ISSN 1819-1894

Asian Journal of Agricultural Research

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Agricultural Research 6 (4): 158-170, 2012
ISSN 1819-1894 / DOI: 10.3923/ajar.2012.158.170

Nitrogen Fertilizer-induced Spatial Variation in the Size of Methane Oxidizing Bacterial Population in Rainfed Rice Cultivars

[1]Bharati Singh, [2]Deepmala Katiyar, [1]Durg Vijay Singh and [3]Amit Jayaswal
[1]Department of Botany, Faculty of Science, Udai Pratap Autonomous College, Varanasi, India
[2]Vitaegen Biotech-Educational and Research Institute, Varanasi, India
[3]Departments of Biotechnology, Faculty of Sciences, Banaras Hindu University, India

Corresponding Author: Bharati Singh, Department of Botany, Faculty of Science, Udai Pratap Autonomous College, Varanasi, Uttar Pradesh, India Tel: +91 9450846045, +91 0542 2277296 Fax: 0542- 2277587

ABSTRACT

This study was conducted to investigate the effect of N fertilization on methane oxidizing bacteria that lead to changes in the microbial communities in rice agroecosystems. Variation in MOB population size due to rice varieties of three different soil type bare, bulk and rhizosphere was investigated in rainfed rice NDR-97 (Narendra-97), Pant Dhan-12 and Vanaprabha. The growth variables (shoot biomass and root biomass) were higher in fertilized plots than unfertilized plots. The ammonium-N, nitrate-N was higher in fertilized plot than unfertilized plots. There were significant differences in MOB population size during the study ($p<0.05$). The highest MOB bacterial population was found in rhizospheric soils. So that, MOB bacterial population range between 19.2×10^6 to 70.54×10^6 cells g^{-1} dry across soil type of varieties and treatment. N-fertilization reduces population size of MOB. Thus the result suggests that MOB population size were varied by different soil type and soil fertilization. This study showed significant impact of N-fertilization on MOB population.

Key words: Rainfed rice cultivar, methane oxidizing bacteria, nitrogen fertilization, rice rhizosphere

INTRODUCTION

Rice fields are the most significant contributors of atmospheric methane accounting for 11-13% of the World's total anthropogenic methane emission (Wang *et al*., 2004). Most of the world's rice is grown in Asia, which represents 90% of the global rice growing area. In many rice-growing countries, rice systems are intensive cropping systems with a total grain production (Dobermann and Witt, 2000). According to an estimate, rice production will need to expand by around 60% over the next 25 years to meet the demand of the World's growing human population (Cassman *et al*., 1998), making rice cultivation a potential major cause of increasing atmospheric methane. Methane-consuming microbes play a vital role in global warming issues, as they are the only biological sink for methane. In dry upland soils they account for approximately 6% of the global sink strength of atmospheric methane (Le Mer and Roger, 2001). Methane-Oxidizing Bacteria (MOB) are strictly aerobic and gram-negative bacteria and are subdivided into types I and II on the basis of phylogeny, physiology, morphology and biochemistry (Bowman, 2000; Hanson and Hanson, 1996). Methanotrophs utilize methane as their sole carbon and energy source and fix methane in a reaction catalysed by methane monooxygenase (Lidstrom *et al*., 1994).

Methanotrophic bacteria play an important role in global methane budget by consuming the potential amount of methane in rice fields. Methanotrophic bacteria are present in the aerobic soil layer, the roots, the soil surrounding the roots, so-called rhizosphere (Dubey and Singh, 2000; Watanabe *et al*., 1997). Methanotrophs associated with the rhizosphere of rice plants oxidize CH_4 with molecular O_2 and use it as the sole source of carbon and energy. Methanotrophic bacteria are present in the aerobic soil layer, the roots, the soil surrounding the roots, so-called rhizosphere (Dubey and Singh, 2000; Gilbert and Frenzel, 1998) and on the stem bases of flooded rice plants (Watanabe *et al*., 1997). The rice rhizosphere appears to be a very heterogeneous habitat for methanotrophs because both methane concentrations and oxygen released by roots are highly variable (Gilbert and Frenzel, 1995).

Nitrogen is one of the most important nutritional elements for the productivity of cereal crops. In the case of rice, up to two-thirds of the N absorbed by the plant is furnished from the soil, even in fertilized fields. Therefore, natural sources of N, transformation and availability processes of N, markedly influence fertility in paddy fields and the efficiency of the fertilizer nitrogen use for high yields. Response of rice varieties to N in rice fields is generally recognized, but crop recovery of applied N is only 20 to 35% due to the losses in several ways. Nitrogen fertilizers are supposed to stimulate methane production by enhancing rice plant growth, thus increasing the carbon supply for methanogens (Schimel, 2000). A direct stimulatory effect of nitrogen fertilization was also observed on methane production (Dan *et al*., 2001). Both methane production and oxidation are biological processes and affected by nitrogen fertilizer directly or indirectly (Schimel, 2000).

In this present communication, we report the dynamics of methane oxidizing bacteria in and their process in rice soil namely bare, bulk and rhizospheric soil, at regular intervals across the cropping season. We have also compares the population size and spatial patterns of methane oxidizing bacteria among rhizosphere, bulk and bare soils in the influence of nitrogen fertilization.

MATERIALS AND METHODS

Site description: The site of present investigation was situated in the Chandauli district of Uttar Pradesh, India (Latitude: 25°26' and Longitude: 83°26') in July, 2007-2009.

Climate: The areas are characterized by dry tropical climate with typical monsoonal character. The temperature in the summer season ranged from (50-60°C) during the day. Warm condition between 30-55°C with high relative humidity (75-95%) prevails during the rainy season. The temperature may fall down from 10-20°C in the winter season.

Soil analyses: The soil is well drained, inceptisol, pale brown, silty loam (sand 30.49%, silt 26.08%) with pH 7-7.2. The percentage of C present in soil was 0.42%. Soil pH was measured using a pH meter equipped with glass electrode.

Experimental design: The experimental field for rainfed rice crop is designed in three replicate plots, each having a dimension of 5×3 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^{-1}, respectively was applied to each plot. A chemical fertilizer in the form of urea was used in the experiments, fertilizer was applied in there split doses at the rate or 40:30:30 kg N ha^{-1}.

Rainfed rice: In the year 2007 (rainy season), spatial distribution (Bare, Bulk and Rhizospheric) and influence of N-fertilizer (urea) on methanotrophic population was investigated employing Narendra-97, Pant Dhan 12 and Vanaprabha as rainfed rice variety. 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×10 m width×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°C for 24 h, 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 dichromate and titrated with ferrous ammonium sulphate. 1,10-ortho-phenanthroline monohydrate was used as indicator.

Total N: Total N was analyzed by macro-Kjeldahl digestion using K_2SO_4, $CuSO_4.5H_2O$, Se and concentrated H_2SO_4 (Jackson, 1958).

Ammonium-N: Extractable soil ammonium-N was estimated colorimetrically by the phenate method (APHA/AWWA, 1985). The method has sensitivity range of 10-500 μg NH_4^+-N L^{-1}.

Nitrate-N: Phenol disulphonic acid (PDSA) method was used for estimation of nitrate-N content and $CaSO_4$ was extracting agent. The optical density was measured at 420 nm.

Population size of MOB: MOB population was enumerated by most probable number technique (Bender and Conrad, 1992). Suspension was prepared by dissolving 5 g soil in the 30 mL sterile nitrate mineral salt medium (Whittenbury *et al.*, 1970) in 100 mL flask and shaken for 24 h 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 milliliter in each. The culture tubes contained 5 mL sterile nitrate mineral medium having KH_2PO_4 (0.54 g L^{-1}); K_2HPO_4 (0.70 g L^{-1}); KNO_3 (1 g L^{-1}); $MgSO_4.7H_2O$ (1 g L^{-1}); $CaCl_2.2H_2O$ (0.20 g L^{-1}) and trace elements (1 mg 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 (Eller and Frenzel, 2001). After five weeks of incubation, the tubes having turbid appearance were marked as positive and MOB population was determined using MPN table.

Statistical analysis: All data was analyzed using ANOVA by Statistical Analysis Systems (SPSS package 13.5) with a 95% significance level. A General Linear Model (GLM) two way analysis of variance with repeated measures was used to analyze the effect of soil type and fertilizer dose on soil process. The differences between the averages were compared using *post hoc*. Methane Oxidizing Bacterial population , Plant growth parameters, NH_4^+-N and NO_3^--N concentration in

different soil types where re-sampling of the same plots on six days interval was treated as repeated measures for each set of data analysis, the three replicate plot were considered as independent plots.

RESULTS AND DISCUSSION

Spatial and temporal scales are important factors for study of microbial populations. The present study sites are spatially separated which have been subjected to analysis by other investigators previously. During the present investigation, the sites were studied in relation dynamics of MOB population. Total C and N content of the soil do not varied significantly across the rice field.

Soil pH: The soil pH ranged from 7.1 to 7.5 in soil. ANOVA indicated significant differences in soil pH due to soil type, fertilization, time and their interaction (Table 2). The soil moisture was significantly different due to soil type and time.

Plant biomass: Variation in root biomass in plots following application of fertilizer has been presented in Fig. 1a and b. The root biomass increased and then decreased at the end of the cultivation period. The maximum root biomass was recorded at 100 DAS (Days After Sowing) while minimum root biomass recorded at 20 DAS. The root biomass ranged from 5.0 to 60.0 g^{-2} in control

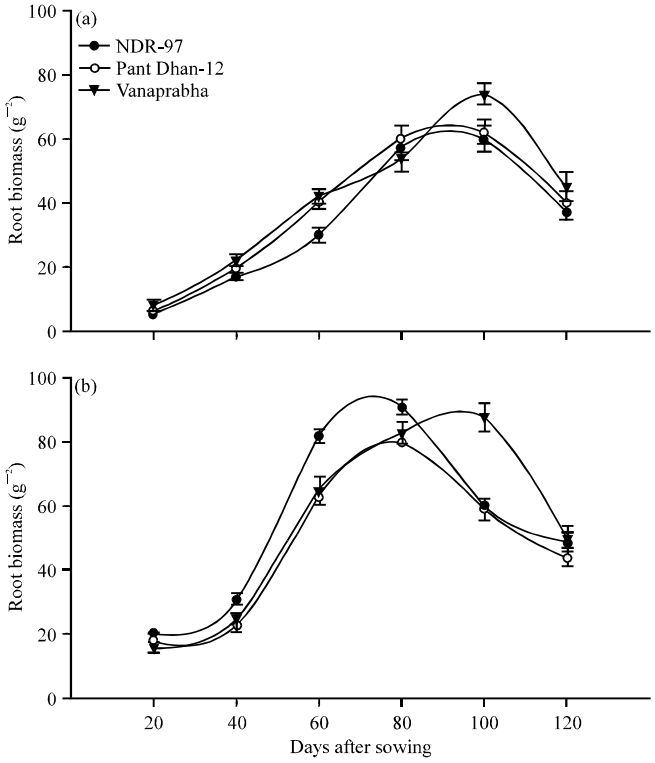

Fig. 1(a-b): Variation in root of the rainfed rice varieties under (a) Control (unfertilized) and (b) Fertilized condition during cultivation period (July to November 2007), Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

(unfertilized). The three rice varieties exhibited sigmoid pattern of growth. Changes in root biomass across the three varieties in fertilized plot have been described in Fig. 1b. Composite analysis of root biomass showed significant difference among the varieties and treatment. ANOVA indicated significant difference due to days ($F_{5, 60}$ = 384.2, $p<0.05$), due to treatment ($F_{1, 12}$ = 399.0, $p<0.05$) and due to varieties ($F_{2, 12}$ = 13.6, $p<0.05$) and their interaction days×variety×treatment also significant (Table 1).

Changes in shoot biomass across three varieties in control (unfertilized) plot have been presented in Fig. 2a. The growth pattern of shoot biomass was also sigmoid. The highest shoot

Table 1: F-ratios and their significance levels for two-way ANOVA with repeated measures for Root Biomass for three rainfed cultivars (NDR-97, Pant Dhan-12 and Vanaprabha) soil type and two fertilization treatments (0 and 100 kg N ha^{-1}) where sampling time was treated as a repeated measures

	Source of variation						
	Within subject			Between subject			
Parameters	C	F	C×F	T	T×C	T×F	T×C×F
Root biomass	13.65*	399.02**	384^{ns}	19.9**	6.09*	25.6**	3.26*
Shoot biomass	63.12*	248.06*	1302.90^{ns}	13.84**	60.57**	84.03**	6.02*

ns: Not significant, *, **Significant at $p<0.05$, and $p<0.01$, respectively, C: Cultivar, F: Fertilizer, T: Time

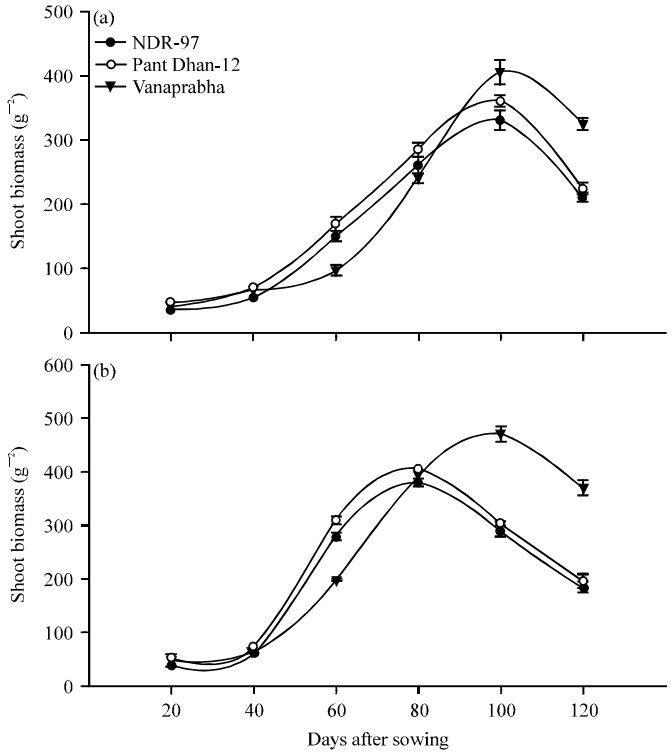

Fig. 2(a-b): Variation in shoot of the rainfed rice varieties under (a) Control (unfertilized) and (b) Fertilized condition during cultivation period (July to November 2007), Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

biomass was attained in all the rice varieties on 100 DAS which decline subsequently. Variation in shoot biomass across three rice varieties in fertilized plots have been presented in Fig. 2b. The composite analysis of data indicated that there was significant difference due to days ($F_{5, 60} = 1302.9$, $p<0.05$), varieties ($F_{2, 12} = 63.1$, $p<0.05$) and treatment ($F_{1, 12} = 248.06$, $p<0.05$) and their interaction days×variety×treatment also significant (Table 1). The growth variable (Root Biomass and Shoot Biomass) were higher in fertilized plots than unfertilized plots.

Ammonium-N: The ammonium content in bare (unfertilized) plots has been presented in Fig. 3. The highest ammonium N content was observed on 40 DAS (5.70±0.17 μg g^{-1} dry soil) in control. The ammonium-N content of vegetated (unfertilized) that is control plots has been described in Fig. 3. Across the rice varieties soil type, rhizospheric soil has the highest value on 20 DAS in control of bare plots whereas in fertilized soil of Pant Dhan-12 rhizospheric soil has highest value (9.1±0.34 μg g^{-1} dry soil).

Our results showed a greater accumulation of NH_4^+-N in bare soil which was followed by bulk and rhizosphere soil and the differences were significant. In the bare, the mean value of unfertilized plots across days ranged from 4.75 to 3.9 μg g^{-1} dry soil and the differences were significant. The differences between the soil types were significant. ANOVA indicated significant differences due to days ($F_{5, 28} = 247.88$, $p<0.05$). The mean value across varieties rhizospheric ranged from 0.60 to 4.1 μg g^{-1} dry soil and differences were significant. The differences among varieties were also significant. ANOVA indicated significant differences due to different soil type of varieties (Table 2).The variation in the ammonium-N content in bare (fertilized) plot has been presented in Fig. 4. The highest ammonium-N content ranged from 5.8 to 11.6 μg g^{-1} dry soil. In the vegetative plots the changes in the ammonium-N content has been showed in Fig. 3. Changes in ammonium-N content in vegetative (fertilized) plots have been showed in Fig. 4. The highest ammonium-N in

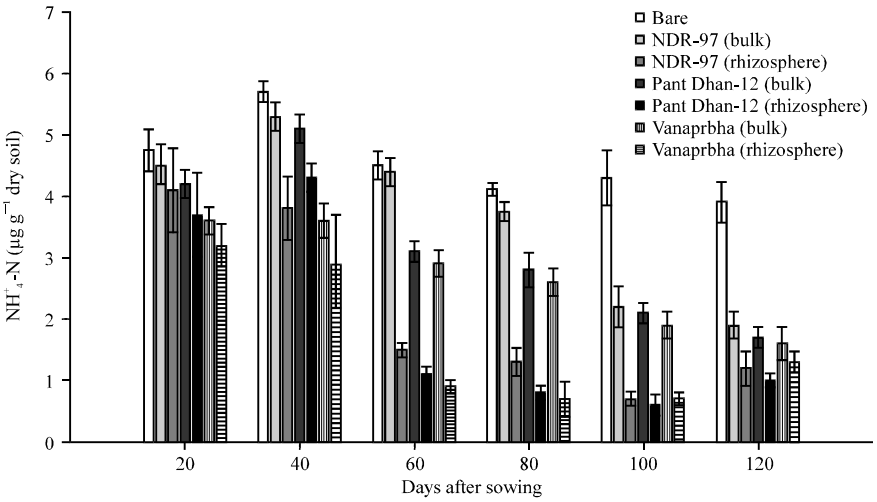

Fig. 3: Variation in ammonium-N content of the bare (unvegetated) and vegetated plots (NDR-97, Pant Dhan-12 and Vanaprabha) soils during July to November 2007 in the rainfed rice field, The graph showed ammonium-N content in different rainfed rice varieties soil types (Bulk and Rhizospheric) in control (unfertilized) condition, Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

Table 2: F-ratios and their significance levels for two-way ANOVA with repeated measures for NH_4^+-N, NO_3^--N and MOB population for three rainfed cultivar's soil type and two fertilization treatments (0 and 100 kg N ha^{-1}) where sampling time was treated as a repeated measures

	Source of variation						
	Between subject			Within subject			
Parameters	ST	F	T	ST×F	T×ST	T×F	T×ST×F
pH	37.9**	32.0**	12.9**	4.8*	6.8*	11.2**	4.3*
Soil moisture	49.0**	1.9^{ns}	0.69^{ns}	12.8**	1.2^{ns}	0.69^{ns}	0.90^{ns}
NH_4+-N	217.2**	2152.2**	5. 8*	247.8**	15.2*	25.1**	8.4*
NO3$^-$-N	181.4**	981.1**	21.3**	94.2**	8.4*	62.65**	4.7*
MOB	234.4**	272.0**	2.6*	259.2**	30.1**	3. 6*	0.73^{ns}

*, ** Significant at p<0.05 and p<00.1, respectively, ns: Not significant, ST: Soil type, F: Fertilizer, T: Time

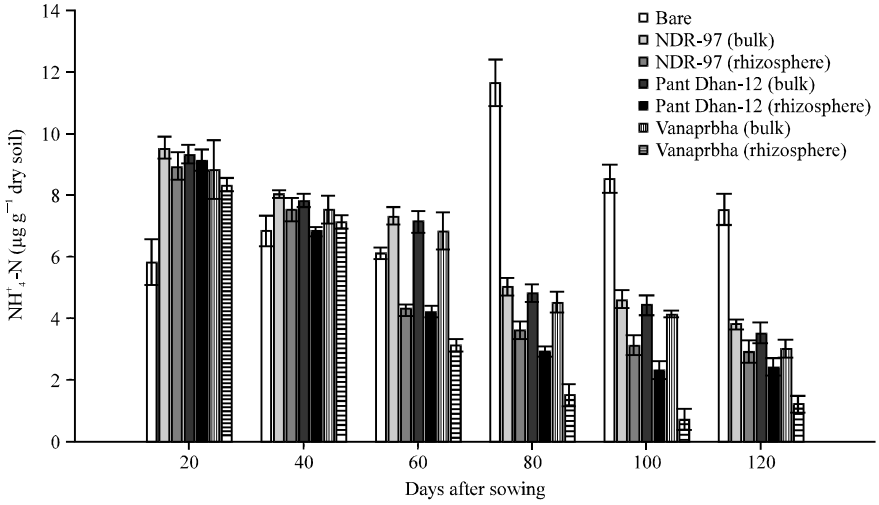

Fig. 4: Variation in ammonium-N content of the bare (unvegetated) and vegetated plots (NDR-97, Pant Dhan-12 and Vanaprabha) soils during July to November 2007 in the rainfed rice field. The graph showed ammonium-N content in different rainfed rice varieties soil types (bulk and rhizospheric) in fertilized condition, Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

vegetative (fertilized) plots was on 20 DAS (NDR-97 bulk 9.5±0.34; Pant Dhan-12 bulk 9.3±0.28; Vanaprabha bulk 8.80±0.98) followed by rhizospheric and bare soil and lowest at 120 DAS. There was consistent decrease in ammonium-N content of soil after 20 DAS. In the case of rhizospheric soil type of varieties, the highest ammonium-N concentration on 20 DAS (NDR-97 rhizosphere 8.9±0.46; Pant Dhan-12 rhizosphere 9.1±0.0.34; Vanaprabha rhizosphere 8.3±0.23) and lowest at 100 DAS. The mean value of vegetative (fertilized) soil type of three varieties across the days ranged from 1.2 to 9.5 µg g^{-1} dry soil and differences of the two were significant. ANOVA indicated significant differences due to days ($F_{5, 28}$ = 2.47.8, p<0.05). The mean values across varieties were significant. ANOVA indicated significant differences due to varieties soil types ($F_{5, 28}$ = 217.8, p<0.05). The interaction due to day's×soil type was also significant (Table 2).

The result revealed that ammonium-N content was all over higher at all days in fertilized plots than in unfertilized plots. Composite analysis revealed significant differences due to days, fertilization, soil type and interaction due to fertilization×days×soil type was also significant (Table 2). The Tukey *post hoc* test revealed that there was significant difference among bare, bulk and rhizospheric soiltype of all varieties in control and fertilized whereas NH4$^+$-N content was not significant among all rhizospheric soil of each varieties.

The application of nitrogenous fertilizers resulted in a higher NH_4^+-N concentration, as a consequence of hydrolysis which proceeds rapidly in warm, moist soils. The low NH_4^+-N in the rhizosphere soil evidently resulted from the continuous uptake by rice and uptake and oxidation by microorganisms such as ammonia oxidizers and MOB (Arth *et al.*, 1998).

The concentration of NH_4^+-N and NO_3^- -N occurred in the soil during the growth stages varied significantly. This showed a greater accumulation of NH_4^+-N in bare soil which was followed by bulk and rhizosphere soil and the differences were significant. Such observations are similar to others on dry rainfed rice (Dubey and Singh, 2000).

Nitrate-N: Variation in soil nitrate N content following three different rice varieties soil type (Bare, Bulk and Rhizospheric) presented in Fig. 5 and 6. The nitrate N content decreased steadily throughout observation period.

The nitrate-N content in bare soil of control has been described in Fig. 5. The nitrate N content in bare fertilized soil was highest (5.9±0.23 μg g^{-1} dry soil) on 80 DAS and lowest (1.8±0.12 μg g^{-1} dry soil) at 20 DAS. On the other hand among the rice varieties rhizospheric soil type, NDR 97 rhizospheric soil has the highest nitrate N content (1.87±0.05 μg g^{-1} dry soil) on 40 DAS and lowest at 120 DAS. ANOVA indicated significant differences due to days ($F_{5, 140} = 94.17$, $p<0.05$). The mean values of fertilized plots across days ranged from 0.9 to 4.6 in bulk soil type and 0.8 to 2.6 in

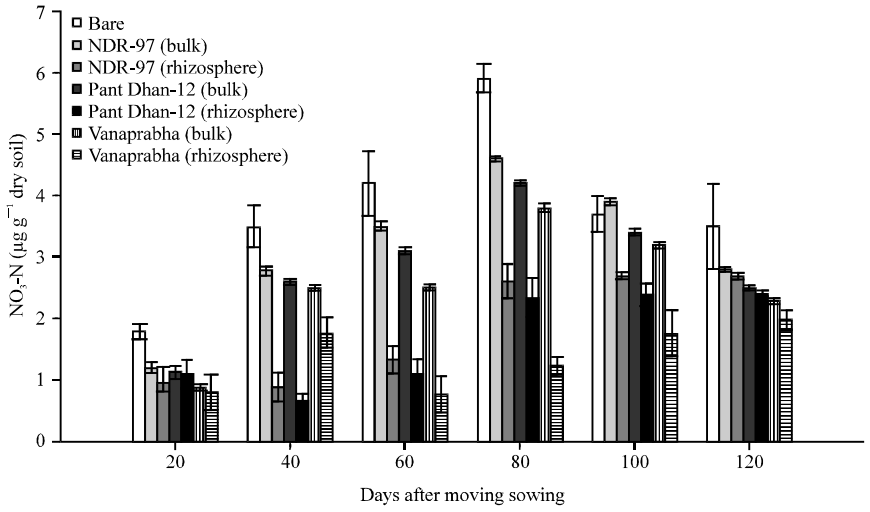

Fig. 5: Variation in nitrate-N content of the bare (unvegetated) and vegetated plots (NDR-97, Pant Dhan-12 and Vanaprabha) soils during July to November 2007 in the rainfed rice field. The graph showed nitrate-N content in different rainfed rice varieties soil types (bulk and rhizospheric) in control condition, Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

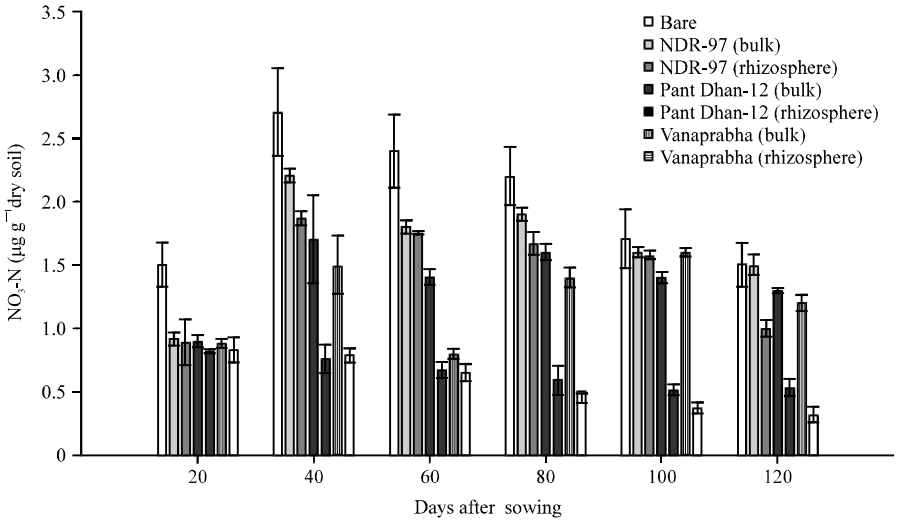

Fig. 6: Variation in nitrate-N content of the bare (unvegetated) and vegetated plots (NDR-97, Pant Dhan-12 and Vanaprabha) soils during July to November 2007 in the rainfed rice field, The graph showed nitrate-N content in different rainfed rice varieties soil types (bulk and rhizospheric) in fertilized condition, Each data point is an average of three replicate plots. Vertical line on each point represents ±SE

rhizospheric soil type of the varieties. The difference among varieties was also significant. ANOVA indicated significant difference due to varieties soil type (Table 2).

The present result showed that the differences between days were also significant ANOVA indicated significant differences due to days ($F_{5, 140} = 94.17$, $p<0.05$) and due to soil type ($F_{2, 28} = 181.4$, $p<0.05$) and interaction between fertilizer and soil type (Table 2).

Comparison of Fig. 5 and 6 exhibited higher nitrate-N in fertilized than unfertilized soil type. Composite analysis of unfertilized and fertilized plots indicated significant differences due to fertilization, days and soil type of varieties (Table 2). The interaction of fertilizer×days×soil type was also significant ($F_{30, 140} = 4.65$, $p<0.05$). The Tukey *post hoc* test revealed that there was significant difference between bare and rhizospheric soiltype of all varieties.

The present study also showed a strong temporal variability in nutrient availability at all the studied in three different varieties soil types is in conformity with the other reports regarding spatial distribution in rice agro-ecosystem (Dubey and Singh, 2000). The concentration of NH_4^+-N and NO_3^--N occurred in the soil during the growth stages varied significantly (Dubey and Singh, 2000). The coupled assimilation/dissimilation of $NO3^-$-N may decrease the soil NO_3^--N concentration during active growth of methanotrophs.

MOB population: The changed in Methane Oxidizing Bacterial (MOB) population of bare soil type (Unfertilized) has been presented Fig. 7. The highest MOB population on 120 days (22.0×10^6 cells g^{-1} dry soil) and lowest on 20 days (7.3×10^6 cells g^{-1} dry soil). The Spatial distribution and variation in MOB population in vegetated (unfertilized) bare and bulk rhizospheric soil presented in Fig. 7. The higher MOB observed on 80 DAS (NDR-97 rhizosphere 58.87×10^6 cells g^{-1} dry soil; Pant Dhan-12 rhizosphere 51.23×10^6 cells g^{-1} dry soil) and lowest on 20 DAS in

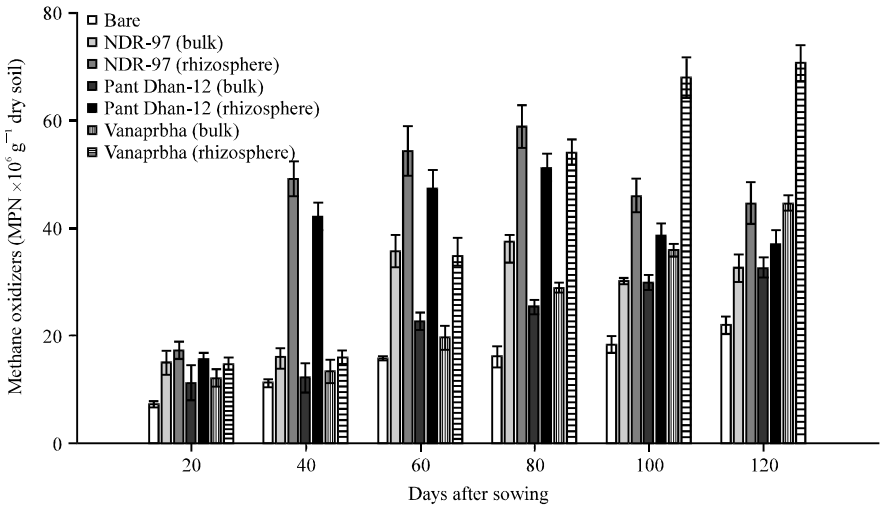

Fig. 7: Variation in MOB population of the bare (unvegetated) and vegetated plots (NDR-97, Pant Dhan-12 and Vanaprabha) soils during July to November 2007 in the rainfed rice field, The graph showed MOB population in different rainfed rice varieties soil types (bulk and rhizospheric) in control condition, Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

rhizospheric soil of three varieties (NDR-97 rhizosphere 17.2×10^6 cells g^{-1} dry soil; Pant Dhan-12 rhizosphere 15.2×10^6 cells g^{-1} dry soil; Vanaprabha rhizosphere 14.2×10^6 cells g^{-1} dry soil). In the bulk soil of all three varieties also exhibited highest MOB population on 80 DAS (NDR-97 bulk 32.5×10^6 cells g^{-1} dry soil; Pant Dhan-12 bulk 32.6×10 cells g^{-1} dry soil; Vanaprabha bulk 44.6×10^6 cells g^{-1} dry soil) and lowest on 20 DAS. Comparison of all three varieties soil type (bare, bulk and rhizosphere) Vanaprabha rhizospheric soil showed highest MOB population (70.54×10^6 cells g^{-1} dry soil) on 120 DAS and lowest (37.01×10^6 cells g^{-1} dry soil) on 80 DAS in Pant Dhan-12 rhizospheric soil. NDR-97 rhizospheric soil was in intermediate position.

Where as in case of vegetated fertilized (Fig. 8) soil type, (bare, bulk and rhizosphere) the highest MOB population observed on 80 to 120 DAS and lowest on 20 DAS. In bare fertilized soil type the maximum MOB was 15.3×10^6 cells g^{-1} dry soil and minimum MOB population 5.2×10^6 cells g^{-1}.

ANOVA indicated significant differences due to days ($F_{5, 140} = 259.2$, $p<0.05$), varieties soil type ($F_{6, 28} = 234.46$, $p<0.05$). The interaction due to day's×soil type was also significant. Two ways ANOVA indicated significant effect due to interaction ($F_{30, 140} = 30.11$, $p<0.05$).

Comparison between bare and vegetated plots revealed that the MOB population was highest in vegetated bulk and rhizospheric soil type and lowest in bare soil while in case of fertilized and unfertilized plots, the MOB population was highest in unfertilized plots. Composite analysis of unfertilized and fertilized plot indicated significant difference due to days, fertilization, soil type and their interaction due to days×fertilization×soil type (Table 2). The Tukey *post hoc* test revealed that there was significant difference among bare, bulk and rhizospheric soiltype of all varieties in control and fertilized. There was also significant difference in MOB population between bulk and rhizospheric soil type of each variety.

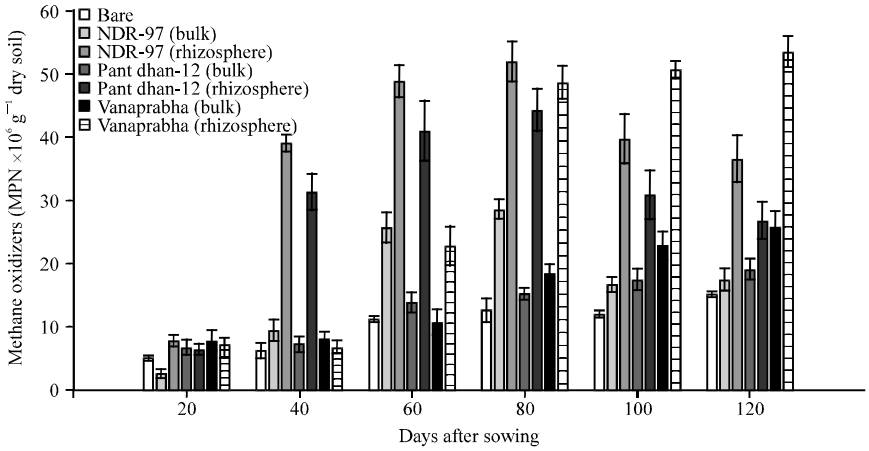

Fig. 8: Variation in MOB population size in the bare (unvegetated) plot and vegetated plots (NDR-97, Pant Dhan-12 and Vanaprabha) soils during July to November 2007 in the rainfed rice field, The graph showed MOB population size in different rainfed rice varieties soil types (bulk and rhizospheric) in fertilized condition, Each data point is an average of three replicate plots, Vertical line on each point represents ±SE

Spatial distribution of methanotrophic population varied across the three soil type. Higher MOB population at the rhizospheric soil in all cultivars found that active Methane Oxidizing Bacteria (MOB) occurred near to root mat because the O_2 supplying potential of plant roots is a major factor for the multiplication, growth and sustenance of methanotrophic bacteria in the rhizosphere (Gilbert and Frenzel, 1998). The plant variables, especially root biomass and shoot biomass representing the conduit and ventilation effects were important for methane oxidation in dryland rice agriculture. Comparisons between the soil type and treatments should be possible and valid (Bosse and Frenzel, 1997). The higher MOB observed on 80 DAS NDR-97 rhizospheric soil types ($58.87\pm3.9\times10^6$ cells g^{-1} dry soil) followed by Pant Dhan-12 rhizosphere ($51.23\pm2.7\times10^6$ cells g^{-1} dry soil). Therefore in the rainfed condition the MOB was significantly different among the varieties soil types (bare, bulk and rhizosphere). The high MOB population tended to get confined to the rhizosphere, attributed to downward diffusion of atmospheric oxygen and the upward diffusion of endogenous methane (Hutsch, 2001). The other factors have been water content, nutrients and soil type (Hanson and Hanson, 1996) and thus periodicity of these factors will likely lead to periodical changes in the maximum viable MOB population. Activity and population size of MOB varied with soil gradient.

CONCLUSIONS

In conclusion, the development of the rhizosphere brings about a spatial pattern in the distribution of MOB population which increases in size during the vegetative period among rhizospheric, bulk and bare soil. Greater O_2 availability due to ventilation by rice plants, lower concentrations of NH_4^+-N due to continuous plant uptake and a larger MOB population make the rice rhizosphere a microsite for intense methane oxidation activity. Thus, we conclude that soil type (bare, bulk, rhizosphere) of different rice cultivars, N fertilization and plant growth affect MOB in rainfed rice field. That may be due to the different ability of rice cultivar in emitting methane gas were mostly related to growth performance.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Head, Department of Botany and Department of Agronomy, Institute of agricultural Sciences Banaras Hindu University, for providing laboratory facilities. We are also grateful to principal, U.P. College, Varanasi for providing necessities of this work to pursue higher studies.

REFERENCES

APHA/AWWA, 1985. Standard methods for the examination of water and waste water quality. J. Environ. Toxicol. Water Qual., 11: 72-82.

Arth, I., P. Frenzel and R. Conrad, 1998. Denitrification coupled to nitrification in the rhizosphere of rice. Soil Biol. Biochem., 30: 509-515.

Bender, M. and R. Conrad, 1992. Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH4 mixing ratios. Federation Eur. Microbiol. Soc. Microbiol. Ecol., 101: 261-270.

Bosse, U. and P. Frenzel, 1997. Activity and distribution of CH4-oxidizing bacteria in flooded rice microcosms and in rice plants (*Oryza sativa*). Appl. Appl. Environ. Microbiol., 63: 1199-1207.

Bowman, J., 2000. The Methanotrophs-the Families Methylococcaceae and Methylocystaceae. In: The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community, Dworkin, M. (Ed.). 3rd Edn., Springer, New York.

Cassman, K.G., S. Pen, D.C. Olk, J.K. Ladha, W. Reichardt, A. Dobermann and U. Singh, 1998. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. Field Crops Res., 56: 7-39.

Dan, J., M. Kruger, P. Frenzel and R. Conrad, 2001. Effect of a late season urea fertilization on methane emission from a rice field in Italy. Agric. Ecosys. Environ., 83: 191-199.

Dobermann, A. and C. Witt, 2000. The Potential Impact of Crop Intensification on Carbon and Nitrogen Cycling in Intensive Rice Systems. In: Carbon and Nitrogen Dynamics in Flooded Soils, Kirk, G.J.D. and D.C. Olk (Eds.). IRRI, Los Banos, Philippines, pp: 1-25.

Dubey, S.K. and J.S. Singh, 2000. Spatio-temporal variation and effect of urea fertilization on methanotrophs in a tropical dryland rice field. Soil Biol. Biochem., 32: 521-526.

Eller, G. and P. Frenzel, 2001. Changes in activity and community structure of methane oxidizing bacteria over the growth period of rice. Applied Environ. Microbiol., 67: 2395-2403.

Gilbert, B. and P. Frenzel, 1995. Methanotrophic bacteria in the rhizosphere of rice microcosms and their effect on pore water methane concentration and methane emission. Biol. Fertil. Soils, 20: 93-100.

Gilbert, B. and P. Frenzel, 1998. Rice roots and CH_4 oxidation: The activity of bacteria, their distribution and the microenvironment. Soil Biol. Biochem., 30: 1903-1916.

Hanson, R.S. and T.E. Hanson, 1996. Methanotrophic bacteria. Microbiol. Rev., 60: 439-471.

Hutsch, B.W., 2001. Methane oxidation in non-flooded soils as affected by crop production. Eur. J. Agron., 14: 237-260.

Jackson, M.L., 1958. Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, NJ., USA.

Le Mer, J. and P. Roger, 2001. Production, oxidation, emission and consumption of methane by soils: A review. Eur. J. Soil Biol., 37: 25-50.

Lidstrom, M.E., C. Anthony, F. Biville, F. Gasser, P. Goodwin, R.S. Hanson and N. Harms, 1994. New unified nomenclature for genes involved in the oxidation of methanol in Gram-negative bacteria. FEMS Microbiol. Lett., 177: 103-106.

Schimel, J., 2000. Global change: Rice, microbes and methane. Nature, 403: 375-377.

Wang, J.S., J.A. Logan, M.B. McElroy, B.N. Duncan, I.A. Megretskaia and R.M. Yantosca, 2004. A 3-D model analysis of the slowdown and interannual variability in the methane growth rate from 1988 to 1997. Global Biogeochem. Cycles., Vol. 18.

Watanabe, I., T. Hashmoto and A. Shimoyama, 1997. Methane oxidizing activities and methanotrophic population associated with wetland rice plants. Biol. Fertil. Soils, 24: 261-265.

Whittenbury, R., K.C. Phillip and J.F. Wilkinson, 1970. Enrichment, isolation and some properties of methane oxidizing bacteria. J. Gen. Biol., 61: 205-218.