and FYM ( $2.5 t ha^{-1}$ ), respectively, when added along with  $1/2$  RDF in winter rice crop. Kimani et al. (2018) observed a decrease in seasonal  $CH_4$  emission from the paddy field by 34.7 % due to *Azolla* cover in comparison to control (without *Azolla*). Compared to rice plants, *Azolla* has a higher photosynthetic rate which could be the probable explanation for lower  $CH_4$  emission from the field in the dual culture of the rice with *Azolla*. Inubushi et al. (2003) and Wang et al. (1993) reported that the net  $CH_4$  emission from the rice ecosystems is governed by the equilibrium between  $CH_4$  flux and  $CH_4$  oxidation and by the redox potential of the system. Moreover, rice plant released only  $60.7 mg O_2 g^{-1}$  plant, and a higher release of  $O_2$  by *Azolla* ( $67.4 mg O_2 g^{-1}$  plant) to the environment was reported (Liu et al., 2008). Hence, higher redox potential and an increase in dissolved oxygen (DO) in the water under *Azolla* treated rice fields caused a reduction in  $CH_4$  emission (Ali et al., 2015; Malyan et al., 2016; Liu et al., 2017; Kimani et al., 2018).  $CH_4$  emission through bubbling and diffusion processes are also impeded due to large floating masses of *Azolla* in submergence paddy soil (Van Der Steen et al., 2003). Hence, decline in  $CH_4$  flux from the paddy field treated with *Azolla* in this study is probably due to two reasons. First, the increasing DO levels in the water due to presence of *Azolla* roots and second, the physical presence of *Azolla* plant floating on the standing water (as a physical obstruction) which partially impedes  $CH_4$  emission from the rice-rice system. Research reports from India (Bharati et al., 2000) and China (Xu et al., 2017) also confirmed a decline in  $CH_4$  emission from rice fields in dual crop with *Azolla*. Moreover, the abrupt rise in  $CH_4$  flux from the winter rice crop supplemented with *Azolla* ( $T_7$ ) at 60 DAT might be ascribed to the incorporation of *Azolla* into the soil at 47 DAT. *Azolla* as a relatively labile organic substrate adds mostly labile C fractions into the soil (Fig. 3). Hence, decomposition of easily degradable fresh *Azolla* supplies fermentable substrates for soil methanogens which enhanced  $CH_4$  emission. In this study, low  $CH_4$  emission from the control plot ( $T_1$ , receiving no manures and fertilizers) might be a consequence of the lack of appropriate reducing substrates and nutrients that inhibit the microbial activity.

The higher N inputs, although helping in increasing crop yields, have negative consequences on the environment at a global scale (Bowles et al., 2018). Several studies have deemed that nitrogenous fertilizer application significantly increased  $N_2O$  emissions (Ji et al., 2021), particularly when urea application is high (Schwenke and Haigh, 2016). In our study,  $N_2O$  emissions increased over the unfertilized control ( $T_1$ ) plot by 126.3 % in winter rice and by 211.7 % in autumn rice when the full RDF ( $T_2$ ) was applied. The rationale behind the higher  $N_2O$  emission might be ascribed to the fact that urea [ $CO(NH_2)_2$ ] hydrolyses to form ammonium ( $NH_4^+$ ) and then its oxidation by the nitrifying bacteria forms nitrate ( $NO_3^-$ ). Thus, the risk of loss through leaching and as  $N_2$  and  $N_2O$  is increased (Jamali et al., 2016). We observed a decline in  $N_2O$  emission with INM in rice crop over RDF. Among the organic sources of nutrients,  $N_2O$  emission was minimum with *Azolla* (used in dual crop with winter rice) compared to FYM and rice stubbles. Recently, Kimani et al. (2018) reported that unlike other symbiotic  $N_2$  fixation plants (emitting more  $N_2O$  to the atmosphere), *Azolla* in dual crop with rice systems did not result in extra  $N_2O$  fluxes. However, the rapid increase in  $N_2O$  emissions after 45 DAT of the winter rice in the *Azolla* treatment could be due to the incorporation of *Azolla* into the soil at 47 DAT, hence decomposition of *Azolla* biomass (containing a higher amount of N) might have increased  $N_2O$  emission in comparison to the other treatments during the subsequent rice-growing season. Our results indicated 13.0 and 6.8 % higher emission of  $N_2O$  from the rice-rice cropping sequence owing to the addition of rice stubbles and FYM, respectively as compared to *Azolla*. Stimulating effect of rice stubbles incorporation on  $N_2O$  flux during rice season was reported earlier (Liu et al., 2019; Wang et al., 2019). Supplementation of N through organic inputs increases SOC stock (Gogoi et al., 2021a) that can induce high  $N_2O$  emissions (Stehfest and Bouwman, 2006).

Generally,  $N_2O$  emission peaks in response to N top dressing in the rice crop (Akiyama et al., 2006; Vilakazi et al., 2021). From our study

(Fig. 5), it was apparent that N<sub>2</sub>O emission peaked first at 30 DAT and secondly at 60 DAT of winter rice (except in the control). Two N<sub>2</sub>O peaks were also observed at 30 and 45 DAT of autumn rice. The second N<sub>2</sub>O emission peak was recorded at different age of plants owing to dissimilarity in the growth durations of the cultivars. After panicle emergence (60 and 45 DAT), the rate of N<sub>2</sub>O flux started to decline and low emissions were recorded at crop maturity stages (105 and 75 DAT) both in winter and autumn rice, respectively (Fig. 5). The peaks of N<sub>2</sub>O found in this study could be ascribed to the organic N contained in the organic materials (such as rice stubble, FYM, and *Azolla*) that mineralize and thus represents a source of N<sub>2</sub>O in soil (Mosier et al., 1995). Earlier research also reported a direct relationship of N<sub>2</sub>O emission with split application of urea i.e., application time (Steinbach and Alvarez, 2006) and rate of N fertilizers (Vilakazi et al., 2021). Enhanced N<sub>2</sub>O emission due to intensive N fertilization may be credited to the shifts of denitrifying microorganisms under anaerobic condition (Li et al., 2021).

Finally, the greenhouse gas emission intensity (GHGI) with positive values has signified the net source of CO<sub>2</sub> equivalents per unit of yield. In the present study, the rice monocropping (rice-rice sequence) managed under INM was a net GHG source. The quantification of emissions per unit of economic output has been significantly higher in case of RDF + RS over other treatments which could be primarily ascribed to higher CH<sub>4</sub> emission from RS addition. Besides, RDF + RS treatment has shown significantly higher global warming potential (1458.7 kg CO<sub>2</sub>-eq. ha<sup>-1</sup>) over others. Mboyerwa et al. (2022) reported GHGI from the rice field (0.42–2.04 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> paddy) which were higher compared to this present study. Our estimated rates of GHGI have been lower both in case of winter and autumn rice (217.7–361.1 and 179.0–236.0 kg CO<sub>2</sub>-eq t<sup>-1</sup> grain yield, respectively) compared to previous studies (Ali et al., 2019; Win et al., 2020; Yu et al., 2021) might be due to dissimilarities in management practices.

#### 4.1. Statistical correlations

The long-term integrated appliance of chemical fertilizers and organic materials improves soil quality contributing to various fractions of C and N in soil (Lal, 2004). The increased contents of C and N enhanced the microbial activities in soil, and ultimately increased CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emission. Previous research also shown that GHG emission pathways are microbially mediated and hence dependent on soil C and N (Spott et al., 2011; Rex et al., 2018). Significant and positive correlations of C and N content in soil with GHG emission from rice fields were reported earlier (Baruah et al., 2010) because of the addition of organic matter that increases the C supply to microorganisms and their activity that in turn enhances GHG flux (Wassmann et al., 1996). Organic C and N compounds are used by soil microorganisms (such as methanogens, nitrifiers, denitrifiers and others) as energy source for their growth (Tiedje et al., 1982; Gogoi et al., 2021b) and that may be the cause for the significant positive association of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O with C and N fractions in this study.

The temporal dynamics and magnitude of GHG emissions depend on key soil properties and microbial activities (Wang et al., 2018). However, the potential recurrent behaviour of key soil properties as well as the possible effects of different management choices could be analyzed through the Recurrence Quantification Analysis (RQA) already applied to study the temporal dynamics of different ecological problems (Zurlini et al., 2018; Petrosillo et al., 2021). The C and N cycles are mediated by soil microbes, mostly by bacterial activity; thus, positive correlations were recorded between microbial populations and GHG emissions from paddy soils. Owing to their lack of the N<sub>2</sub>O-reductase enzyme (that reduces N<sub>2</sub>O to N), fungi does not entirely complete the N-cycling process (Mothapo et al., 2015; Maeda et al., 2015); this is probably the reason why fungi population was significantly and positively correlated with N<sub>2</sub>O emission in our study. Significant and positive correlation of dehydrogenase (DH) enzyme with CO<sub>2</sub> and N<sub>2</sub>O emission could be attributed to the fact that DH takes part in the electron transport system

of O<sub>2</sub> metabolism; DH is also present in all active microbial cells, which indicates the soil microbiological activity (Nannipieri et al., 2011). Several enzymes (*viz.*, esterases, lipases and proteases) can hydrolyze the fluorescein diacetate (FDA) and produce the fluorescent compound fluorescein, and FDA hydrolysis is measured an excellent indicator of soil biological property (Gogoi et al., 2021b). In our study, FDA activity increased with the integrated appliance of biomaterials with the reduced doses of inorganic fertilizers leading to an increase in soil C and N status and microbial activities. Consequently, FDA hydrolysis showed a significant positive correlation with GHG emission from paddy soils (Nayak et al., 2007). Urease activity commonly indicates the soil quality resulting from the nutrient management practices (Diaz-Marcote et al., 1995). A higher C and N concentration in soil stimulated the urease activity (Gogoi et al., 2021b) reflecting significant positive correlations with the emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O from soil (Mandal et al., 2018).

## 5. Conclusions

Systemic and comprehensive comparisons among the greenhouse gases (GHGs) from rice fields under different management practices are of immense importance for efficient emission reduction measures to deal with global climate change. Integrated application of organic materials (farmyard manure, rice stubbles and *Azolla*) and chemical fertilizers significantly influenced GHG emission from the rice-rice system, highlighting that both quantitative and qualitative aspects of organic inputs significantly influenced the GHG emissions. Likewise, further research is required in the other predominant cropping sequences for addressing the ecological contribution of agricultural management on GHG emissions and climate change. Adjusted management of agroecosystems can play a crucial role in optimizing cropping systems to increase soil ecosystem services like soil carbon sequestration and mitigate the GHGs emissions (Babu et al., 2023), contributing to climate action and, in general, to sustainability (Fusco et al., 2023). In this sense, green cropping systems can represent a suitable climate change mitigation strategy at local scale. However, as in the case of land conversion (Huang et al., 2023), more detailed applications taking into consideration the spatial effect on the results is needed.

Finally, within the context of Agenda 2030 for sustainable development goals (SDGs), it is evident how management choices at local scale can affect the achievement of a real sustainable development. This research has shown the interrelated effects of different management practices on climate change mitigation. However, the reduction of chemical fertilizers could affect positively the quality of soil and biodiversity in agroecosystems, while guaranteeing food security. Therefore, management practices represent the main agroecological approach towards the achievement of different SDGs in developing countries.

#### CRedit authorship contribution statement

**Bhabesh Gogoi:** Conceptualization. **Ranjan Das:** Resources. **Dhruba Jyoti Nath:** Resources. **Samiron Dutta:** Resources. **Monisha Borah:** Formal analysis. **Lipika Talukdar:** Formal analysis. **Dilip Kumar Patgiri:** Project administration. **Kalyan Pathak:** Project administration. **Donatella Valente:** Writing – review & editing. **Irene Petrosillo:** Writing – review & editing. **Nilay Borah:** Supervision.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.



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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2024.111876>.

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