Spatio-temporal variation and effect of urea fertilization on methanotrophs in a tropical dryland rice field

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Abstract

Population size of methanotrophs in a dryland field planted to Oryza sativa L. variety Narendra-118 was quantified over a period of 13 weeks. Methanotroph numbers were higher in control plots (52.9–736.6 $10^5$ cells g$^{-1}$ dry soil) than in plots treated with urea (43.8–676.0 $10^5$ cells g$^{-1}$ dry soil), and were highest in the rhizosphere soil (499.8–736.6 $10^5$ cells g$^{-1}$ dry soil) followed by bulk (451.4–684.1 $10^5$ cells g$^{-1}$ dry soil) and bare (43.8–67.5 $10^5$ cells g$^{-1}$ dry soil) soil. The concentrations of NH$_4^+$-N were significantly ($P < 0.001$) lower in the rhizosphere (3.1–6.4 µg g$^{-1}$ soil) than in bulk (4.1–8.3 µg g$^{-1}$ soil) and bare soils (5.1–10.7 µg g$^{-1}$ soil). The study suggests that the development of the rice rhizosphere brings about a spatial pattern in the distribution of methanotrophic bacteria which increases in size, over time, within the rhizosphere and adjoining bulk soil, and that the rhizosphere is a potential microsite of intense CH$_4$ oxidation activity. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Dryland rice; Methanotroph population; Rhizosphere; Urea fertilization

1. Introduction

A major anthropogenic source of atmospheric methane, a greenhouse gas, is wetland rice agriculture. The atmospheric concentration of CH$_4$ is expected to increase further due to expansion of rice cultivation (Singh and Singh, 1995). Although the chemical reaction of CH$_4$ with hydroxyl radicals in the atmosphere is considered to be the major sink (Crutzen, 1991), uptake of ambient methane by soils could be an additional sink, representing 1–15% of that oxidized by reaction with hydroxyl radicals (Born et al., 1990). Methane oxidation by soils in temperate ecosystems, tropical forest, desert, savanna and dryland rice fields has been reported (for review see King, 1992; Dubey et al., 1996; Topp and Pattey, 1997). Methane oxidizing bacteria (gram-negative, aerobic bacteria belonging to the family Methylcoccaceae) are ubiquitous in nature and are believed to reduce CH$_4$ flux to the atmosphere from sediments and soils (Oremland and Culbertson, 1992).

A recent investigation on the dynamics of CH$_4$ flux demonstrated that tropical dryland rice fields are potential net CH$_4$ sinks, and urea application reduced their capacity for CH$_4$ oxidation (Singh et al., 1999). Singh et al. (1998a) reported that the rice plant was a part of this sink. Our goal was to investigate whether the methane oxidizing bacteria (MOB) in the rhizosphere are responsible for the CH$_4$ sink strength of dryland rice and whether population changes are responsible for the urea effect on the sink strength. In the present investigation, we have measured the population size of methanotrophs from the rhizosphere, and bulk soil in a dryland rice field planted to the rice variety Narendra-118, which exhibited highest CH$_4$ oxidation activity in the earlier investigation (Singh et al., 1999). The population of methanotrophs in bare soil was also estimated.
2. Materials and methods

2.1. Experimental site

Our study was carried out on the dryland rice field of the experimental Botanical Garden, Banaras Hindu University, at Varanasi, India (25°18′N 83°3′E, 129 m above mean sea level). The climate is dry tropical and monsoon-marked by a cold winter (November–February), a hot summer (March–June) and a warm rainy season (July–September). During the experiment, minimum temperatures ranged from 14 to 27°C and the maximum from 22 to 38°C. The annual rainfall was 1208 mm of which 970 mm fell during the rainy monsoon from the southwest. The soil is a well drained Inceptisol, pale brown, silty loam (sand 32%, silt 65% and clay 3%) with pH 7–7.8.

2.2. Experimental design and rice cultivation

The experimental field consisted of 12 plots each measuring 5 × 3 m. The experiment was laid down in a completely randomized block design. A 0.5-m strip separated plots. Basal treatment of KCl + P2O5 + farmyard manure was applied at a rate of 60:60:1000 kg ha−1, to all plots during plowing. Six plots were fertilized with urea and the remaining served as control. In the fertilized plots, urea was applied in three split doses, at the time of tillering, flowering and grain filling stages at the rates of 40, 30 and 30 kg N ha−1, respectively. Among the 12 plots, six plots (three with and three without urea) were sown to rice while the other six (three with and three without urea) were maintained as bare soil. Thus the experiment had three plots each for bare control, bare fertilized, vegetated and fertilized treatments. Seeds of rice (Oryza sativa L., cultivar Narendra-118) were sown by dibbling on 10 July 1997, at a spacing of 15 cm (hill-to-hill) by 20 cm (row-to-row) in the plots designated as vegetated plots. No irrigation was provided throughout the experiment and the sole source of water was rainfall.

2.3. Soil sampling and analysis for NH4+-N

Samples of bulk (between the plant rows) and bare (bare plots) soil were collected, separately for each plot, from 0–10 cm depth using a 5-cm dia soil corer. The 0–10 cm soil depth was chosen because observation indicated that ≥92% roots are concentrated in this soil layer. The rhizospheric soil was collected by tapping the roots on a plastic sheet (Lee et al., 1997). The soil samples were sieved (2 mm), and fine roots were removed. One part of each sample was weighed and oven dried at 105°C to determine the moisture content. Field moist samples, stored at 4°C, were used for chemical analyses and methanotrophic population counts within 2 days after sampling. The soil sampling was carried out at 40 and 90 days after sowing (DAS). Ammonium nitrogen (NH4+-N) was measured by the phenate method (APHA, 1985) in an extract with 2 M KCl.

2.4. Plant biomass

On each sampling date, one rice hill was harvested from each experimental plot with soil as a block (15 × 20 × 15 cm depth) using a rectangular open-top plastic chamber. Roots were washed with water. After counting the tillers, root and shoot materials were dried at 60°C to constant weight.

2.5. Population of methanotrophs

The numbers of methanotrophic bacteria were enumerated by the MPN (most probable number) technique as described in Bender and Conrad (1992). In brief, fresh soil (5 g) was suspended in 15 ml of a modified ammonium mineral salts medium (AMS) and shaken for 12 h at 4°C in the dark. This suspension served as the inoculum. The modified AMS medium (Heyer et al., 1984) contained 1 l−1 distilled water (pH 6.9); 10 mmol NH4Cl, 0.4 μmol MgSO4·7H2O, 4 mmol K2HPO4, 0.1 μmol CaCl2 and 1 ml trace element solution (Widdel and Bak, 1992). Instead of microtiter plates, culture tubes were used (Espiritu et al., 1997).

Dilution was carried out from 10−1 to 10−9, as described by Espiritu et al. (1997). Each dilution, 1 ml was inoculated into tubes containing 3 ml AMS medium. There were six replicates for each dilution. After inoculation under aseptic conditions, the tops of the tubes were closed with sterilized cotton plugs. The tubes were incubated under 20% methane in air at 25°C in the dark in atmosbags (Sigma, USA) for 3 weeks. For control, culture tubes were prepared without soil inoculum (Espiritu et al., 1997). In tests we had used control with sterilized soil and found that control without soil was as good as a control with sterilized soil. A more appropriate control would be culture tubes with soil inoculum under CH4-free air. Further, perhaps a more reliable method to enumerate cultivable MOB would be MPN in tubes (6–8 weeks incubation) with measurement of CH4 consumption (Escoffier et al., 1997).

Data were checked for normality and homogeneity of variances and subjected to analysis of variance (ANOVA) and regression analysis according to Snedecor and Cochran (1989).
Table 1
Soil moisture (mg g⁻¹ soil) and NH₄⁺-N concentration (µg g⁻¹ soil) in rhizosphere, bulk and bare soils from control and fertilized (100 kg N ha⁻¹) planted to rice variety Narendra-118, determined on two sampling dates. (DAS = days after sowing) (mean ± 1 S.E.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>DAS</th>
<th>Rhizosphere</th>
<th>Bulk</th>
<th>Bare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>fertilized</td>
<td>control</td>
</tr>
<tr>
<td>NH₄⁺-N</td>
<td>40</td>
<td>3.3 ± 0.7</td>
<td>4.7 ± 0.6</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>Moisture</td>
<td>40</td>
<td>163.3 ± 6.6</td>
<td>176.7 ± 8.8</td>
<td>90.7 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>81.7 ± 7.3</td>
<td>100.0 ± 11.5</td>
<td>80.0 ± 5.8</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. Soil moisture and NH₄⁺-N

Soil moisture ranged from 70 to 187 mg g⁻¹ soil. ANOVA indicated significant differences in soil moisture due to days (F₁, 26 = 54.69, P = 0.000) but differences due to treatment and soil origin (rhizosphere, bulk and bare) were not significant. Nevertheless, moisture content was consistently higher for bare soil than for bulk or rhizosphere soil. The ammonium-N concentration in the soil was higher for the fertilized plots compared to control plots (F₁, 26 = 53.61, P = 0.000) and was higher on 40 DAS compared to 90 DAS (F₁, 26 = 5.56, P = 0.026) (Table 1). The NH₄⁺-N concentration declined from bare to bulk or bare to rhizosphere soil, both in control and fertilized soil, with the differences between soil positions being significant (F₂, 26 = 13.64, P = 0.000). The range of NH₄⁺-N concentration observed in the present investigation for bulk soil was similar to that reported earlier for the dryland rice fields in this region (Singh and Singh, 1994; Jha et al., 1996). Evidently, the low NH₄⁺-N in the rhizosphere resulted from the continuous uptake by rice plants.

3.2. Plant biomass

In the present study we measured number of tillers, root biomass and shoot biomass as affected by urea fertilization and growth stage (Table 2). There was a significant effect of urea treatment on these growth characteristics (P ranging from 0.000 to 0.002). The values observed for plant growth parameters were within the range reported earlier for the same rice variety (Singh et al., 1999).

3.3. Population of methanotrophs

The size of the methanotroph community ranged from 52.9 to 736.6 x 10⁵ cells g⁻¹ in control and from 43.8 to 676.0 x 10⁵ cells g⁻¹ in fertilized soils (Table 3). There is a lack of information on the population size of methanotrophs in dryland rice fields. Joulian et al. (1997) sampled 22 rice paddies at the end of the crop cycle from five different countries and found that the methanotroph population ranged from 1.5 x 10⁵ to 3.5 x 10⁷ cells g⁻¹. The overall range in the population size observed in this dryland rice field is thus higher than the range reported for paddy soils. The present range of population size is also higher than the range (2.4 x 10⁵–3.6 x 10⁶ cells g⁻¹) reported for a variety of soils from cultivated and natural ecosystems (Bender and Conrad, 1992).

Our results indicated that the population size decreased significantly in plots amended with urea (F₁, 26 = 40.33, P = 0.000). Bender and Conrad (1994) demonstrated that population size of methanotrophs was lower in rice paddy soils incubated with NH₄⁺ (1.1 x 10⁵–2.9 x 10⁵ counts g⁻¹ dw) than in control soil (2.2 x 10⁵–8.0 x 10⁵ counts g⁻¹ dw), indicating that NH₄⁺ inhibits the soil methanotrophs. A decline in methanotroph population in fertilized plots is in accord with the observation that CH₄ consumption was lower in fertilized than in unfertilized dryland rice plots (Singh et al., 1998a, 1999). The inhibitory effect of NH₄⁺ on methane oxidation has also been demonstrated by Bronson and Mosier (1994), King and Schnell (1994), Hutsch et al. (1996) and Gulledge et al. (1997).

There was an inverse relationship between the size of methanotroph population and NH₄⁺-N concentration in soil (Table 4). However, according to this relationship, variability in NH₄⁺-N concentration accounted for only 38% of the variability in the population size of methanotrophs, indicating involvement
of additional factors. Plant biomass was much higher in the urea fertilized treatment which can lead to higher carbon input and thus higher oxygen consumption by heterotrophs. Although there is no empirical evidence from this study, oxygen could well be a limiting factor, particularly during periods of high soil moisture.

Our data indicate that the population size of methanotrophs was significantly higher \( \left( F_{1, 26} = 4.89, P = 0.036 \right) \) on 90 DAS as compared to 40 DAS in both the control and fertilized soils. Such a difference in population size may be related to soil moisture since it was higher on 40 DAS \( \left( 8 = 169.2 \text{ mg g}^{-1} \text{ soil} \right) \) than on 90 DAS \( \left( 8 = 88.7 \text{ mg g}^{-1} \text{ soil} \right) \) which, in turn, affected the amount of aeration. Negative exponential relationships between \( \text{CH}_4 \) oxidation rates and soil moisture have been reported for natural ecosystems \( \left( \text{Singh et al., 1997, 1998a, 1999} \right) \) as well as for dryland rice agriculture \( \left( \text{Singh et al., 1999} \right) \). The differences in the rhizospheric methanotroph population size between 40 DAS and 90 DAS could be related to plant development, more specifically to aerenchyma development with age. Frenzel and Gilbert \( \left( \text{1998a, 1998b} \right) \) also observed an increase in cell numbers of methanotrophs with time in the rice rhizosphere.

In this study there were significant differences \( \left( F_{2, 26} = 550.63, P = 0.000 \right) \) in methanotroph population size among the rhizosphere, bulk and bare soils (Table 3). The lowest population size occurred in bare soil \( \left( 43.8–67.5 \times 10^5 \text{ cells g}^{-1} \text{ soil} \right) \) and the highest in rhizospheric soil \( \left( 499.8–736.6 \times 10^5 \text{ cells g}^{-1} \text{ soil} \right) \). The bulk soil had an intermediate population size \( \left( 451.4–684.1 \times 10^5 \text{ cells g}^{-1} \text{ soil} \right) \). Differences in methanotroph population size between bulk and rhizosphere soils were also reported by Espiritu et al. \( \left( \text{1997} \right) \) for a wetland rice field. Rice plant roots had a stimulatory effect on total soil bacteria including methanotrophs, and the number of methanotrophs was one order of magnitude higher in the rhizosphere than in bulk soil \( \left( \text{Gilbert and Frenzel, 1995} \right) \). The association of methanotrophs with roots \( \left( 5 \times 10^4–8 \times 10^5 \text{ CFU g}^{-1} \text{ dry matter} \right) \) and stem bases \( \left( 7 \times 10^4–2 \times 10^5 \text{ CFU g}^{-1} \text{ dry matter} \right) \) in two wetland rice varieties was observed by Watanabe et al. \( \left( \text{1997} \right) \), who concluded that methane oxidizing activity was mainly associated with roots. Evidently the increased number of methanotrophs in the vicinity of rice plant roots is most probably the result of a higher \( \text{O}_2 \) partial pressure due to aeration of the rhizosphere. In an elegant microcosm study simulating flooded rice field, Frenzel and Gilbert \( \left( \text{1998} \right) \) found that active \( \text{CH}_4 \) oxidizing bacteria \( \left( \text{MOB} \right) \) occurred near to the root mat, and that in the bulk soil no \( \text{O}_2 \) was detected below 2 mm depth. In the present dryland rice field the MOB population was much higher in the bulk soil compared to the bare soil, indicating that the bulk soil was not entirely free from the influence of roots.

The soil of dryland rice field also gets periodically saturated due to heavy rainfall events when \( \text{CH}_4 \) emission instead of net consumption occurs \( \left( \text{Singh et al., 1998a, 1999} \right) \). Under such a situation, the \( \text{O}_2 \)-supplying potential of plant roots is a major factor for the multiplication, growth and sustenance of methanotrophic bacteria in the rhizosphere. The aerenchymatous tissue of rice plant serves as a conduit to transport \( \text{CH}_4 \) from the anoxic soils to the atmosphere \( \left( \text{Mariko et al., 1991} \right) \) and oxygen from the atmosphere to the rhizosphere \( \left( \text{Frenzel et al., 1992} \right) \). The supply of both \( \text{CH}_4 \) and oxygen would thus be more favorable for the

### Table 3
Distribution of methanotroph population size \( \left( 10^5 \text{ cells g}^{-1} \text{ soil} \right) \) in rhizosphere, bulk and bare soils from control and fertilized \( \left( 100 \text{ kg N ha}^{-1} \right) \) plots on dryland rice field planted to the variety Narendra-118, determined on two sampling dates \( \left( \text{DAS} = \text{days after sowing} \right) \) (mean \( \pm 1 \text{ S.E.} \))

<table>
<thead>
<tr>
<th>DAS</th>
<th>Rhizosphere</th>
<th>Bulk</th>
<th>Bare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>fertilized</td>
<td>control</td>
</tr>
<tr>
<td>40</td>
<td>691.0 ± 5.6</td>
<td>499.8 ± 8.6</td>
<td>579.5 ± 6.2</td>
</tr>
<tr>
<td>90</td>
<td>736.6 ± 19.3</td>
<td>676.0 ± 5.5</td>
<td>684.1 ± 4.8</td>
</tr>
</tbody>
</table>

### Table 4
Relationship between population size of methanotrophs \( \left( Y, 10^5 \text{ cells g}^{-1} \text{ soil} \right) \) and \( \text{NH}_4^+ - \text{N} \) concentration \( \left( X, \mu \text{g g}^{-1} \text{ soil} \right) \) in a dryland rice field, according to \( Y = a + bX \)

<table>
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<tr>
<th>DAS</th>
<th>Regression parameters</th>
<th>Significant statistics</th>
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<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>40 (n = 18)</td>
<td>771.8</td>
<td>−59.1</td>
</tr>
<tr>
<td>90 (n = 18)</td>
<td>891.8</td>
<td>−80.8</td>
</tr>
<tr>
<td>Pooled for both DAS (n = 36)</td>
<td>825.3</td>
<td>−67.8</td>
</tr>
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</table>
methanotroph population to develop in rhizosphere than in the bulk or bare soil. The view that supply of both CH4 and O2 is essential for methanotroph population is supported by the findings that the population size in paddy soils exposed to air enriched with 20% methane, increased to 2.3 × 10^6 cells g^-1 in comparison to control soil (4.2 × 10^6 cells g^-1) (Bender and Conrad, 1992). Singh et al. (1998a, 1999) found that plant variables, specially the number of tillers, root volume and root porosity, representing the conduit and ventilation effects were important for CH4 oxidation in dryland rice agriculture.

In conclusion, the development of the rhizosphere brings about a spatial pattern in the distribution of methanotrophic population which increases in size during the vegetative period, and within the rhizosphere and adjoining bulk soil as compared to the bare soil. Greater O2 availability due to ventilation by rice plants, lower concentrations of NH4^+-N due to continuous plant uptake and a larger methanotroph population make the rhizosphere a microsite for intense CH4 oxidation activity. We thus demonstrate that plant, plant age and fertilization affect MOB in dryland rice field.

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References


