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Combined influence of blue green algae and azolla on dissolved oxygen, redox status and methane emission potential of systems of rice cultivation

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Abstract
Blue Green Algae and *Azolla* are aerobic photosynthetic organisms. In the medium of their growth, they release a lot of O2 during photosynthesis. As a result, when they grow in rice fields they make the standing water highly oxygenated. Its directly influence the dissolved oxygen and redox potential ultimately reduces the emission of methane from rice filed. The field experiment was conducted at Ponnaniar Basin, Thirchirapalli District, Tamil Nadu, India in *rabi* season of 2013. Redox status of soil and dissolved oxygen content in the standing water are indirect indicators of methane flux from rice ecosystem and higher the redox and dissolved oxygen, lower would be the methane flux. Among the different systems of rice cultivation, System of Rice Intensification recorded higher dissolved oxygen and redox potential than other cultivation systems. BGA and *Azolla* application individually and in combination enhanced the dissolved oxygen concentration and redox in the standing water in all growth stages while the dissolved oxygen concentration was minimum in plots applied only with recommended dose of fertilizer. The mean methane flux in only fertilizer applied plot was higher, while the flux was reduced to 22.3 per cent, due to application of BGA and *Azolla.*

Keywords: blue green algae, azolla, dissolved oxygen, redox and methane

Introduction
Rice is the world's most important food crop and cultivated in different methods. Among different methods of rice cultivation, transplanting method is the most dominant and traditional method in irrigated low land rice, which not only consumes more water but also causes severe wastage of water and causes environmental pollution by emitting methane under anaerobic condition (Suresh Naik *et al.*, 2015) [1]. Methane (CH_4) is primarily a biogenic gas, which is implicated in global warming. Although its production in the anoxic conditions is regulated by several edaphic factors,. Among the edaphic factors, dissolved oxygen concentration (DO) and redox potential (Eh) are the most important, which largely determines the action of methanogenic bacteria. However, rice cultivation contributes to the emissions of the most influential greenhouse gases (GHGs), particularly CH_4 and N_2O. Approximately 30 per cent and 11 per cent of global agricultural CH_4 and N_2O emissions are attributed to rice fields (USEPA, 2006) [2]. Methane is emitted through the rice field in response to organic manure and inorganic nutrient application increases emission. But source, type and method of application may also have direct effects. Use of slow releasing nitrogenous fertilizers and humified organic manure reduces methane emission by inhibiting methanogenic bacterial activity in soil and sulfate reducing bacteria compete with methanogens for the limited hydrogen. Minimizing the methane emission from rice cultivation is considered as an important climate change mitigation strategy. In the global CH_4 cycle biological process consumes substantial amount of CH_4 the only known biological sink for atmospheric oxidation of aerobic soils by Methanotrophs or Methane Oxidizing bacteria, which can contribute upto 1 per cent to the total global CH_4 destruction. The photosynthetic system such as Blue Green Algae and Azolla are also known to minimize the global warming potential in rice by enhancing the dissolved oxygen content in the soil water interface that ultimately suppresses the activity of methanogens. This nitrogen fixing biological systems can be used to reduce methane flux from flooded rice ecosystem besides their ability in supplementing nitrogen to the rice crop (Lakshmanan *et al.*, 2010) [3]. Therefore, this study was undertaken to find out a relation between methane emission and edaphic factors in the field conditions such as dissolved oxygen concentration and redox status.

Materials and Methods

Treatments consisted of different rice cultivation methods viz., system of rice intensification (I_1), alternate wetting and drying method (I_2) conventional (I_3) and direct wet seeded (I_4) cultivation with recommended dose of fertilizer (RDF-T_1), RDF + *Azolla* (T_2), RDF+ Blue green algae (BGA-T_3) and RDF+ *Azolla* + BGA (T_4), in three replications. All the agronomic practices for conventional method and direct puddle sowing method were followed as per the standard recommendations given in Crop Production Guide for Tamil Nadu (2012) [4]. The data on various parameters studied during the course of investigation were statistically analyzed as suggested by Gomez and Gomez (2010) [5]. Wherever the treatment differences were found significant, critical difference (CD) were worked out at the 5 per cent level of significance with mean separation by least significant difference.

Chemical Analysis

Measurements for redox potential were done with each set of Green House Gas (GHG) flux measurement. The redox potential (Eh) of the field soil was measured by inserting a combined water proof ORP/ redox meter (Eutech Instruments, USA) to the soil and measuring the potential difference in mV (Satpathy, 1997) [6]. The Eh of soil was measured (rhizosphere to bulk soil interface) in the morning and afternoon at different points near the flux measurement setup and averaged for the day. Dissolved oxygen concentration at the soil–floodwater was measured using an Azide modification iodimetric (Wringler) method and expressed as mg l^{-1}.

Methane Flux Measurements

Plant-mediated CH_4 emission flux from the experimental plots was measured by closed chamber method of Khosa *et al.* (2010) [7]. For measuring CH_4 emission, rice hills were covered with a locally-fabricated transparent polycarbonate sheet chamber (50 cm length, 50 cm width and 100 cm height). A battery-operated fan was fixed for air circulation (avoid plant suffocation) to mix the air inside the chamber and draw the air samples into air-sampling bags (Tedlar®).Each chamber was placed on the soil surface with 4-5 cm inserted into the soil, 10 minutes prior to each sampling for equilibration to reduce the disturbance so as to minimize the disturbance to the sampling site. After covering the plants with the chamber, four air samples were collected in Tedlar bags starting with zero time and subsequent sampling at an interval of 15 minutes using syringe and one way valve pump. As described by Jayadeva *et al.* (2009) [8], the air samples were collected in the morning (09:00-10:00 hours) and in the evening (14:00-15:00 hours) and the average of morning and evening fluxes were used as the flux value for the day. Gas samples were collected continuously for a week at critical stages of crop growth *viz.,* active tillering, panicle Initiation, flowering and maturity stages and the average of the seven days were reported as the average daily methane and nitrous emission rate for the respective stage

CH_4 estimation

The CH_4 was estimated in a Shimadzu GC-2014 gas chromatograph (GC) equipped with flame ionization detector. The gas samples were introduced into the analyzer by filling the fixed loop (1.0 ml) on the sampling valve. Samples were injected into the column system by starting the analyzer which automatically activated the valve and back flush the samples according to the time programmed. The retention time of CH_4 was between 4 to 4.17 min. The GC was calibrated before and after each set of measurements using 1ppm, 2.3ppm and 5ppm of CH_4 (Chemtron® science laboratories Pvt. Ltd., Mumbai) as primary standard curve linear over the concentration ranges used. The minimum detectable limit for CH_4 was 1 ppm. CH_4 flux was expressed as mg m^{-2} hr^{-1} using the equation given by Lantin *et al*. (1995) [9].

Results and Discussion

Influence on dissolved oxygen concentration

Dissolved oxygen in the root region of the rice had showed, interaction effect between systems of rice cultivation and levels of treatments were found significant except the panicle initiation stage. The average dissolved oxygen concentration in the experimental plots showed decreasing trend as growth stages progressed. The lowest dissolved oxygen concentration of 2.67 mg L^{-1} was noticed during maturity stage. In the present study, the BGA and *Azolla* application individually and in combination enhanced the dissolved oxygen concentration in the standing water in all growth stages while the dissolved oxygen concentration was minimum in plots applied only with fertilizer without *Azolla* and BGA. *Azolla* and BGA are aerobic photosynthetic organisms and in the medium of their growth, they release a lot of oxygen during photosynthesis. As a result when they grow in rice fields they make the standing water highly oxygenated. When there is profuse growth of *Azolla* and BGA, the surface layer of the soil absorbs enough oxygen through diffusion to become aerobic in nature and prevents the development of highly reduced conditions (Sethunathan *et al*., 2000) [10]. Mandal *et al*. (1998) [11] reported similar findings that BGA application increased the dissolved oxygen content in the standing water of rice field. The SRI system of rice cultivation recorded significantly higher dissolved oxygen concentration at all critical stages of crop growth. Among the treatments I_1T_4 (SRI+ RDF + *Azolla*+ BGA) registered mean maximum dissolved oxygen concentration of 3.93 mg L^{-1}. The plots under Conventional system + RDF (I_3T_1) recorded the lowest dissolved oxygen concentration of 1.71 mg l^{-1}. Prasanna *et al.* (2002) [12] also reported the beneficial effect of cyanobacteria in decreasing the headspace concentration of methane due to higher dissolved oxygen concentration that enhanced the methane oxidation at source and results in nitrous oxide emission. This fact of enhanced oxygenation due to Blue Green Algal and *Azolla* application in rice fields has been confirmed by the results of current field trials.

Influence on soil redox potential (Eh)

Redox status of soil is an indirect indicator of methane flux pattern from rice ecosystem (Wang *et al.,*1993) [13] and soils with lower redox potential are usually associated with high methane flux. Hence the redox potential was measured in all the treatments during different crop growth stages. Systems of rice cultivation and different levels of treatment applications had marked effect on soil Eh, the soil redox potential values of SRI were significantly higher than other cultivation systems. The measured values of the oxidation - reduction potential under intermittent irrigation conditions were always distinctly higher than those under submerged. Normally, the oxidation-reduction potential decreases over rice growing with submerged condition and it increased with that the soil aeration was improved (Peng *et al.,*1997) [14]. Conventional system recorded lower mean redox potential (-104, -139, -123 and -78 mV) while the higher value (-24, -51, -42 and -28 mV) was recorded in SRI at active tillering, panicle initiation, heading and maturity stages. Application of *Azolla* + BGA and cultivation systems significantly influence the soil redox potential (Eh) regardless of stages of observation. Among the stages, the reduction was the highest at panicle initiation and the lowest at maturity stages. The interaction effect was significant. The increase in soil redox potential due to the application of *Azolla* and BGA was more in SRI and AWD systems. The redox potential value was the lowest in treatments applied with recommended dose of fertilizer (RDF) alone. Methane production mostly occurs in the soil microenvironment where the redox status is expected to be lower (Neue, 1993) [15]. Bharati and Mohanty (2000) [16] found that *Azolla* dual cropping in rice registered a higher redox potential leading to low methane flux under Blue green algal application in rice fields.

Influence on methane emission rate

Methane flux pattern exhibited significant variation among different systems of cultivation and various levels of treatments. Methane emission rate was significantly high in conventional system than DWS, AWD and SRI, throughout the crop growth. In conventional system, the methane emission rate ranged from 1.93 to 2.6 mg m^{-2} hr^{-1}. Methane emission rate in SRI ranged from 0.48 to 0.68 mg m^{-2} hr^{-1}. The methane emission among the treatment was low (0.48 mg m^{-2} hr^{-1}) in SRI applied with *Azolla* and BGA in both the seasons.

Table 1: Influence of BGA and *Azolla* on different rice cultivation systems with Azolla and BGA on methane flux (mg m^{-2} hr^{-1})

Treatments		Active Tillering	Panicle Initiation		Heading		Maturity		Mean
I_1	T_1	0.66	0.76		0.71		0.60		0.68
	T_2	0.54	0.66		0.65		0.57		0.60
	T_3	0.44	0.62		0.58		0.54		0.55
	T_4	0.44	0.51		0.50		0.48		0.48
I_2	T_1	0.70	1.00		0.90		0.83		0.86
	T_2	0.48	0.82		0.79		0.74		0.70
	T_3	0.47	0.74		0.71		0.69		0.65
	T_4	0.46	0.70		0.63		0.59		0.59
I_3	T_1	0.75	3.35		3.28		3.03		2.60
	T_2	0.69	3.28		2.71		2.46		2.29
	T_3	0.64	2.92		2.58		2.42		2.14
	T_4	0.61	2.84		2.21		2.04		1.93
I_4	T_1	0.68	3.29		3.10		2.89		2.49
	T_2	0.65	2.91		2.67		2.38		2.15
	T_3	0.62	2.78		2.57		2.36		2.08
	T_4	0.62	2.48		2.26		2.15		1.88
Mean		0.59	1.85		1.68		1.55		1.42
	SED	CD	SED	CD	SED	CD	SED	CD	
I	0.011	0.028	0.016	0.040	0.033	0.082	0.016	0.039	
T	0.006	0.012	0.015	0.032	0.012	0.026	0.018	0.037	
I x T	0.015	0.036	0.031	0.068	0.040	0.093	0.035	0.075	
T x I	0.012	0.025	0.031	0.064	0.025	0.053	0.036	0.074	

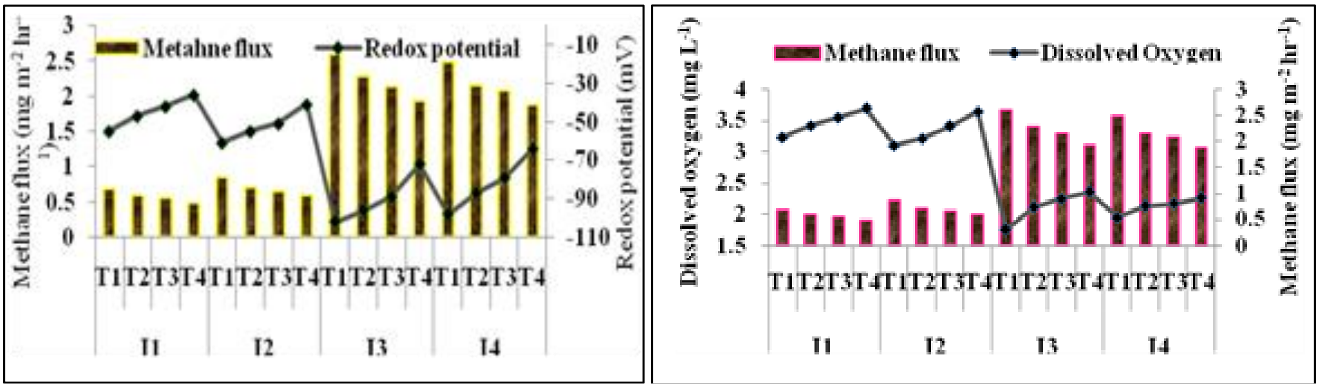

Fig 1: Influence of dissolved oxygen and redox potential on methane flux

The emission rate was high (2.60 and 2.53 mg m^{-2} hr^{-1}) in Conventional cultivation applied with RDF without *Azolla* and BGA in both the seasons. Methane emission rate was high when RDF was applied without *Azolla* and BGA and application of *Azolla* and BGA lowered methane emission under RDF. Methane oxidation in rice paddy soils may consume at least 90 per cent of the potential methane (Holzapfel-Pschorn *et al.*, 1986) [17]. Prassana *et al.*, (2012) [18] reported that the cyanobacterium was the most effective in retarding methane concentration in rice by 10 to 20 fold over that in controls without cyanobacteria. Prasanna *et al.*,(2002) [13] reported that the photo synthetically generated O_2 is of crucial importance in the maintenance of redox potential and oxygenic conditions of soil despite frequent flooding. Shankar (2011) [19] reported that the mean methane flux in farmyard manure and green leaf manure applied plot was 2.43 mg m^2 hr^{-1}, while the flux was reduced to 20 percent due to BGA and *Azolla* application (1.93 mg m^2 hr^{-1}). Bharati and Mohanty (2000) [16] indicated that dual cropping of *Azolla* reduced methane flux and yet increased grain yield similar that to that of urea application. The decrease in methane efflux in plots with dual crop of *Azolla* could be related to the release of oxygen in the standing water leading to less reduced conditions in the soil.

Conclusion

The results of the present investigation found that application of Blue green algae and *Azolla* in rice cultivation as biofertilizers minimize methane flux by enhancing the dissolve oxygen concentration and soil redox that is unfavorable to methane generating methanogens. This practice may significantly reduce methane (CH_4) emissions, a potent greenhouse gas.

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