Mitigation of greenhouse gas intensity by supplementing with *Azolla* and moderating the dose of nitrogen fertilizer

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ABSTRACT

Integrated use of biological-chemical nitrogen application during rice cultivation is the way forward for sustainable rice production. Experiments were performed to evaluate the effects of *Azolla* (blue green algae) application along with reduced dose of recommended N fertilizer (urea) application on emissions of nitrous oxide (N_2O), methane (CH_4), global warming potential (GWP) and greenhouse gas intensity (GHGI) in rice. The study consisted of nine treatments on growing three rice cultivars Pusa 1509, Pusa RH-10, and Pusa-44 under recommended fertilizer (120 kg N ha^{-1} by Urea), reduced fertilizer along with *Azolla* application (90 kg N ha^{-1} by urea $+50$ g m^{-2} *Azolla*), and low fertilizer along with *Azolla* application (60 kg N ha^{-1} by urea $+50$ g m^{-2} *Azolla*). The lowest cumulative seasonal emissions of N_2O, CH_4, and GWP were observed in LN1509 (81.55 mg N_2O m^{-2}), RN1509 (2.476 g CH_4 m^{-2}), and RN1509 (110.0 g CO_2 eq. m^{-2}), respectively. Application of *Azolla* along with the reduced dose of urea did not significantly decrease the yield of rice and the GHGI reduced by 18.41%, 16.88%, and 17.97%, respectively, in Pusa 1509, Pusa RH-10, and Pusa-44 as compared to the recommended fertilizer treatment. The findings of this study suggested that the use of *Azolla* in rice might help in reducing the application of urea fertilizer by 25% in rice without affecting the rice yields. This will be a win-win situation for the farmer who will be saving on the cost of fertilizer and for the policy makers as well by mitigating the greenhouse gas emissions.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important global cereal crops mainly cultivated across Asia (Bhatia et al., 2012a). Continuous standing water throughout the crop growth period is generally practiced for higher rice production (Suryavanshi et al., 2013). However, continued flooding of water in rice creates an anaerobic environment in soil, which results in lowering the soil redox potential (Eh) (Dubey, 2005). Lower Eh increases the population of methanogenic bacteria (Jiang et al., 2019). Methanogens consume the soil organic matter as a carbon source and emit methane (CH_4) as a byproduct gas to the

atmosphere (Malyan et al., 2016a). According to the latest data published by FAO (2019), the global paddy production in 2017 was around 769 million tonnes and India contributed about 168 million tonnes to the total global production (FAO, 2019). Rice is a cereal crop for more than half of the world population and its demand is increasing as the world population is increasing (Yang et al., 2019; Ranjan and Yadav, 2019). To achieve higher production goals, high yielding rice varieties are cultivated, which require higher amounts of nitrogenous (N) fertilizers as compared to low yielding rice cultivars (Pathak et al., 2014). The amount, type, mode of N fertilizer application for rice significantly affect the emission rate of CH_4 and nitrous oxide (N_2O) from the rice

fields to the atmosphere (Bhattacharyya et al., 2018; Malyan, 2017; Gupta et al., 2016; Hussain et al., 2015; Bhatia et al., 2005, 2012b; Malla et al., 2005). The mitigation strategies promulgated for each of these gases result in a trade-off relationship between CH_4 and N_2O emissions from rice fields wherein decrease in the emission of one gas may result in an increase in the emission of the other (Bhatia et al., 2010; Gupta et al., 2015). Therefore, mitigation of N_2O and CH_4 during rice cultivation is of primary concern to all the rice researchers. *Azolla* is a free floating water fern, which occurs in symbiotic association with the cyanobacteria *Anabaena azollae* and fixes atmospheric N_2 (Bhuvaneshwari and Singh, 2015; Ali et al., 2012; Pabby et al., 2003). Beneficial uses of *Azolla* such as nitrogen fixation, rich organic content, and weed control have been documented earlier in a few studies (Singh and Strong, 2016; Abraham et al., 2015; Yadav et al., 2014; Pabby et al., 2003). *Azolla* with its nitrogenfixing capacity and ability to scavenge nutrients from soil and water is the most commonly used green manure for rice crop in Asia. Increase in plant height, number of effective tillers, dry mass and nitrogen content of rice plants with the use of *Azolla* and N-fertilizers alone and other combinations have been reported in literature (Bhuvaneshwari and Singh, 2015; Ali et al., 2012). *Azolla* doubles its biomass in a few days (Watanabe et al., 1989) and can supply more than half of the required nitrogen to the rice crop (Pabby et al., 2003) and other beneficial impacts such as preventing rise in pH, reducing water temperature, curbing NH_3 volatilization, suppressing weeds and mosquito proliferation (Kollah et al., 2016; Cisse and Vlek, 2003). Previous studies have shown that *Azolla* application in the rice could decrease (Ali et al., 2012; Bharati et al., 2000) or increase (Ying et al., 2000; Chen et al., 1997) CH_4 emissions from rice soils. The results may vary due to different rice cultivars, soil types and other experimental conditions.

N_2O emission from rice paddy along with *Azolla* application has earlier been studied by Chen et al. (1997) and Ma et al. (2012). They reported an increase in N_2O emission from rice paddies due to N_2-fixation by *Azolla*, providing a source for N_2O production through nitrification and denitrification. However, the effect of *Azolla* on emission of both CH_4 and N_2O together in rice is very limited and has only recently been reported by Kimani et al. (2018), who observed a significant reduction in CH_4 emission without any significant lowering in N_2O emission. However, as *Azolla* in rice has been used in Asia as a green manure for its N2 fixation potential, its application along with inorganic N fertilizer may lead to a decrease in the dose of inorganic N fertilizer applied, thereby, affecting the N_2O emissions from rice. This may lead to a reduced greenhouse gas intensity (GHGI) due to a lower global warming potential per tonne of rice yield. The effects of different rice genotypes on global warming potential (GWP) and greenhouse gas intensity (GHGI) are not well documented (Gorh and Baruah, 2019). This study was thus carried out to quantify the effects of *Azolla* application on three rice cultivars of different durations with recommended and reduced doses of N fertilizer on emissions of CH_4 and N_2O, global warming potential and greenhouse gas intensity.

2. Materials and methods

2.1. Experimental site

Plastic crates were used to conduct experiments at the research farms of the Indian Agricultural Research Institute (IARI), New Delhi. This study site is located in the Pusa Campus, New Delhi (28°38′23″N, 77°09′27″E, altitude: 228 m), Northern India (Fig. 1). The region lies in the Indo-Gangetic Plain, which has a humid subtropical climate and is characterized by dry winter and prolonged hot summer and maximum rainfall occurs during late June to mid-September of year. The region has an annual rainfall around 700 mm and an annual mean temperature of 25 °C, which varies from 46 °C in summer to 0 °C in winter. Weather conditions such as maximum, minimum air temperature and rainfall in the experimental farms were collected from the metrological

observatory of IARI and represented in Fig. 2. The soil used in this study was collected from the upper 15 cm depth of rice research farm and the soil was sandy loam in texture as being developed from alluvial deposits. The initial properties of the experimental farm soil (0–15 cm depth) were pH (1:2.5) 8.3, bulk density $1.39\,g\,cm^{-3}$, EC $0.28\,dS\,m^{-2}$, organic C 0.53%, available N $250\,kg\,ha^{-1}$, available P $31.9\,kg\,ha^{-1}$ and available K $366\,kg\,ha^{-1}$.

2.2. Treatment details and management practices

The experiment consisted of nine treatments: N1509- rice cultivar 1509 with recommended N fertilizer ($120\,kg\,N\,ha^{-1}$), RN1509- rice cultivar 1509 with reduced N fertilizer ($90\,kg\,N\,ha^{-1}$) + *Azolla* ($50\,g\,m^2$), LN1509- rice cultivar 1509 with low N fertilizer ($60\,kg\,N\,ha^{-1}$) + *Azolla* ($50\,g\,m^2$), NPRH10- rice cultivar Pusa RH-10 with recommended N fertilizer, RNPRH10- rice cultivar Pusa RH-10 with reduced N fertilizer ($90\,kg\,N\,ha^{-1}$) + *Azolla* ($50\,g\,m^2$), LNRH10- rice cultivar Pusa RH-10 with low N fertilizer ($60\,kg\,N\,ha^{-1}$) + *Azolla* ($50\,g\,m^2$), NP44- rice cultivar Pusa-44 with recommended N fertilizer, RNP44- rice cultivar Pusa-44 with reduced N fertilizer ($90\,kg\,N\,ha^{-1}$) + *Azolla* ($50\,g\,m^2$) and LNP44 rice cultivar Pusa-44 with low N fertilizer ($60\,kg\,N\,ha^{-1}$) + *Azolla* ($50\,g\,m^2$) (Table 1). Single Superphosphate for P (@ $60\,P_2O_5\,kg\,ha^{-1}$) and Muriate of Potash (MOP) for K (@ $40\,K_2O\,kg\,ha^{-1}$) were applied during all the treatments at the time of sowing. P and K were applied as basal while N was applied in three splits: ½ N as basal application, ¼ N as first split at the tillering stage and ¼ as second split at the panicle initiation stage. Thirty-three day old rice seedlings of Pusa-1509, PRH-10, and Pusa-44 were transplanted on 25 June 2015. Fresh *Azolla* was broadcast 12 days after transplantation into the standing water surface at a rate of $50\,g\,m^2$ (Table 1). The applied *Azolla* multiplied vegetatively and thus, it was maintained throughout the crop. Standing water from the plastic crates was drained 20 days before harvesting of the crop. No pesticide and herbicide was applied and weeding was done manually whenever required. Irrigation was applied whenever required for maintaining flooded soil conditions in the crates.

2.3. Gas sampling and analysis

Closedchamber was used to measure CH_4 and N_2O emissions from rice crates (Bhatia et al., 2011). Acrylic chambers (15 cm × 15 cm x 100 cm; length x width x height), fitted with thermometer, battery operated fan and rubber septa on the top were used for sampling of gases. Gas samples were collected once in a week between 9 and 11 a.m. using a 20-ml syringe fitted with a three-way stopcock, at 0, 30, and 60 min after chamber closure. Gas samples were also collected on 0 and 1 days after transplanting (DAT), followed by a seven day regular interval. For each treatment, sampling was carried out in triplicates.

CH_4 and N_2O concentrations in the collected sample were analyzed by a Gas Chromatograph (GC: Hewlett Packard 5890) having a stainless steel column (Porapak N) fitted with a flame ionization and electron capture detector. The cumulative amount of CH_4 and N_2O emissions were determined by linear interpolation of two adjacent intervals of measurements carried out on the sampling days assuming that GHGs emissions followed a linear trend during the periods when no sample was taken (Pathak et al., 2003, 2013).

The emissions of CH_4 and N_2O from soil were calculated by the following equation:

$$F = \rho \times (V/A) \times (\Delta c / \Delta t) \times (273 / T)$$

Where, F was the CH_4/N_2O flux (mg CH_4 $m^{-2}\,h^{-1}$/µg N_2O $m^{-2}\,h^{-1}$), ρ was the gas density, V was the volume of the close chamber (m^3), 'A' was the surface area of the closed chamber (m^2), $\Delta c/\Delta t$ was the rate of increase of CH_4/N_2O gas concentration in the chamber (mg/µg $m^{-3}\,h^{-1}$) and T (absolute temperature) was calculated as 273 + mean
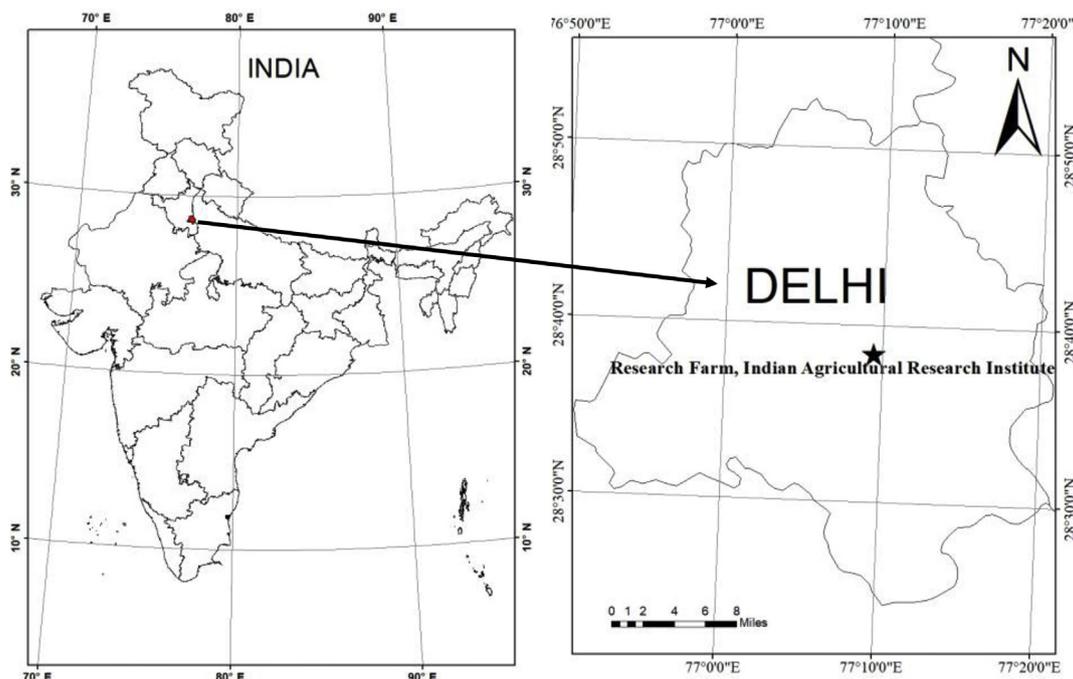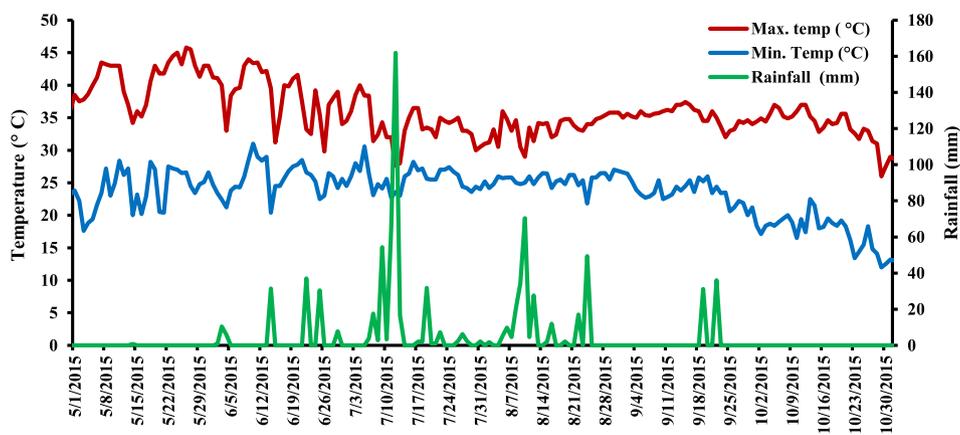
Fig. 1. Map showing the study site.

Fig. 2. Weather conditions in the experimental farm during the crop cultivation period.

temperature (°C) of the chamber. Total CH_4/N_2O flux for the entire cultivation period was computed by linear interpolation (Singh et al., 1999) using the following equation:

Total gas flux = Σ_i^n (Ri x Di)

Where, R_i was the CH_4/N_2O emission flux (g m^{-2} d^{-1}) on the ith sampling interval, Di was the number of days in the ith sampling interval, and n was the number of sampling intervals.

Table 1
Treatment details of the study.

Rice cultivar (Type)	Treatment	N Source (g N m^{-2})		Remarks
		Urea	Azolla	
Pusa-1509 (Short duration variety)	N1509	12	NA	Recommended dose (12 g N m^{-2}) of N fertilizer through urea
	RN1509	9	3*	Reduced dose (9 g N m^{-2}) of N fertilizer by Urea
	LN1509	6	3*	Low dose (6 g N m^{-2}) of N fertilizer by Urea
Pusa RH-10 (Medium duration variety)	NPRH-10	12	NA	Recommended dose (12 g N m^{-2}) of N fertilizer through urea
	RNPRH-10	9	3*	Reduced dose (9 g N m^{-2}) of N fertilizer by Urea
	LNPRH-10	6	3*	Low dose (6 g N m^{-2}) of N fertilizer by Urea
Pusa-44 (Long duration variety)	NP-44	12	NA	Recommended dose (12 g N m^{-2}) of N fertilizer through urea
	RNP-44	9	3*	Reduced dose (9 g N m^{-2}) of N fertilizer by Urea
	LNP-44	6	3*	Low dose (6 g N m^{-2}) of N fertilizer by Urea

NA-not applied; *50 g m^{-2} fresh Azolla was applied 12 days after transplanting (had the capacity to fix 3 g N m^{-2} per season).

2.4. Global warming potential (GWP), grain yield, and greenhouse gas intensity (GHGI)

GWP is the quantification of warming potential of a mole of trace gas released into the atmosphere relative to a mole of CO_2 as the standard gas. GWP of CH_4 was taken as 34 and that of nitrous oxide as 298 on a 100 year' time horizon (Yang et al., 2019; Malyan et al., 2018; Gupta et al., 2016). The Global warming potential (GWP), and the yield scaled emission in terms of greenhouse gas intensity (GHGI) i.e carbon emitted per unit of grain yield of rice were estimated using the following equations:

$$\text{GWP (g } CO_2 \text{ equivalent } m^{-2}) = \text{seasonal } CH_4 \text{ emission (g } CH_4\ m^{-2})^* \ 34 + \text{ seasonal } N_2O \text{ emmission (g } N_2O\ m^{-2})^*\ 298 \qquad\qquad 298$$

$$\text{Greenhouse gas intensity (GHGI)} = \text{GWP (g } CO_2 \text{ eq. } m^{-2})/ \text{ grain yield (g } m^{-2})$$

2.5. Statistical analysis

Data were analyzed by applying the technique of 'analysis of variance (ANOVA)' for Randomized Block Design using SPSS (version 17.0) software. ANOVA with Duncan's Multiple Range Test (DMRT) at 5% level of significance was carried out to test whether the differences between means were statistically significant or not.

3. Result and discussion

3.1. Nitrous oxide emission

The first major N_2O peak occurred just after the application of the first split of N fertilizer (basal dose) (Fig. 3). N_2O flux ranged from 86 to 1358 $\mu g\ m^{-2}\ day^{-1}$, 71–1293 $\mu g\ m^{-2}\ day^{-1}$, and 49–1344 $\mu g\ m^{-2}\ day^{-1}$ in Pusa-1509 (Fig. 3A), Pusa RH-10 (Fig. 3B), and Pusa-44 (Fig. 3C) respectively, during rice growth. The second highest peak of N_2O emission from Pusa-1509, Pusa RH-10, and Pusa-44 were observed at 50, 43, and 36 days after transplanting (DAT) respectively. The peak N_2O flux was observed one day after each N fertilizer application due to hydrolysis of the fertilizer. The highest cumulative N_2O flux was found in NP-44 (125.7 mg $N_2O\ m^{-2}$) followed by NPRH-10 (122.6 mg $N_2O\ m^{-2}$), N1509 (111.9 mg $N_2O\ m^{-2}$), RNPRH-10 (104.6 mg $N_2O\ m^{-2}$), RNP-44 (103.9 mg $N_2O\ m^{-2}$)), LNP-44 (92.42 mg $N_2O\ m^{-2}$), RN1509 (91.14 mg $N_2O\ m^{-2}$), LNPRH-10 (88.88 mg $N_2O\ m^{-2}$) and LN1509 (81.55 mg $N_2O\ m^{-2}$) as shown in Table 2. In all the three rice cultivars

Table 2
Grain yield, nitrous oxide, methane, global warming potential (GWP), and greenhouse gas intensity (GHGI) of rice.

Treatment	Yield (g m^{-2})	N_2O (mg m^{-2})	CH_4 (g m^2)	GWP (g CO_2 eq. m^{-2})	GHGI
N1509	426d*	111.9c	3.113d	139.2d	0.327d
RN1509	418d	91.14g	2.476g	111.4g	0.266f
LN1509	318g	81.55i	2.521f	110.0g	0.346a
NPRH-10	473c	122.6b	3.585b	158.4b	0.335c
RNPRH-10	481b	104.6d	3.025d	134.0e	0.278e
LNPRH-10	368f	88.88h	2.904e	125.2f	0.340b
NP-44	518a	125.7a	4.161a	179.0a	0.346a
RNP-44	515a	103.9e	3.360c	145.2c	0.284e
LNP-44	401e	92.42f	3.321c	140.5d	0.350a

*Different letters are significantly different (at p < 0.5) by DMRT in a column.

(Pusa-1509, Pusa RH-10, and Pusa-44), the lowest cumulative N_2O flux was observed in the low dose of N fertilizer application. N_2O emissions from rice under *Azolla* application have not been very well documented. Kimani et al. (2018) in a recent study reported that *Azolla* application in rice did not have any significant impact on N_2O emission and they observed a very low N_2O flux (0.01 $\mu g\ N\ m^{-2}\ h^{-1}$) for up to four weeks after transplanting. In the present study, N_2O emission for the first two weeks in all the treatments was relative higher and very different from that reported by Kimani et al. (2018). Kimani et al. (2018) observed that the low concentration of nitrate (NO_3^--N) in the soil solution was responsible for the low N_2O flux as high NO_3–N in soil is the main substrate for denitrification process, which emits the N_2O gas (Kimani et al., 2018). The type of fertilizer applied was probably nitrate based (as not specified in paper). It has been reported that the application of N fertilizer in rice enhances the N_2O emissions because the hydrolysis of urea to ammonium occurs (Gupta et al., 2016). Ammonium produced from urea under anaerobic conditions, as in this study, acts as a substrate for autotrophic ammonia-oxidiser microorganisms and produces N_2O through ammonia oxidation followed by denitrification (Malyan, 2017; Kumar and Malyan, 2016; Majumdar, 2003). In the present study, urea applied was hydrolyzed to ammonium, which was then nitrified and denitrified producing high fluxes of N_2O emission.

The application of lower doses of urea in RN and LN treatments reduced N_2O emission significantly. The substitution of 30 kg N ha^{-1} (RN1509) and 60 kg N ha^{-1} (LN1509) with *Azolla* in short duration rice variety 1509 reduced the N_2O flux by 18.56% and 27.13%, respectively over the control treatment (N1509) Table 3. In the medium duration rice variety, substitution of 30 kg N ha^{-1} and 60 kg N ha^{-1} by *Azolla* in
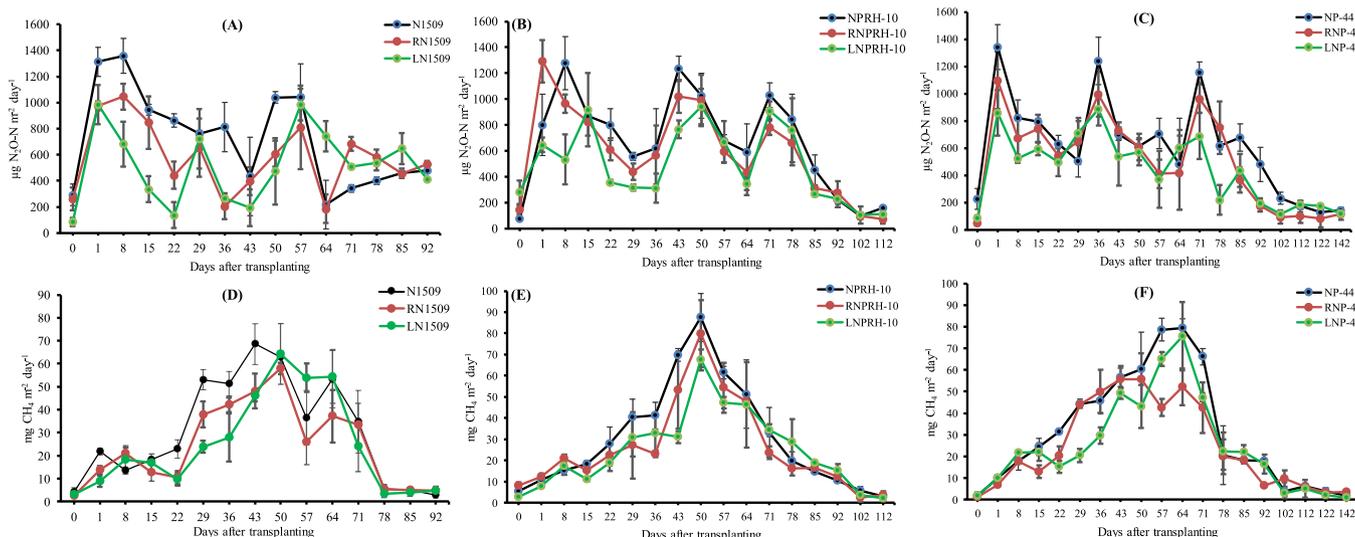

Fig. 3. Temporal emission of N_2O in (A) Pusa-1509 (B) PRH-10 (C) Pusa-44; Temporal emission of CH_4 in (D) Pusa-1509 (E) PRH-10 (F) Pusa-44.

Table 3
Comparison of greenhouse gas (CH_4 and N_2O) emission from this study with other relevant studies.

Study site (References)	Treatment	Nitrogen fertilizer	CH_4 (kg CH_4 ha^{-1}) [mitigation%]	N_2O (mg N_2O m^{-2}) [mitigation%]
New Delhi, India (This study)	N1509	120 kg N ha^{-1} by Urea (U)	31.13[C^l]	111.9 [C]
	RN1509	90 kg N ha^{-1} by U + 30 kg N ha^{-1} by fresh *Azolla*	24.76[20.46%]	91.14[18.56%]
	LRN1509	60 kg N ha^{-1} by U + 30 kg N ha^{-1} by fresh *Azolla*	25.21[19.03%]	81.55[27.13%]
	NPRH-10	120 kg N ha^{-1} by U	35.85[C]	122.6[C]
	RNRH-10	90 kg N ha^{-1} by U + 30 kg N ha^{-1} by fresh *Azolla*	30.25[15.63%]	104.6[14.70%]
	LNRH-10	60 kg N ha^{-1} by U + 30 kg N ha^{-1} by fresh *Azolla*	29.04[18.99%]	88.88[27.49%]
	NP-44	120 kg N ha^{-1} by U	41.61[C]	125.7[C]
	RNP-44	90 kg N ha^{-1} by U + 30 kg N ha^{-1} by fresh *Azolla*	33.60[19.26%]	103.9[17.36%]
	LNP-44	60 kg N ha^{-1} by U + 30 kg N ha^{-1} by fresh *Azolla*	33.21[20.19%]	92.42[26.50%]
Hunan Province, China (Yang et al., 2019)$^&$	CFN2A0	0.9 g N pot^{-1} by U (continues flooding)	2.31 g CH_4 pot^{-1} [C]	Not study (NS)
	CFN2A2	0.9 g N pot^{-1} by U + fresh *Azolla* (continues flooding)	1.56 g CH_4 pot^{-1} [32.47%]	NS
	CFN0A0	0 g N pot^{-1} by U (continues flooding)	2.71 g CH_4 pot^{-1} [C]	NS
	CFN0A2	0 g N pot^{-1} by U + fresh *Azolla* (continues flooding)	1.92 g CH_4 pot^{-1} [29.15%]	NS
	MSAN2A0	0.9 g N pot^{-1} by U (midseason aeration)	1.31 g CH_4 pot^{-1} [C]	NS
	MSAN2A2	0.9 g N pot^{-1} by U + fresh *Azolla* (midseason aeration)	0.87 g CH_4 pot^{-1} [33.59%]	NS
	MSAN0A0	0 g N pot^{-1} by U (midseason aeration)	1.53 g CH_4 pot^{-1} [C]	NS
	MSAN0A2	0 g N pot^{-1} by U + fresh *Azolla* (midseason aeration)	1.18 g CH_4 pot^{-1} [22.88%]	NS
Yamagata, Japan (Kimani et al., 2018)$^&$	Control	183.84 kg N ha^{-1} (fertilizer not specified)	73.87* [C]	4.27[C]**
	Azolla	183.84 kg N ha^{-1} + *Azolla* (5 g pot^{-1})	48.27*[34.66%]	4.07[4.68%]
Hunan Province, China (Xu et al., 2017)	RAN0	*Azolla* with +0 kg N ha^{-1}	376.2	NS
	RMN0	0 kg N ha^{-1}	319.7	NS
	RAN1	100 kg N ha^{-1} by U + *Azolla*	497.9	NS
	RMN2	200 kg N ha^{-1} by U + *Azolla*	428.3	NS
Hunan Province, China (Zhang et al., 2017)	NPK	100 kg N ha^{-1} by U	198.1 [C]	NS
	NPK + *Azolla*	100 kg N ha^{-1} by U + *Azolla* (0.76 kg m^{-2})	175.9 [11.21%]	NS
Mymensingh, Bangladesh (Ali et al., 2012)	Urea (upland site)	250 kg N ha^{-1} by U	105.5 [C]	NS
	U + *Azolla* (upland site)	187.5 kg N ha^{-1} by U + *Azolla* (1 t ha^{-1})	91.3 [13.46%]	NS
	Urea (lowland site)	250 kg N ha^{-1} by U	129.0 [C]	NS
	U + *Azolla* (lowland site)	187.5 kg N ha^{-1} by U + *Azolla* (1 t ha^{-1})	114.5 [11.24%]	NS
Cuttack, India (Bharati et al., 2000)	Urea-N	60 kg N ha^{-1} by U	155.28 [C]	NS
	U + *Azolla*	30 kg N ha^{-1} by U + 30 kg N ha^{-1} by *Azolla*	149.37 [3.81%]	NS
	U + *Azolla* dual cropping	30 kg N ha^{-1} by U + 30 kg N ha^{-1} by *Azolla*	89.29 [42.50%]	NS
	Azolla + *Azolla* dual cropping	60 kg N ha^{-1} by *Azolla*	105.64 [31.97%]	NS

$^&$Pot study; C^l-control; * Result converted from g C m^{-2} to kg CH_4 ha^{-1}; ** Result converted from mg N m^{-2} to mg N_2O m^{-2}.

RNPRH-10 and LNPRH-10 treatments resulted in 14.70% and 27.49% less cumulative N_2O emission, respectively as compared to NPRH-10 treatment during the crop growth period (Table 3). The cumulative N_2O emissions in long duration variety were also reduced in lower doseRNP-44 (17.36%) and LNP-44 (26.50%) as compared to NP-144 (Table 3).

3.2. Methane emission

In the current study, the crop growth duration (transplanting to harvesting) for rice cultivars Pusa-1509, Pusa RH-10, and Pusa-44 was 92, 112 and 142 days respectively. CH_4 flux was low for the first three weeks in all the treatments and the pattern of fluxes is shown in Fig. 3. The increase in CH_4 flux started around 29 DAT in all the three rice cultivars. The highest peak of CH_4 flux was observed on 43 DAT, 50 DAT, and 64 DAT in Pusa-1509 (Fig. 3D), Pusa RH-10 (Fig. 3E), and Pusa-44 (Fig. 3F) respectively, when the rice plants were at the stem elongation stage. In the cultivars Pusa-1509, Pusa RH-10, and Pusa-44, the CH_4 flux ranged from 2.61 to 68.53 (Figs. 3D), 2.05 to 87.52 (Figs. 3E), and 0.95–79.32 mg CH_4 m^{-2} day^{-1}(Fig. 3F), respectively during the crop growth period. The cumulative CH_4 emission per season was the lowest in RN-1509 (g 2.476 CH_4 m^{-2}) and the highest in NP-44 (4.161g CH_4 m^{-2}) treatment (Table 2). The cumulative CH_4 emission was higher in Pusa-44 as compared to the other rice varieties (Pusa-1509 and Pusa-44) due to longer crop duration and higher root and shoot biomass. The root biomass is the most important substrate for methanogenic bacteria (Le Mer and Roger, 2001), which might have resulted in higher CH_4 emissions by this variety.

The rate of CH_4 emission from the soil depends upon the availability of soil organic matter, redox potential (Eh), above/belowground crop biomass, duration of crop and water management (Xu et al., 2017; Gupta et al., 2016; Le Mer and Roger, 2001). It may also be affected by the type, method, and dose of nitrogen fertilizer application (Kumar and Malyan, 2016; Hussain et al., 2015; Majumdar, 2003; Le Mer and Roger, 2001). In the current study, the low CH_4 fluxes in all the treatments during the first week were due to the high soil Eh. The higher soil Eh impeded the CH_4 production rate in soil and hence, resulted in lower CH_4 emissions (Malyan, 2017; Xu et al., 2017). Subsequently for the next two weeks, lower emissions could be associated with low substrate availability for the methanogens and as ebullition was the major pathway for CH_4 emission from rice soil. As the plant became bigger and aerenchyma started to develop, higher CH_4 emissions were observed. (Malyan et al., 2016a). In all the rice varieties, the CH_4 flux for all the treatments (full N dose, reduced N and low N) was observed to be the highest during the midseason (Fig. 3). The reason for high CH_4 emission could be associated with standing water, which reduced the soil Eh and the greater availability of carbon substrate due to higher root exudation around maximum tillering to booting stage, which might have resulted in higher CH_4 production by methanogens (Malyan et al., 2016b; Serrano-Silva et al., 2014; Shi et al., 2006). Among the three cultivars, the lowest cumulative CH_4 emission under reduced N fertilizer along with *Azolla* cover was observed in Pusa-1509 cultivar while the lowest cumulative CH_4 emission under low N fertilizer along with *Azolla* cover were observed in Pusa RH-10 and Pusa-44 cultivars (Table 2). The reason for low cumulative CH_4 emission could be that *Azolla* application liberated oxygen in standing water, which upsurged the soil Eh and lowered the CH_4 production (Xu et al., 2017; Hussain et al., 2015; Bharati et al., 2000). In all the three cultivars, (Pusa-1509, Pusa RH-10, and Pusa-44), *Azolla* application with reduced N fertilizer decreased the seasonal CH_4 emission by 20.46%, 15.63%, and 19.26%, respectively as compared to the recommended N treatment (Table 3). The results of this study were also supported by pervious studies (Yang et al., 2019; Kimani et al., 2018; Zhang et al., 2017; Xu et al., 2017; Ali et al., 2012; Bharati et al., 2000) (Table 3). Yang et al. (2019) reported that *Azolla* inoculation significantly decreased cumulative CH_4 emissions in both continuous flooding and midseason aeration in rice (Table 3). Bharati et al. (2000) found that a 42.50% reduction in

seasonal CH_4 emission under *Azolla* dual cropping over urea alone in the rice fields of Cuttack, India (Table 3). Ali et al. (2012), quantified the impact of *Azolla* cover with urea in lowland and upland site of Mymensingh, Bangladesh and they obtained about 13.46% and 11.24% reduction in CH_4, respectively, over control (Table 3). In an earlier study, Bharati et al. (2000) used *Azolla* as a supplement N source in rice field due to its nitrogen fixing capacity. In a rice field, *Azolla* on photosynthesis liberated oxygen in standing water and increased the amount of dissolved oxygen (Xu et al., 2017; Malyan et al., 2016a,b). Under high dissolved oxygen in the standing water of rice, the CH_4 emissions would be reduced by suppression of the methanogenic activity and enhancement of the methanotrophic activity. Zhang et al. (2017), observed that *Azolla* reduced CH_4 emission by 11.21% over control, which was similar to the present study(Table 3). The rice variety Pusa-1509 of shorter duration resulted in lower cumulative CH_4 emissions as compared to PRH-10 and Pusa-44 varieties.

3.3. Grain yield, global warming potential (GWP) and greenhouse gas intensity (GHGI)

Rice grain yield varied from 318 to 518 g m^{-2} among the treatments (Table 2). The grain yield was the highest in Pusa 44 variety followed by Pusa RH-10 and Pusa1509. The highest yield was observed during the recommended fertilizer treatment in Pusa-44 (518 g m^{-2}) and the lowest was observed during low N dose treatment in LN1509 (318 g m^{-2}) (Table 2). In the Pusa RH-10 variety, the rice yield was higher in the reduced N treatment with *Azolla* (RNPRH-10) as compared to the recommended N fertilizer treatment (NPRH-10), however, the increase was not significant. In the present study, *Azolla* application with a reduced dose of urea fertilizer (90 kg ha^{-1}) led to at par yields with the recommended N fertilizer treatment in all the three rice varieties (Table 2). However, the yield with low N fertilizer along with *Azolla* treatment was significantly lower as compared to the full N dose treatment in all the three rice varieties (Table 2). The results of this study showed that the chemical N fertilizer could be substituted up to 30 kg N ha^{-1} without affecting the grain yield but lowering N fertilizer further has led to a decline in the rice yield. Cisse and Vlek (2003) have reported that *Azolla* in rice had a capacity to fix 0.6–0.7 kg N ha^{-1} daily. This might be the reason for no significant loss in grain yield even when the fertilizer was reduced by 30 kg N ha^{-1} in the current study. In the present study, 76–80% and 20–24% GWP were contributed by CH_4 and N_2O, respectively (Fig. 4).

In Pusa-1509 cultivar, *Azolla* application with reduced (RN1509) and low (LN1509) N fertilizer decreased the GWP by 20.00% and 20.97%, respectively as compared to the recommended N fertilizer (Fig. 4). *Azolla* application in RNPRH-10, and LNPRH-10 reduced the GWP by 15.63% and 20.95%, respectively over NPRH-10 (Table 2). Ali
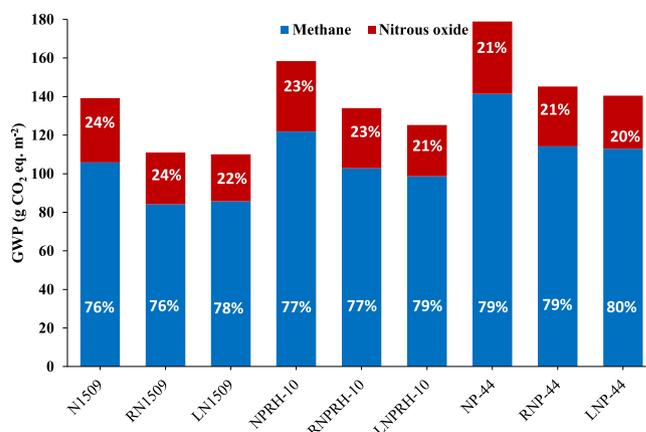

Fig. 4. Percentage contribution of methane and nitrous oxide to the total global warming potential.

et al., 2012 reported 11.24–13.46% mitigation in GWP as compared to the control with *Azolla* application to rice in Bangladesh. The yield-scaled greenhouses gas emission was represented as greenhouse gas intensity (GHGI). GHGI was the highest in LNP-44 and the lowest in RN1509 (Table 2). *Azolla* application in reduced N fertilizer treatment decreased the GHGI by 18.41%, 16.88%, and 17.97% over the recommended fertilizer in Pusa-1509, PRH-10 and P-44, respectively (Table 2). The lower yield–scaled CH_4 emission by the application of *Azolla* in rice have earlier been reported by Bharati et al. (2000) and Xu et al. (2017), which were similar to the present findings. Lowering the N fertilizer by $60 \, kg \, N \, ha^{-1}$ per season (LN1509 and LNP-44) in rice along with *Azolla* cover, however, led to increased GHGI due to reduced yields in the low N treatment (Table 2).

4. Conclusions

Azolla application along with the reduced dose of urea fertilizer into the standing water of rice significantly reduced the yield-scaled greenhouse emission as compared to the recommended dose of N application. Among the cultivars, Pusa 1509 had the lowest global warming potential per kg of rice yield. The GWP of rice cultivars viz., Pusa-1509, Pusa RH-10, and Pusa-44 could be significantly reduced by 20.00%, 15.63%, and 18.87% respectively, on application of *Azolla* along with $90 \, kg \, N \, ha^{-1}$ per season as compared to the recommended $120 \, kg \, N \, ha^{-1}$. Thus, *Azolla* application with a reduced amount of N fertilizer can be suggested for not only reducing the global warming potential of rice but also for better air quality. The findings of this study suggest that the use of *Azolla* in rice may help in reducing the application of urea fertilizer by 25% without deteriorating the rice yields.

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