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Effect of continuous and alternate water regimes on methane efflux from rice under greenhouse conditions

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Abstract In a greenhouse study, the effect of moisture regimes (continuously flooded, continuously nonflooded, alternately flooded) on methane efflux from an alluvial soil planted to rice was studied using the closed chamber method. Methane efflux was almost 10 times more pronounced under continuously flooded conditions than under continuously nonflooded conditions. Intermittently flooded regimes (alternately flooded and nonflooded cycles of 40 or 20 days each) emitted distinctly less methane than the continuously flooded system. A significant negative correlation was found between methane emission under different water regimes and rhizosphere redox potential. Extractable Fe^{2+} , readily mineralizable carbon (RMC) and root biomass presented a significant positive correlation with cumulative methane emission. The correlation of methane emission with other plant parameters and microbial biomass was not significant. Our results further suggest the possibility of reduced methane emissions through appropriate water management in a rainfed rice ecosystem.

Key words Methane efflux · Rice paddy · Alternate flooding · rainfed lowland · Plant microbe interactions · Greenhouse effect · Redox potential

Introduction

The increasing atmospheric concentration of methane, a radiatively important trace gas, can be attributed to man's agricultural activities (Crutzen 1995) in general and to intensive rice cultivation in particular (Houghton et al. 1995). Methane emission from rice paddies is the net ef-

fect of a series of complex processes involving plant-microbe interactions (Rennenberg et al. 1992; Conrad 1993; Banker et al. 1995). The quantum of methane available for release to the atmosphere is determined by the rates of methanogenesis and methane oxidation. While methane is exclusively produced by strictly anaerobic methanogenic bacteria, methane oxidation is essentially an aerobic process.

In a rainfed rice ecosystem which constitutes 54% of global lowlands planted to rice (Garrity et al. 1986), intermittent flooded and dry situations are of common occurrence as a result of precipitation punctuated by drought. Heavy precipitation leads to flooding and creates conditions conducive for soil reduction promoting methanogenesis. On the other hand, during dry cycles, the return of aerobic conditions inhibits methanogenesis and favors methane oxidation. Mid-season drainage, a common agronomic practice in Japan to alleviate soil-related toxicity to rice plants in anaerobic soils, also reduces methane emissions (Yagi and Minami 1990). However, frequent flooding and drying cycles in a crop season under a rainfed ecosystem can adversely affect crop growth and yield, in addition to influencing methane emission (Nugroho et al. 1994; Husin et al. 1995). Practically no information is available on the influence of fluctuating water regimes on methane emission from rainfed or irrigated systems under tropical Indian conditions. In a greenhouse experiment, we studied the effect of continuous and alternately flooded and nonflooded regimes on methane efflux from an alluvial soil planted to rice. In addition, we studied the interrelationship between selected plant growth parameters, certain soil (physicochemical and biochemical) properties and methane emission.

Materials and methods

Greenhouse study

Earthenware pots measuring 25.5 cm (height) × 9.5 cm (width) filled with 5 kg of an alluvial soil from the experimental farm of the Central

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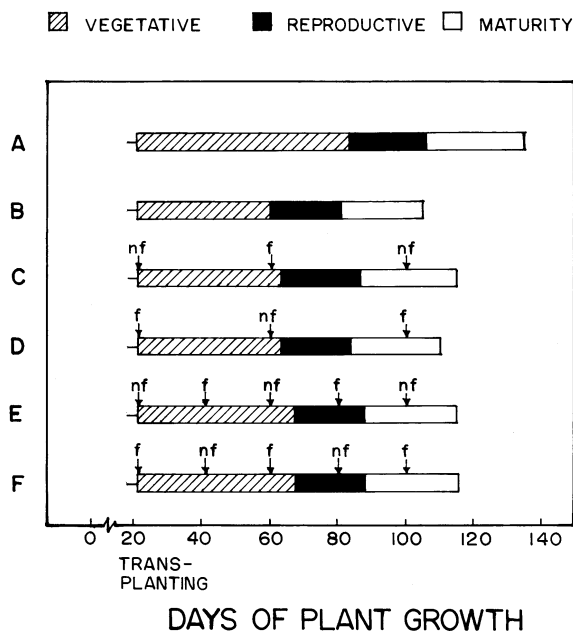


Fig. 1 Typical growth stages of rice plants (cv. Lalat) grown in pots under different water regimes (A continuously nonflooded, B continuously flooded, C nonflooded (40 days) → flooded (40 days) → nonflooded until maturity, D flooded (40 days) → nonflooded (40 days) → flooded until maturity, E nonflooded and flooded cycles alternating with each other at 20-day intervals, F flooded and nonflooded cycles alternating with each other at 20-day intervals). Arrows on bars indicate introduction of flooding (f) or drainage (nf) cycles for the pots under specific treatment

Rice Research Institute, Cuttack, were used for the study. The soil was a typical Haplaquept (deltaic alluvium) with a sandy clay loam texture (25.9% clay, 21.6% silt and 52.5% sand), pH (1:1.25 H₂O) 6.4, organic carbon 0.65% and total N 0.06%. Rice seedlings (21 days old; cv. Lalat) were transplanted at four hills of one plant each per pot. Phosphorus as single superphosphate and potassium as KCl were applied (40 kg h⁻¹) to all the pots as a basal dressing. Nitrogen was applied (80 kg h⁻¹) as urea to all the pots, 50% as a basal dressing and 25% each at the maximum tillering and panicle initiation stages of the crop. The pots were maintained under appropriate nonflooded or flooded regimes up to maturity. Plant growth stages including panicle primordium initiation (beginning of reproductive phase) were followed as per Mohanty et al. (1990). Typical growth stages of rice plants under different water regimes are shown in Fig. 1.

Water regimes

In order to study the influence of continuous and alternate flooded regimes on methane emission from rice plants, six treatments were included. The treatments comprised: (1) continuously nonflooded until maturity; (2) continuously flooded until maturity; (3) nonflooded for the first 40 days followed by flooded for 40 days and then a return to nonflooded until maturity; (4) flooded for the first 40 days followed by nonflooded for 40 days and a return to flooded until maturity; (5) nonflooded and flooded cycles alternating with each other at 20-day intervals; and (6) flooded and nonflooded cycles alternating with each other at 20-day intervals. There were three uniform replicate pots for each treatment for methane emission measurements. Another set of six uniform replicate pots under each treatment was used for soil and plant analysis.

Nonflooded pots were maintained at 40% of water-holding capacity (WHC) while flooded pots were maintained with a standing water depth of 3 cm. To achieve nonflooded conditions in a flooded pot, standing water was drained and the pots were allowed to stand until

40% WHC was attained. The moisture content of the drained soil reached 40%, within 4–6 days after draining, which corresponded to the moisture content of the continuously nonflooded soil. Nonflooded pots were returned to the flooded condition by adding sufficient water to provide a standing water depth of 3 cm. The moisture contents of the nonflooded pots were maintained at the respective levels for nonflooded conditions by periodical replenishment of water required at 7-day intervals to compensate for the evaporation loss.

Methane emission measurement

Methane fluxes were measured by the closed chamber method (Sass et al. 1990; Adhya et al. 1994) at 5-day intervals from the day of transplanting until maturity. Sampling for methane efflux measurement was done in the morning (0900 hours) and in the afternoon (1500 hours) and the average of the morning and evening fluxes was used as the flux value for the day. For measurement of methane emission, individual planted pots were placed on a tray and covered with a locally fabricated Perspex chamber (53×37×71 cm: length×width×height). The tray was filled with water to a depth of 2 cm, which acted as an air seal when the Perspex box was placed on the tray. A battery-operated air circulation pump with air displacement of 1.5 l min⁻¹ (M/s Aerovironment Inc., Monrovia, CA, USA) connected to polyethylene tubing was used to mix the air inside the chamber and draw the air samples into Tedlar air-sampling bags (M/s Aerovironment Inc., USA) at fixed intervals of 0, 15 and 30 min (Adhya et al. 1994). The air samples from the sampling bags were analyzed for methane in a Varian 3600 gas chromatograph equipped with FID (flame ionization detector) and a column packed with a 0.5-nm molecular sieve. Column, injector and detector were maintained at 80°, 90° and 100°C, respectively. The gas chromatograph was calibrated before and after each set of measurement using 9.03 µl ml⁻¹ CH₄ in N₂ obtained from M/s Altech Associates, USA, and used as a primary standard. Under these conditions, the retention time of CH₄ was 1.35 min and the minimum detectable limit was 0.5 µl ml⁻¹. Methane concentration in the soils was expressed as mg pot⁻¹ day⁻¹.

Soil analysis

Extractable Fe²⁺ was measured by extracting the soil samples with NH₄OAC: HCl (pH 2.8) and analyzed by colorimetry after reaction with orthophenanthroline (Pal et al. 1979). The redox potential of the rhizosphere soil was measured by inserting a combined platinum-calomel electrode (Barnant Co., IL, USA) to the root region and measuring the potential difference in millivolts (Pal et al. 1979). The pH of the soil and water was monitored with a portable pH meter (Barnant Co., IL, USA). Readily mineralizable carbon (RMC) was measured by extracting soil samples with 0.5 M K₂SO₄ and titrating the extract with ferrous ammonium sulfate after wet digestion with chromic acid (Vance et al. 1987). Microbial biomass carbon was measured by the fumigation-extraction method (Inubushi et al. 1991). After scraping off the surface soil layer (1–2 cm) with a spatula, subsurface soil (equivalent to 20 g on an oven-dry basis) was sampled from each of three replicate pots from the same treatment and incubated separately in 100-ml glass beakers. The soil samples in glass beakers were fumigated with alcohol-free chloroform in a desiccator and microbial biomass carbon estimated by extracting the fumigated soil with K₂SO₄ and organic carbon measured by dichromate digestion (Vance et al. 1987).

Plant parameters

Mean aerial biomass (fresh and dry weights) was measured by harvesting above-ground portions on each day of methane sampling. Root biomass was measured only at maturity by harvesting the roots after gently removing all the soils under the tap, drying the roots in an oven at 105°C and taking the weight. Grain and straw yields from individual pots were measured at maturity and the harvest index calculated.

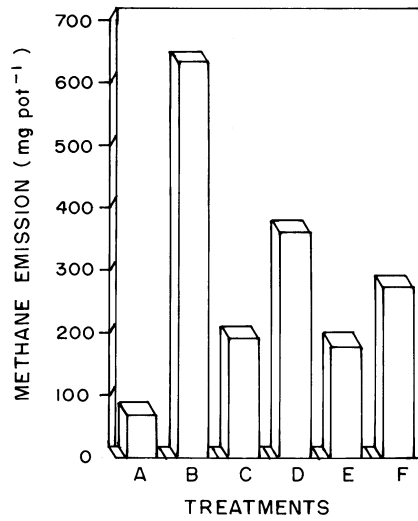


Fig. 2 Cumulative methane emission from pots planted to rice (cv. Lalat) under different water regimes. Treatments are the same as detailed in the legend of Fig. 1

Statistical analyses

Individual character data sets were statistically analyzed and the mean comparison between treatments was established by Duncan's multiple range test using Irristat (version 3.1; International Rice Research Institute, Philippines). Simple and multiple correlation analysis between cumulative methane emission and select plant and soil parameters was established to find out the effect of individual water regimes on select characters vis-à-vis methane emission.

Results and discussion

Appreciable methane flux was observed in all the pots under greenhouse conditions. Cumulative methane emission from continuously nonflooded pots planted to rice (cv. Lalat) during the entire crop growth period of 120 days was always low ($73.8 \text{ mg CH}_4 \text{ pot}^{-1}$) and was around one-tenth of that from continuously flooded pots ($632.5 \text{ mg CH}_4 \text{ pot}^{-1}$) (Fig. 2). Methane emission reached a peak at maturity stage of the rice crop under continuously flooded conditions, while no visible peak was noticed under continuously nonflooded conditions. Under flooded conditions an additional peak was noticed at the vegetative (maximum tillering stage: 30 days after transplantation) stage (Fig. 3). Intermittent flooding caused a distinct decrease in methane efflux as compared to that under the continuously flooded regime. Generally, methane emission increased during flooded cycles and decreased during nonflooded cycles under the intermittently flooded regime. Interestingly, the nonflooded (40 days) → flooded (40 days) → nonflooded (40 days) regime for this 110-day duration crop was the most effective in reducing methane emission of the four versions of the intermittently flooded regime. Probably, nonflooded conditions especially at the maturity stage (last 40 days) of the crop were not conducive to methane production in the soil. However, the maximum amount of methane is produced at the maturity stage of

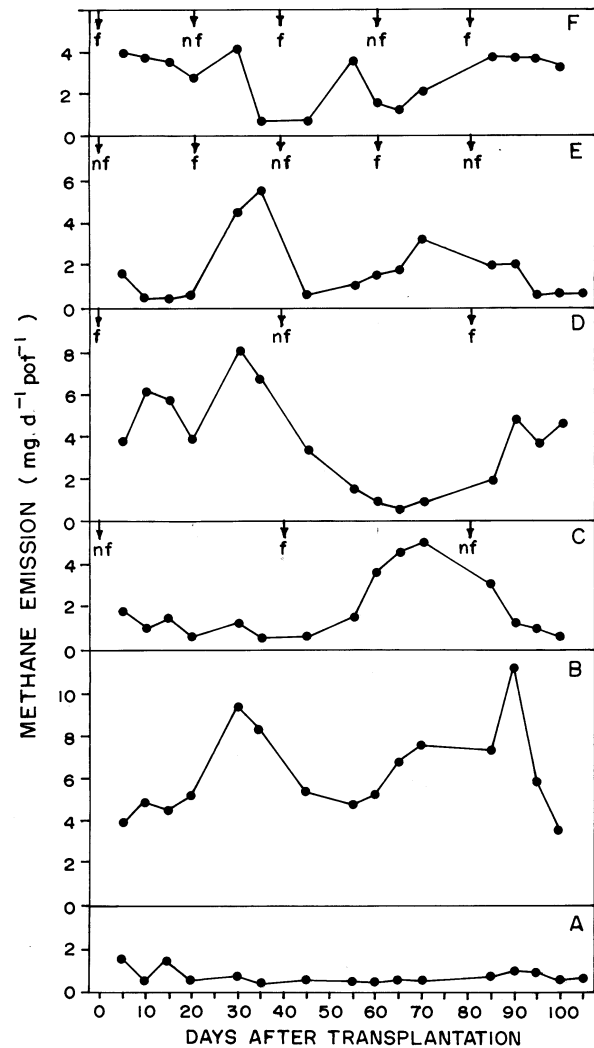


Fig. 3 Effect of continuous and alternate flooded regimes on methane efflux from rice (cv. Lalat) grown under greenhouse conditions. Treatments are the same as detailed in the legend of Fig. 1. Vertical arrows indicate introduction of flooded (*f*) or drainage (*nf*) cycles for the pots under specific treatments

the crop if flooded conditions are provided at this stage. This would explain the 70% less emission of methane in the nonflooded (40 days) → flooded (40 days) → nonflooded (40 days) regime than in the continuously flooded regime. Moreover, overall methane emission is governed by the duration of soil submergence in a crop season. Data suggest that methane emission increased with increasing duration of soil submergence during the 110-day duration. Thus, the flooded (40 days) → nonflooded (40 days) → flooded (40 days) regime with a total of 80 days of soil submergence emitted more methane (a twofold increase) than that under the nonflooded (40 days) → flooded (40 days) → nonflooded (40 days) regime with 40 days of submergence. Evidently, duration of soil submergence and water regime especially at maturity seem to have a profound influence on methane emission from flooded rice soils.

Table 1 Variation in redox potential in the rhizosphere of rice grown under continuous and alternating flooded regimes (*F* flooded, *NF* nonflooded, *ND* not determined)

Treatment	Redox potential (mV)									
	10	20	30	45	55	65	85	95	105	115
	(days after transplantation)									
NF	+127 ^{c*}	+152 ^d	+211 ^b	+318 ^a	+321 ^b	+331 ^b	+331 ^a	+258 ^b	+193 ^a	+182 ^a
F	+127 ^c	+60 ^e	+45 ^e	-53 ^d	-18 ^f	-112 ^e	-128 ^e	-155 ^d	ND	ND
NF (40 d) → F (40 d) → NF (40 d)	+224 ^a	+236 ^b	+196 ^b	+167 ^c	+40 ^e	-4 ^d	+31 ^d	+301 ^a	ND	ND
F (40 d) → NF (40 d) → F (40 d)	+183 ^b	+179 ^c	+134 ^d	+289 ^b	+278 ^c	+318 ^a	+229 ^b	-18 ^c	ND	ND
NF (20 d) → F (20 d) → NF (20 d) → F (20 d) → NF (20 d)	+153 ^c	+264 ^a	+167 ^c	+280 ^b	+360 ^a	+105 ^b	+336 ^a	+320 ^a	ND	ND
F (20 d) → NF (20 d) → F (20 d) → NF (20 d) → F (20 d)	+154 ^c	+149 ^d	+244 ^a	+180 ^c	+101 ^d	+56 ^c	+92 ^c	-10 ^c	ND	ND

Means within a column followed by the same letter do not differ significantly ($P < 0.05$) by Duncan's multiple range test

* Mean of three replicate observations

Table 2 Correlation between select plant and soil characters and cumulative methane emission under alternate flooded and nonflooded conditions

Variable	<i>r</i> value
Soil redox potential ($n=6$)	-0.889 *
Reduced iron content ($n=6$)	0.935 **
Readily mineralizable carbon ($n=4$)	0.938 *
Microbial biomass carbon ($n=4$)	0.665
Mean aerial plant biomass ($n=6$)	0.177
Root biomass ($n=6$)	0.888 *
Grain yield ($n=6$)	0.701
Straw yield ($n=6$)	0.204
Harvest index ($n=6$)	0.755

* Significant at 5% level

** Significant at 1% level

That water management practices affect methane emissions from rice paddies is well known (Yagi and Minami 1990; Sass et al. 1990, 1992; Bouwman 1991). Methanogenesis is essentially a biological process exclusively mediated by anaerobic microorganisms. Anaerobiosis created by submergence in rice paddies promotes methane production (Lindau et al. 1993). Aerobic conditions upon drainage of water from rice fields would not only retard the production of methane, but also promote the oxidation of methane, produced in the preceding flooded regime, by methanotrophs. Thus in the present greenhouse study, all the four versions of the intermittently flooded regime emitted less methane than the continuously flooded regime.

We studied the relationship between methane emission and some important soil and plant variables. Redox potential, an important soil parameter governing methane emission, was always high under continuously nonflooded conditions, but dropped rapidly within a few days after submergence (Table 1). In intermittently flooded regimes, redox potential declined rapidly with a shift from nonflooded to flooded conditions and vice versa. A simple correlation analysis (Table 2) showed a significantly negative relationship ($r = -0.889^*$) between redox potential and methane emission under different water regimes, indicating higher methane emission under more reduced conditions.

The iron redox system plays a key role in the thermodynamic sequence of reduction of a flooded rice soil (Takai and Kamura 1966). We studied the variation in the Fe^{2+} content of soils as affected by different water regimes (Table 3). A highly significant positive correlation ($r = 0.935^*$) existed between methane emission and accumulation of Fe^{2+} under different water regimes. Furthermore, a significant negative correlation ($r = -0.828^*$) existed between Eh and Fe^{2+} content of the soil.

Methane emission is governed by the decomposition of organic matter to make substrates available for methanogenesis. Decomposition of organic substrates could be affected by the alterations in the water regime (Van Gestel et al. 1993). We studied the effect of water regimes on the readily mineralizable carbon (RMC) content in the soil. RMC content increased with incubation under both continuously flooded and nonflooded conditions and reached a maximum at the late vegetative stage (Table 4). This increase was more pronounced in flooded soil than in non-

Table 3 Variation in soil Fe²⁺ content under different moisture regimes (*F* flooded, *NF* nonflooded, *ND* not determined)

Treatment	Fe ²⁺ content (µg g ⁻¹ soil)									
	10	20	30	45	55	65	85	95	105	115
	(days after transplantation)									
NF	25.2 ^{c*}	81.3 ^c	26.5 ^{c^d}	5.6 ^b	1.4 ^{a^b}	1.4 ^c	1.7 ^b	1.4 ^b	2.8 ^a	2.1 ^a
F	160.0 ^a	175.2 ^b	240.1 ^b	289.1 ^a	493.9 ^a	932.5 ^a	1302.9 ^a	376.2 ^a	ND	ND
NF (40 d) → F (40 d) → NF (40 d)	32.1 ^b	19.9 ^c	15.9 ^d	16.9 ^b	25.4 ^b	91.4 ^b	49.8 ^b	1.4 ^b	ND	ND
F (40 d) → NF (40 d) → F (40 d)	48.1 ^b	356.8 ^a	526.5 ^a	17.9 ^b	18.5 ^b	10.6 ^b	4.6 ^b	5.4 ^b	ND	ND
NF (20 d) → F (20 d) → NF (20 d) → F (20 d) → NF (20 d)	34.4 ^b	48.8 ^c	89.4 ^c	7.8 ^b	1.9 ^b	25.5 ^c	21.4 ^b	1.3 ^b	ND	ND
F (20 d) → NF (20 d) → F (20 d) → NF (20 d) → F (20 d)	43.5 ^b	175.9 ^b	52.1 ^{c^d}	5.1 ^b	15.5 ^b	8.9 ^c	5.7 ^b	10.5 ^b	ND	ND

Means within a column followed by the same letter do not differ significantly ($P < 0.05$) by Duncan's multiple range test

* Mean of three replicate observations

Table 4 Changes in readily mineralizable carbon (RMC) content in the soil under different moisture regimes maintained under greenhouse conditions (*F* flooded, *NF* nonflooded, *ND* not determined)

Treatment	Readily mineralizable carbon (µg C g ⁻¹)									
	10	20	30	45	55	65	85	95	105	115
	(days after transplantation)									
NF	0.60 ^{b*}	2.02 ^a	3.47 ^b	2.31 ^a	2.55 ^b	0.43 ^c	0.84 ^a	1.19 ^a	0.95 ^a	0.67 ^a
F	1.68 ^a	2.56 ^a	4.16 ^a	5.08 ^c	4.81 ^a	3.10 ^a	1.02 ^{a^b}	1.04 ^a	ND	ND
NF (40 d) → F (40 d) → NF (40 d) →	0.80 ^b	1.98 ^a	2.33 ^c	3.49 ^b	2.91 ^b	0.63 ^c	0.55 ^a	0.96 ^a	ND	ND
F (40 d) → NF (40 d) F (40 d)	2.01 ^a	2.27 ^a	2.73 ^c	4.16 ^b	3.38 ^b	1.82 ^b	0.34 ^a	0.93 ^a	ND	ND

Means within a column followed by the same letter do not differ significantly ($P < 0.05$) by Duncan's multiple range test

* Mean of three replicate observations

flooded soil. RMC content in intermittently flooded regimes of nonflooded (40 days) → flooded (40 days) → nonflooded (40 days) and flooded (40 days) → nonflooded (40 days) → flooded (40 days) was statistically on par but was less than in the continuously flooded regime. Interestingly, in the continuously flooded regime the RMC level declined during the reproductive stage with a concomitant increase in methane emission. This would suggest that the available RMC was utilized for methane production. The correlation between RMC contents in soils and methane emission under different water regimes was highly significant ($r = 0.938^*$), indicating RMC to be one of the most important factors influencing methane emission from rice paddies.

Moisture status of the soil has a profound influence on microorganisms and their activities. The effect of water regime on microbial biomass C, as an indicator of microbial activity in the soil, was therefore examined. Like RMC content, microbial biomass C increased under both continuously flooded and nonflooded conditions, but to a greater extent in the former (Table 5). Microbial biomass in nonflooded (40 days) → flooded (40 days) → nonflooded (40 days) and flooded (40 days) → nonflooded (40 days) → flooded (40 days) cycles was statistically on par. Correlation between microbial biomass C and methane emission was not significant, indicating that the build-up of microbial biomass under flooded conditions may not be directly related to methanogenesis. Flooding

Table 5 Alterations in microbial biomass carbon content in soil under different moisture regimes (*F* flooded, *NF* nonflooded, *ND* not determined)

Treatment	Microbial biomass carbon ($\mu\text{g C g}^{-1}$)									
	10	20	30	45	55	65	85	95	105	115
	(days after transplantation)									
NF	0.46 ^{c*}	0.68 ^c	1.74 ^c	1.82 ^c	1.76 ^d	1.53 ^c	1.60 ^c	1.14 ^b	1.19 ^a	1.05 ^a
F	2.84 ^a	3.74 ^a	5.18 ^a	7.39 ^a	5.70 ^a	4.70 ^a	4.62 ^a	3.55 ^a	ND	ND
NF (40 d) → F (40 d) → NF (40 d)	1.36 ^b	1.54 ^b	2.95 ^b	4.19 ^b	4.72 ^b	4.98 ^a	3.64 ^b	2.74 ^a	ND	ND
F (40 d) → NF (40 d) → F (40 d)	1.57 ^b	2.35 ^b	4.80 ^a	3.71 ^b	3.53 ^c	3.30 ^b	4.19 ^{ab}	3.21 ^a	ND	ND

Means within a column followed by the same letter do not differ significantly ($P < 0.05$) by Duncan's multiple range test

* Mean of three replicate observations

Table 6 Variation in select plant parameters under different water regimes

Treatment	Mean aerial biomass (g)	Mean root biomass (g)	Grain yield (g pot ⁻¹)	Straw yield (g pot ⁻¹)	Harvest index (%)
Nonflooded	6.69 ^a	27.84 ^a	2.55 ^a	25.55 ^a	9.07
Flooded	6.68 ^a	85.52 ^b	21.29 ^e	27.13 ^a	43.96
NF (40) → F (40) → NF (40)	4.72 ^a	44.92 ^a	16.21 ^d	38.62 ^b	29.56
F (40) → NF (40) → F (40)	4.49 ^a	36.41 ^a	5.55 ^b	38.62 ^b	12.56
NF (20) → F (20) → NF (20) → F (20) → NF (20)	5.26 ^a	38.52 ^a	6.16 ^b	39.93 ^b	13.36
F (20) → NF (20) → F (20) → NF (20) → F (20)	6.03 ^a	51.88 ^a	12.62 ^c	38.41 ^b	24.73

Means within a column followed by the same letter do not differ significantly ($P < 0.05$) by Duncan's multiple range test

the soil limits the growth of strict aerobes and promotes proliferation of anaerobes (Ponnamperuma 1972). The methanogenic population seldom increases upon soil submergence (Fetzer et al. 1993; Asakawa and Hayano 1995). It is possible that the microflora build-up under flooded conditions consists of zymogenous heterotrophs that help in the mineralization of polymeric carbon to enhance substrate availability for methanogenic consortia. This is also evidenced by a significant increase in the RMC content.

With regard to plant variables versus methane emission, aerial biomass under different water regimes was almost identical (Table 6). In contrast, root biomass significantly differed with the water regime. Root biomass under continuously flooded conditions showed a three fold increase over that under continuously nonflooded conditions and under intermittently flooded cycles. Methane emission was correlated significantly with root biomass ($r = 0.888^*$). Rice grain yield was greatly affected by the water regime. Grain yield was highest under continuously flooded conditions and least under continuously nonflooded conditions. Water stress at panicle development and the flowering stage results in yield reduction (Stansel 1975). In the case of continuously nonflooded, flooded (40 days) → nonflooded (40 days) → flooded (40 days) and nonflooded

(20 days) → flooded (20 days) → nonflooded (20 days) → flooded (20 days) → nonflooded (20 days) treatments, nonflooded situations prevailed during the reproductive phase (Fig. 1). It is possible that yields in these three treatments were least because of this critical condition.

The nonflooded (40 days) → flooded (40 days) → nonflooded (40 days) treatment recorded a significantly higher grain yield than other alternating moisture treatments. However, the cumulative methane emission value was around 70% lower in this treatment than the continuously flooded treatment. Drying and wetting of soil occurs naturally and frequently in rainfed rice ecosystems with alternating drought and rainy periods. Our studies clearly suggest the possibility of reducing methane emission under such an ecosystem, without a drastic reduction in yield levels, through appropriate water management where water does not become limiting during the critical growth stages.

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