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Role of Photosynthetic Diazotrophs in Reducing Methane Flux from Rice Soil Ecosystem

CLIMARICE: "Testing Climate uncertainties and validating selected technologies on farmers fields"

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This Technical brief is a short summary of the results obtained from the field trials conducted at Anbil Dharmalingam Agricultural College and Research Institute, Trichy, India during Rabi (2010-2011) to evaluate the role of photosynthetic diazotrophs in reducing methane flux from rice soil ecosystem as a part of climarice project. Global warming induced by increasing concentration of greenhouse gases (GHGs) in the atmosphere is a matter of great environmental concern. Methane, carbon dioxide, nitrous oxide and chlorofluorocarbon are the GHGs which have strong infrared absorption bands and trap part of the thermal radiation from the earth surface. Rice fields have to be considered as a significant source of greenhouse gases and rice field eco systems account for about 60 Tg methane per year or about 12 per cent of the global annual methane emission. The purpose of this study is to explore the potential of blue green algae and azolla in minimizing methane flux at source in paddy field ecosystem. In the present study, combined application of organics and blue green algae not only recorded higher yield, but also found to emit less methane in paddy cultivation than the application of organics alone.

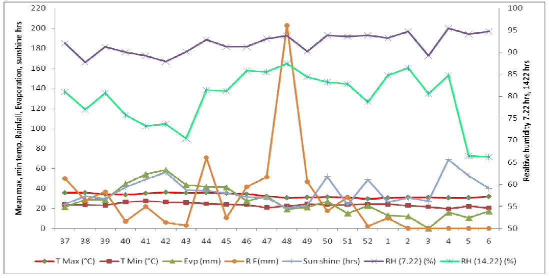
CH_4 emission from rice fields

The rice fields have to be considered as a significant source of greenhouse gases and rice field eco systems accounts for about 60 Tg methane per year or about 12 per cent of the global annual methane emission (IPCC, 1992). The emission of green house gases from rice ecosystem is likely to increase in the days to come due to intensification of rice cultivation. Methane production and consumption in soil are the biological-mediated processes and therefore influenced by the prevalent weather condition, water regime, soil properties and various cultural practices like irrigation and drainage, organic amendments, fertilization and rice cultivars. Temperature, irrigation, redox potential, fertilization, available carbon and seasonal variations are among the factors that influence production of methane in soil (Allen *et al.*, 2003).

Experimental details

The field experiment was carried out in the `A1C' block of farm of Anbil Dharmalingam Agricultural College and Research Institute, Trichy. The farm is situated at 10° 45'N latitude, 78° 36'E longitude and at an altitude of 85 m above mean sea level. The experiment location having the climate of Semi-Arid Tropics experiences a mean annual rainfall of 843 mm distributed over 48 rainy days. The mean maximum temperature and minimum temperature are 34.8°C and 24.7°C respectively. The relative humidity ranged from 87 to 96 per cent in the forenoon and 66 to 87 per cent in the afternoon.

Fig.1. Weather prevailed during crop growing period



The soil of the experimental field was sandy clay loam, taxonomically classified as isohyperthermic Vertic Ustropet, having 191 kg ha^{-1} of available nitrogen, 27.5 kg ha^{-1} of available phosphorus and 240 kg ha^{-1} of available potassium.

Fig.2. Over view of experimental field

The rice variety TNAU (R) TRY1 was chosen for the study and it has duration of 135 days. The Green manure *Sesbania aculeata* was raised in a separate field and incorporated in the field as green leaf manure before planting as per the treatment.

Fig. 3. Experimental plots applied with Green Leaf Manure (T_5) and Farm Yard Manure (T_4)

Treatments involved *viz.*, T_1- Control, T_2-Blue Green Algae, T_3-*Azolla*, T_4-Farm Yard Manure, T_5-Green Leaf Manure, T_6-Blue Green Algae+*Azolla*, T_7-Farm Yard Manure + Green Leaf Manure, T_8- Blue Green Algae + *Azolla* + Farm Yard Manure + Green Leaf Manure. A seed rate of 40 kg ha^{-1} of rice variety (TRY1) was used for the experiment. The seeds were treated with Carbendazim @ 2 g kg^{-1} of seeds for protection against seed borne diseases. After 24 hours of fungicidal treatment, the seeds were treated with *Azospirillum* @ 600 g ha^{-1} of seeds.The treated seeds were soaked in water for 24 hours to induce sprouting. The sprouted seeds were sown uniformly in the well prepared nursery maintaining thin film of water.

Soil temperature and water temperature were measured in each treatment during the entire crop period. Soil temperature reading was taken with mercury in glass thermometers (15 cm depth) which were placed in each treatment and water temperature was measured with ordinary thermometer. Soil and water temperatures were recorded at 10.00 and 15.00 hrs and averaged for the day.

Fig. 4. Experimental field with methane collection chamber

Measurement of methane emission

Plant-mediated CH_4 emission flux from the experimental plots was measured by closed chamber method of Adhya *et al*., (1994) at regular intervals from transplanting to harvest. Samplings for CH_4 flux measurements were made at 09:00-10:00 hours and 15:00-16:00 hours and the average of morning and evening fluxes were used as the flux value for the day. For measuring CH_4 emission, eighteen rice hills were covered with a locally-fabricated transparent acrylic sheet chamber (59.3 cm length, 59.3 cm width and 87.8 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®). The air samples from the sampling bags were analyzed for CH_4. 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 to the sampling site.

The CH_4 was estimated in a Shimadzu GC-2014 gas chromatograph equipped with FID. 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 was automatically activates 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 1 ppm, 2.3 ppm and 5 ppm 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 determined by peak area and CH_4 flux was expressed as mg m^{-2} day^{-1} using the equation given by Lantin *et al*. (1995).

Fig. 5. Colonization of Blue Green Algae and azolla in experimental plot

Soil analyses

Measurements for redox potential and dissolved oxygen concentration were done with each set of CH_4 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 root region and measuring the potential difference in mV (Satpathy *et al.*, 1997). 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 method and expressed as mg l^{-1}. Soil chemical components were analyzed from field soils sampled by inserting auger (2 cm diameter) to a depth of 5-7 cm in between two rice hills.

Table.1. Physiochemical properties of the experimental field

Parameters	Value
A. Mechanical analysis	
Clay (%)	29.1
Silt (%)	13.1
Sand (%)	57.0
Textural class	Sandy clay loam
B. Physical analysis	
Field capacity (%)	43.25
Permanent wilting point (%)	32.51
Available soil moisture (%)	10.74
Bulk density (Mg m^{-3})	1.39
C. Chemical analysis	
pH (1: 2 of soil : water)	9.1
Electrical conductivity (dS m^{-1})	0.35
Organic carbon (%)	0.49
Available nitrogen (kg ha^{-1})	191.0
Available phosphorus (kg ha^{-1})	27.5
Available potassium (kg ha^{-1})	240.0
D. Biological properties	
Bacteria (10^8 CFU g^{-1} of soil)	16
Fungi (10^4 CFU g^{-1} of soil)	6
Actinomycetes (10^3 CFU g^{-1} of soil)	3
Dehydrogenase (µg of TPF g^{-1} of soil)	5.8
Phosphatase (µg of PNPP g^{-1} of soil)	11.2
Urease (µg NH_4-N g^{-1} of soil h^{-1})	8.8

Influence of organic manure and photosynthetic systems on soil /water temperature and methane flux

The field experiment was conducted to study the influence of temperature (air, soil and water) under different organic amendments on methane flux in rice cultivation. As redox potential, soil temperature and dissolved oxygen in the flooded rice soil are major factors influencing the methane flux; their concentrations were monitored in all treatments throughout the growth stages to derive the correlation between temperature/ redox/ dissolved oxygen and methane flux. Minimizing CH_4 flux in rice cultivation is an important climate change mitigation strategy and hence the influence of photosynthetic systems such as blue green algae (BGA) and *Azolla* on soil redox, dissolved oxygen and CH_4 emission was studied. As BGA and *Azolla* supply nitrogen and other growth regulators to the rice crop besides CH_4 emission reduction, their role in enhancing the yield in rice cultivation was also quantified in the field experiment.

Results

The plots applied with farm yard manure and green leaf manure separately (T_4 and T_5) and also in combination (T_7) recorded higher soil and water temperature. The decomposition of organics and mineralization processes would have enhanced the soil temperature in these plots. The plots treated with BGA and *Azolla* registered lower soil and water temperature and the same trend was also noticed during all growth stages such as maximum tillering, flowering and maturity stages. The BGA and *Azolla* form a mat over the water surface and minimize the penetration of solar radiation. More over BGA and *Azolla* being photosynthetic systems releases oxygen into soil water interface that ultimately minimizes the water and soil temperature in the experimental plots. Prasanna *et al.* (2002) reported that cyanobacteria releases oxygen during photosynthesis into the standing water that can diffuse into soil.

The plots applied with farm yard manure and green leaf manure separately (T_4 and T_5) and also in combination (T_7) recorded higher soil and water temperature. Marginal reduction in soil and water temperature in BGA and *Azolla* applied plots as a result of higher oxygen diffusion would be one among the factors that contributed to low methane flux.

The air temperature surrounding the top of the rice plant had relatively small effect on conductance of methane in to atmosphere by rice plants. The higher methane flux in all treatments was recorded between 60 and 75 days after transplanting, which also registered high mean maximum and minimum air temperature.

Influence of organic manure and photosynthetic systems on soil redox potential (Eh) of experimental field

Redox status of soil is an indirect indicator of methane flux pattern from rice ecosystem (Wang *et al.,* 1993) 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. The combined application of BGA and *Azolla* recorded higher redox potential followed by the application of BGA and *Azolla* individually in all growth stages of rice in the experimental plots. The redox potential value was the lowest in treatments applied with farm yard manure and green leaf manure (T_7) which are evident by the low mean redox potential of -107mV in T_7. Methane production mostly occurs in the soil microenvironment where the redox status is expected to be lower (Neue, 1993). Bharati and Mohanty (2000) 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. The results of the present investigation are in line with the earlier findings that BGA and *Azolla* application in rice cultivation as biofertilizers minimize methane flux by enhancing the soil redox that is unfavorable to methane generating methanogens. Dissolved oxygen is important parameter that plays a major role in methane emission from rice field. The lower level of flooded water dissolved oxygen is associated with higher methane emission. In the present investigation significant variation in dissolve oxygen concentration was observed between treatments. The dissolved oxygen concentration showed a decreasing trend in all treatments during growth stages and this might be due to the enhanced microbial activity in rice soil rhizosphere and this is in line with the earlier results of Sethunathan *et al.* (2000).

Influence of organic manure and photosynthetic systems on dissolved oxygen and methane flux

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 farm yard manure and green leaf manure applied plots. BGA and *Azolla* 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 BGA and *Azolla*, the surface layer of the soil absorbs enough oxygen through diffusion to become aerobic in nature and prevents the development of highly reduced conditions underneath it. Mandal *et al.* (1998) and Lakshmanan *et al.* (1994) reported similar findings that BGA application increased the dissolved oxygen content in the standing water of rice field. Prasanna *et al.* (2002) 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.

Effect of organic manure and photosynthetic systems on rice yield

Rice yield was significantly higher in the plots applied with organic manure (FYM and GLM) and biofertilizers (BGA and *Azolla*). Even though the methane flux is found to be high due to organics, application of organic manure is encouraged in rice cultivation due to higher yield and soil health. In the present study, combined application of organics and blue green algae not only recorded higher yield, but found to emit less methane in paddy cultivation than the application of organics alone. The mean methane flux in farm yard manure and green leaf manure applied plot was 58.54 mg m^2 day^{-1}, while the flux was reduced to 20 per cent due to BGA and *Azolla* application (46.37 mg m^2 day^{-1}). Bharati *et al.* (2000) emphasized that application of BGA and *Azolla* reduced methane flux without reducing rice yields and can be used as a practical mitigation option for minimizing the global warming potential of rice ecosystem.

Fig.6. Redox potential and methane flux in different treatments during growth stages

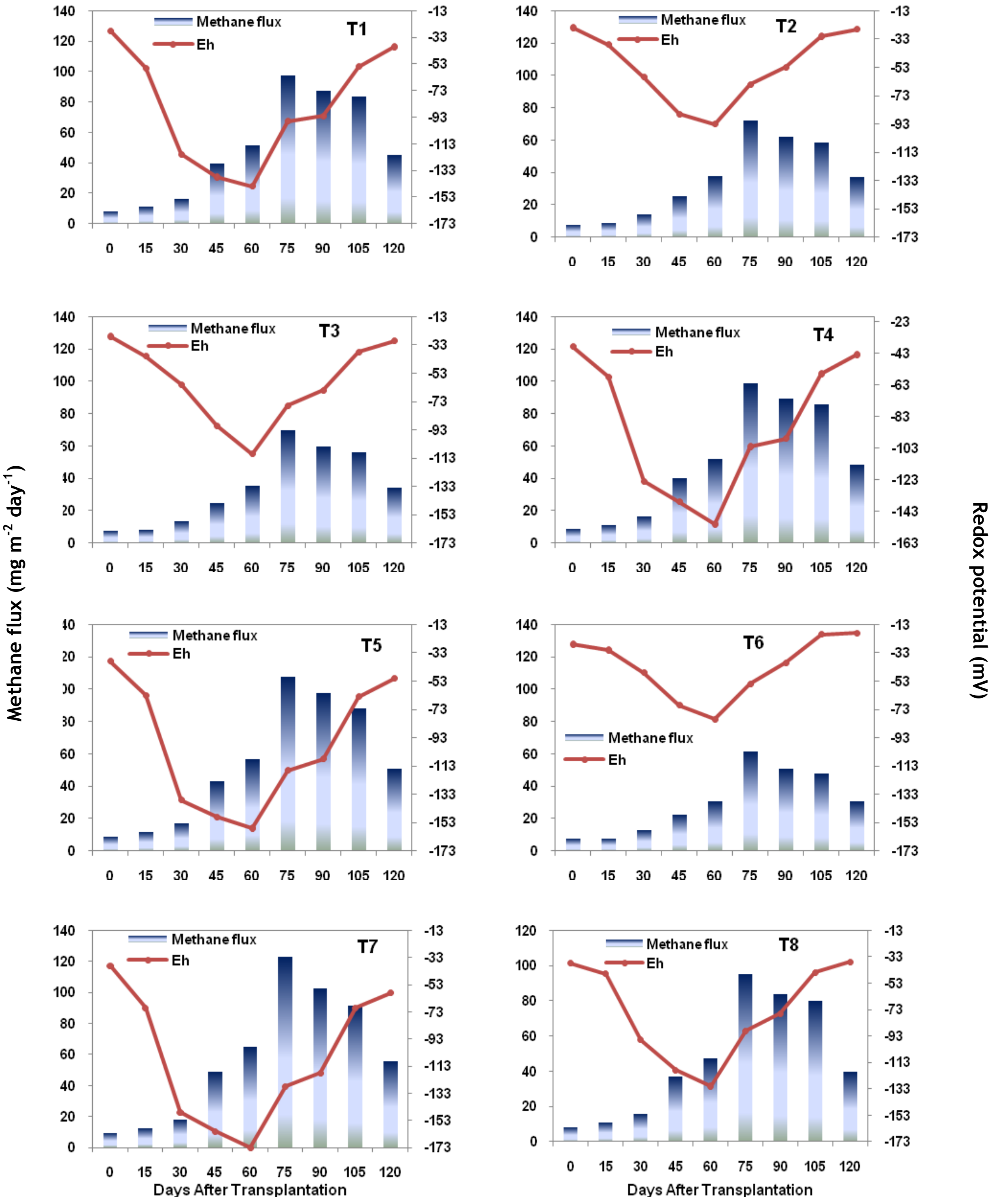


Table 2. Mean CH_4 flux, grain yield (at 14% moisture content), straw yield and harvest index of experimental field

Tr No	Mean CH_4 flux (mg m^{-2} day^{-1})	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest Index
T_1	48.81	3040	4668	39.4
T_2	35.92	3646	5307	40.7
T_3	34.35	3287	5172	38.9
T_4	50.08	3255	5099	39.0
T_5	53.49	3188	5013	38.9
T_6	30.03	3685	5551	39.9
T_7	58.54	3581	5250	40.5
T_8	46.37	3847	5778	40
SEd		**40.3**	**42.1**	**0.38**
CD (P=0.05)		**86.5**	**90.2**	**0.81**

Fig. 7. Mean methane flux and crop yield

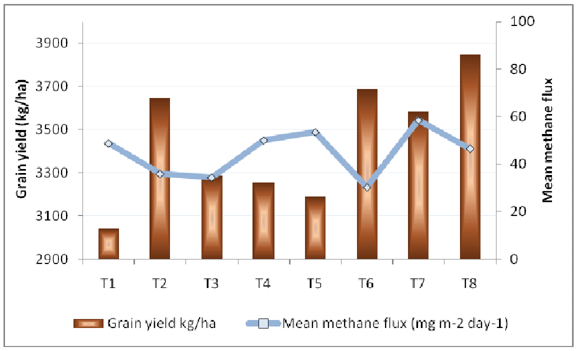
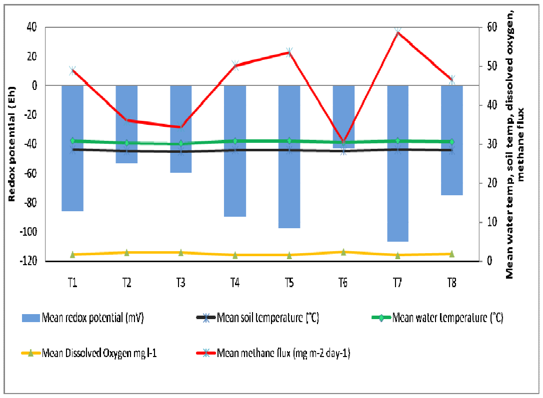


Fig.8. Influence of temperature, redox potential and dissolved oxygen on methane flux.



Conclusion

In the present study, combined application of organics and blue green algae not only recorded higher yield, but also found to emit less methane in paddy cultivation than the application of organics alone. The present field study reiterates that biofertilization of paddy fields with blue green algae and *Azolla* is a potential climate change mitigation strategy due to their effect in minimizing methane emission, besides yield enhancement by nitrogen fixation.

The CLIMARICE Project (2010-2012)

ClimaRice **is** an integrated project that aims to assess the climate variability and its impacts on the water availability and rice production systems in the Cauvery and Krishna river basin of Tamil Nadu, India. The overall goal is to contribute to the regional and national adaption strategies to sustain rice production and ensure food security amidst changing climate. The partners are:

- Bioforsk - Norwegian Institute for Agricultural and Environmental Research (Project Co-ordinator)
- Tamil Nadu Agricultural University, Coimbatore, India
- International Pacific Research Institute, Hawaii, USA
- International Water Management Institute, IWMI, Hyderabad, India.

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