Differential Effects of Nitrogen based Fertilizations on Population of Methane-Consuming Microbes in Soil Planted with Rainfed Rice Cultivar NDR-97

Bharati Singh, Deepmala Katiyar* and Rajveer Singh Chauhan

VitaeGen Biotech-Educational and Research Institute, Varanasi, India.

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Nitrogen is the single most limiting factor for rice production. Detailed knowledge on nitrogen dynamics in rice fields is therefore of major importance for developing sustainable rice production. A field trial experiments was conducted in rainfed rice fields planted to three rice (*Oryza sativa*) cultivar; *NDR-97* to investigate the influence of rice cultivars on nitrogen based fertilization on population size. Urea, DAP, Ammonium chloride, Ammonium nitrate were the fertilizers applied, at a rate of 100 kgNha^{-1} in three split doses. The experiment was laid out in a randomized complete block design with three replicate plots for each cultivar and treatment. The most probable number of methane oxidizing bacteria was estimated on six dates within the cropping period. It was observed that the NH_4^+-N μg g^{-1} dry soil content in soil was highest in urea fertilized *NDR-97* at 20 DAS while NO_3^--N content had highest at 80 DAS. Control soils (no N-fertilization) exhibited higher MCM population size than N-fertilized soils. Above conclusions were supported by measurements of MCM (methane consuming microbes) population size. The highest ammonium-N content was observed in urea (8.5 ± 0.71 μg g^{-1} dry soil) and lowest ammonium-N content in ammonium chloride treated plots (6.7 ± 1.5 μg g^{-1} dry soil) on 20 DAS. ANOVA indicated significant differences due to days fertilization and their interaction due to days × fertilization was also significant. The MCM population size was highest in control on 80 DAS (37.4 × 10^6 Cells g^{-1} dry soil) followed NH_4Cl, NH_4NO_3, and urea treated soils. In case of fertilized plots the highest MCM population was highest on 80 (Urea 28.6 × 10^6 Cells g^{-1} dry soil; DAP 29.7 × 10^6 Cells g^{-1} dry soil; Ammonium nitrate 31.6 × 10^6 Cells g^{-1} dry soil and ammonium chloride 32.9 × 10^6 Cells g^{-1} dry soil) DAS and lowest on 20 DAS

Key words: Methane consuming microbes, Nitrogen fertilizers, Rice cultivars, NDR-97.

Rice (*Oryza sativa* L.) is a major staple food of the people of South-East-Asia and at present more than half of the world population subsists on this crop. One third of the South and South-East Asian rice lands are grown in rainfed lowland ecosystems which remain submerged with water for most of the cultivation period [1]. Generally, nitrogen fertilizer efficiency in flooded rice fields is low [2]. While upland variety uses 40-60% of the applied N, flooded crops are capable of using only 20-40%. Rice paddies are among the most prominent sources in the global CH_4 budget. About 10% of the global annual emission of this greenhouse gas originates from rice cultivation areas [3]. The input of nitrogenous fertilizer may become an important controlling factor in methane emission from rice agriculture. The already high nitrogen applications to rice will have to increase, since this is the limiting factor in rice productivity [4]. This may affect important processes involved in the methane budget of rice paddies i.e., methanogenesis, methane oxidation, nitrification and also plant growth. Studies investigating fertilizer effects on

* To whom all correspondence should be addressed.
TEl.: +91 9452267383; Fax: 0542 2277587;
E-mail:deepmala.katiyar@gmail.com

these processes have yielded contradictory results. After fertilization with urea or $(NH4)_2SO_4$, lower [5,6]. Methane emissions were observed and attributed to direct inhibition of methanogenesis. However, higher CH_4 emissions were also detected from rice paddies after fertilization with urea & $(NH_4)_2HPO_4$ [7,8]. The consumption of CH_4 by methanotrophs is generally thought to be inhibited by ammonium based fertilization, by as yet unraveled mechanisms reviewed by Gulledge et al. (1997) [9]. The obligate aerobic methane oxidizers proliferate in the vicinity of oxygen-releasing roots of rice and other wetland plants [10,11] and may oxidize up to 10–65% of the potentially emitted methane [12,13]. In dry upland soils they account for approximately 6% of the global sink strength of atmospheric methane [14]. Methane-oxidizing bacteria (gram-negative, aerobic bacteria belonging to the family Methylococcaceae) are ubiquitous in nature and utilize methane as their sole carbon and energy source [15] and are subdivided into types I and II on the basis of phylogeny, physiology, morphology, and biochemistry[16].

There is hardly any information about the effects of fertilizer regime on methanotrophic bacteria in these environments and of the impact on methane emission. Input of fertilizer ammonium may stimulate nitrifying bacteria [17]. Which convert it to nitrite and nitrate. Elevated concentrations of these compounds due to high N input may inhibit methane production [18] as well as methane oxidation [19]. Besides this, ammonia oxidizers are capable of oxidizing methane [20] and methanotrophs may convert ammonium to nitrite [21]. The effects of fertilization on nitrification and the relative contributions of ammonia and methane oxidizers to methane and ammonia oxidation in rice paddies have not been amply investigated. The present study aimed at assessing the effect of ammonium based fertilization on microbial processes involved in methane emission from rice soil. We therefore studied the dynamics of methane production, methane oxidation and nitrification in compartmented microcosms fertilized with urea or diammonium phosphate. In addition, we assessed the effect of fertilization on the contribution of methane and ammonia oxidizing bacteria to methane and ammonia oxidation in the rice rhizosphere. For this reason the present research work undertaken to study the yield performance of *NDR-97* rice variety, under different Nitrogen levels and to determine effect N dose on MCM population.

Since fertilizer is an expensive input, an economical and appropriate method of application needs to be determined to enhance productivity and profit of the growers under given situation. At present the world is facing shortage of major fertilizer nutrients especially nitrogen. This problem is more serious for developing countries because the fertilizer production in these countries is expensive and less than its demand. At the same time the importance of increasing its use efficiency cannot be underestimated. The application of nitrogen fertilizer either in excess or less than optimum rate, affects both yield and quality of rice to a remarkable extent, hence proper management of crop nutrition is of immense importance. The most appropriate time of N application to rice is panicle initiation, which produced maximum plant height, number of spikelets (grains) per panicle and grain yield [22]. In two-split nitrogen application, higher grain yield of rice was obtained by applying nitrogen at tillering and panicle initiation [23] at transplanting and tillering [24] as well as at transplanting and panicle initiation [25].

MATERIALS AND METHODS

The experiments were conducted during July to November in year 2010. The rice variety selected for experiment was *NDR-97*. The observation summarized below were made during 20 to 120 days after sowing (DAS) of seeds. The rates and schedules of different kinds of fertilizers have been mentioned in materials and methods.

Experimental Design

The experimental field for rainfed rice crop NDR-97 is designed in three replicate plots, each having a dimension of 5 x 3 m. A strip of 0.5 m was left to separate each plot. The experiment was laid down in completely randomized block design. Basal treatment of KCL + P_2O_5 + farmyard manure at the rate of 60: 60: 1000 kg ha^{-}, respectively was applied to each plot (P_2O_5 was applied in the form of single super phosphate). Chemical fertilizers in the form of Urea, Diammonium phosphate, Ammonium nitrate, Ammonium chloride were used in the experiments, fertilizers were applied in there split doses at the rate or 40: 30: 30 kg N ha^{-1}.

Rainfed rice

In the year 2010 (rainy season), spatial distribution (Bare, Bulk & Rhizospheric) and influence of four fertilizer on methanotrophic population was investigated employing Narendra –97. The fertilizers were applied in three split doses, as indicated above, at the time of sowing, active tillering and flowering, respectively.

Soil Sampling

Soil monoliths (10 cm length x10m width x 15 cm depth) were removed between the rows in the vegetated plots. Similar random samplings were conducted in the bare plots. The samples were collected at regular intervals of 20 to 120 days during the cropping periods.

Plant Biomass

The root and shoot were separated with knife then dried at 105^0 C for 24 hrs, weighed to determine the root and shoot biomass. The data were computed at the rate of per hectare.

Soil parameter

Organic Carbon

Soil samples were oxidized in acidic dicromate and titrated with ferrous ammonium sulphate. 1, 10 Orthro-phenathroline monohydrate was used as indicator.

Total N

Total N was analyzed by macrokjaldal digestion using K_2SO_4, $CuSO_4.5H_2O$ and concentrated H_2SO_4.

Ammonium N

Extractable soil ammonium N was estimated colorimetrically by the phenate method. The method has sensitivity range of 10- 500µg NH_4+-N/ liter.

Nitrate N

Phenol disulphonic acid (PDSA) method was used for estimation of nitrate N content and CaSO4 was extracting agent. The optical density was measured at 420nm.

Population size of MCM

MCM population was enumerated by most probable number technique [26]. Suspension was prepared by dissolving 5g soil in the 30 ml sterile nitrate mineral salt medium [27] in 100 ml flask and shaken for 24 hrs on gyratory shaker (60 rpm) at 4°C. The suspension was considered as 10^{-1} dilution and subsequent serial dilutions were prepared. Taking out 5 ml suspension from a dilution, 5 replicate culture tubes were inoculated by one ml in each. The culture tubes contained 5ml sterile nitrate mineral medium having KH_2PO_4 (0.54 gl^{-1}); K_2HPO_4 (0.70 gl^{-1}); KNO_3 (1 gl^{-1}); $MgSO_4.7H_2O$ (1 g l^{-1}); $CaCl_2.2H_2O$ (0.20 gl^{-1}) and trace elements (1mg l^{-1}). Inoculated culture tubes, closed with sterile cotton plugs under aseptic conditions, were incubated in the environment of 20% methane in air at 25 °C in the dark in Atmosbag. Inoculated tubes incubated in synthetic air without CH_4 were used for control [28]. After five weeks of incubation, the tubes having turbid appearance were marked as positive and MCM population was determined using MPN table.

RESULTS

Ammonium-N content of soil

Variation in ammonium-N contents in soil following application of three different fertilizers has been presented in (Fig 1). The ammonium-N content was observed at 20 to 120 DAS in control and vegetative plots. The ammonium-N content was the highest in urea fertilized plots followed by DAP, ammonium nitrate and ammonium chloride. The ammonium-N content range from 3.04 to 3.7 µg g^{-1} dry soil in control, 4.12 to 8.5 µg g^{-1} dry soil in urea, 3.9 to 8.1 µg g^{-1} dry soil in DAP, 3.7 to 7.8 µg g^{-1} dry soil in ammonium nitrate and 3.2 to 6.7 µg g^{-1} dry soil in ammonium chloride. The differences between the days were significant. Variation in ammonium-N content with respect to four different fertilizers in vegetated plots has been illustrated in fig. 1. The highest value observed on 20 DAS and lowest value observed on 80 to 100 DAS. The highest ammonium-N content was observed in urea (8.5 ± 0.71 µg g^{-1} dry soil) and lowest ammonium-N content in ammonium chloride treated plots (6.7 ± 1.5 µg g^{-1} dry soil) on 20 DAS. ANOVA indicated significant differences due to days ($F_{5, 50}$ =29.84 $p < 0.05$) due to fertilization ($F_{4, 10}$ =10.24 $p < 0.05$) and their interaction due to days × fertilization was also significant ($F_{20, 50}$ = 1.48 $p < 0.05$).

Nitrate-N content of soil

The difference in Nitrate -N contents in soil following application of three different fertilizers has been presented in (Fig 2). The Nitrate -N content was the highest in urea fertilized plots followed by DAP, ammonium nitrate and ammonium chloride. The control (unfertilized) vegetative plots

showed the highest nitrate-N contents on 80 Das and lowest on 20 DAS while fertilized plots showed the highest nitrate-N on 80 DAS and lowest on 20 days. Among fertilizers, ammonium nitrate showed the highest nitrate-N content followed by urea DAP and ammonium chlorides. The nitrate-N content across days ranged from 0.82 to 3.79 µg g^{-1} dry soil in urea, 0.76 to 3.2 µg g^{-1} dry soil in DAP, 1.04 to 4.5 µg g^{-1} dry soil in ammonium nitrate, 0.71 to 2.10 µg g^{-1} dry soil in ammonium chlorides and 0.69 to 2.2 µg g^{-1} dry soil in control. The differences between days were significant. ANOVA indicated significant differences due to days fertilization ($F_{5, 50}$ =238.89 $p < 0.05$, $F_{4, 10}$ =33.50 $p < 0.05$) and their interaction due to days × fertilization was also significant ($F_{20, 50}$ = 10.44 $p < 0.05$).

Methanotrophs population size of soil

Changes in MCM population with respect to four fertilizers in vegetated plots has been presented in Fig. 3 control (unfertilized) exhibited the highest population size on 80 DAS (37.4 × 10^6 Cells g^{-1} dry soil) and showed lowest value (15.0 × 10^6 Cells g^{-1} dry soil) at 20 DAS. In case of fertilized plots the highest MCM population was highest on 80 (Urea 28.6 × 10^6 Cells g^{-1} dry soil; DAP 29.7 × 10^6 Cells g^{-1} dry soil; Ammonium nitrate 31.6 × 10^6 Cells g^{-1} dry soil and ammonium chloride 32.9 × 10^6 Cells g^{-1} dry soil) DAS and lowest on 20 DAS (Urea 2.7 × 10^6 Cells g^{-1} dry soil; DAP 3.1 × 10^6 Cells g^{-1} dry soil; Ammonium nitrate 6.5 × 10^6 Cells g^{-1} dry soil and ammonium chloride 8.6 × 10^6 Cells g^{-1} dry soil). The differences of the two were significant. The differences among fertilizer also significant. ANOVA indicated significant differences due to

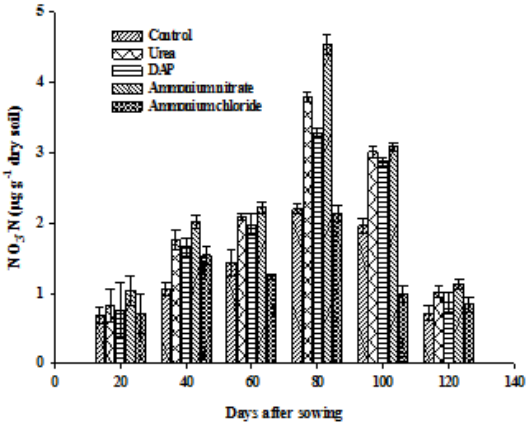

Fig. 2. Changes in nitrate N content of soil during July to November 2010 in vegetated plots planted to rice variety NDR-97. The fertilizers Urea, DAP, Ammonium-Nitrate and Ammonium chloride were applied to plots at a rate of 40:30:30 Kg N/ha-1. Unfertilized plot served as control. Each bar represents average value of three replicate plots. Vertical lines on each bar designate ± S.E

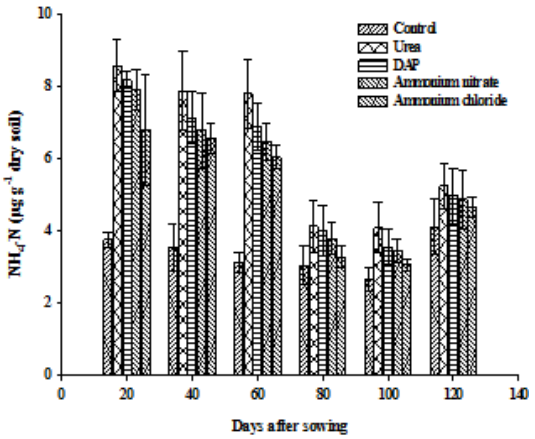

Fig. 1. Changes in ammonium N content of soil during July to November 2010 in vegetated plots planted to rice variety NDR-97. The fertilizers Urea, DAP, Ammonium-Nitrate and Ammonium chloride were applied to plots at a rate of 40:30:30 Kg N/ha-1. Unfertilized plot served as control. Each bar represents average value of three replicate plots. Vertical lines on each bar designate ± S.E

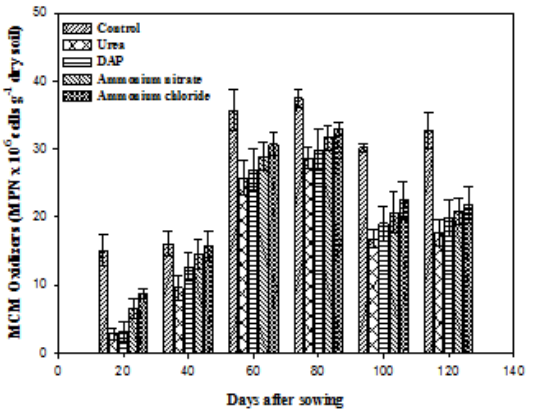

Fig. 3. Changes in ammonium N content of soil during July to November 2010 in vegetated plots planted to rice variety NDR-97. The fertilizers Urea, DAP, Ammonium-Nitrate and Ammonium chloride were applied to plots at a rate of 40:30:30 Kg N/ha-1. Unfertilized plot served as control. Each bar represents average value of three replicate plots. Vertical lines on each bar designate ± S.E.

days ($F_{5, 50}$ = 104.24 p < 0.05, appendix table) due to fertilization ($F_{4, 10}$ =17.4 p < 0.05, appendix table 13) and their interaction due to days × fertilization was also significant ($F_{20, 50}$ = 0.60 p < 0.05).

DISCUSSION

The input of nitrogenous fertilizer may become an important controlling factor in methane emission from rice agriculture. The already high nitrogen applications to rice will have to increase, since this is the limiting factor in rice productivity [4]. This may affect important processes involved in the methane budget of rice paddies i.e., methanogenesis, methane oxidation, nitrification and also plant growth. Studies investigating fertilizer effects on these processes have yielded contradictory results. After fertilization with urea or $(NH_4)_2SO_4$, lower [6] methane emissions were observed and attributed to direct inhibition of methanogenesis. However, higher CH_4 emissions were also detected from rice paddies after fertilisation with urea, $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$ [8]. The consumption of CH_4 by methanotrophs is generally thought to be inhibited by ammonium-based fertilisation, by as yet unraveled mechanisms reviewed by Gulledge et al. (1997) [9]. However, results often are contradictory for inhibition [29], as well as no effects [30] has been observed. The obligate aerobic methane oxidisers proliferate in the vicinity of oxygen-releasing roots of rice [10] and may oxidise up to 10–65% of the potentially emitted methane [13].

The present study aimed at investigating the effect of ammonium-based fertilization (Urea, DAP, Ammonium nitrate & Ammonium chloride) on MCM population from rice soil. I therefore studied the dynamics of methane oxidizer in different fertilizers treated plots. Data from the above results showed the significant differences in NH_4 concentration and MCM population as well. Urea has Highest effect on MCM population while MCM population was lowest in Ammonium chloride and Ammonium nitrate fertilized plots and DAP has intermediate effect on MCM population.

Detailed knowledge on the influence of the soil microbial community on rice crop performance is crucial in order to improve field management to obtain higher yields and sustainable rice production.

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