CH_4 AND N_2O FLUX FROM ANDOSOL WITH DIFFERENT FORMS OF APPLIED FERTILIZER AND CHARACTERISTICS OF METHANOTROPHIC BACTERIA

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ABSTRACT
Methane and nitrous oxide emissions were measured in Andosol fields with application of ammonium sulphate at 150 kg N ha^{-1} yr^{-1} (CF), cow manure compost at 150 kg N ha^{-1} yr^{-1} (CM), or without fertilizer (Con) for two years. CH_4 flux was always negative during the experiment. The average methane uptake rates of Con, CM, and CF were -0.47, -0.62 and -0.68 mg C $m^{-2}d^{-1}$, respectively. Methane uptake rates decreased over three weeks after fertilization, as compared to the rates before applications. On the other hand, N_2O emission increased immediately after fertilizer application. The CH_4 uptake and N_2O emission corresponded with changes in the soil water potential, soil temperature (10cm). The total amount of N_2O emissions from Con, CM and CF were 34.92, 80.17 and 93.58 mg N m^{-2}, respectively, and corresponded to 0.62, 0.53% of applied N in CM and CF, respectively.

In the CM and Con, methanotrophic population level was 10^3 g^{-1}, but in the CF, it was 10^2 g^{-1}. It was therefore suggested that methanotrophic bacteria in soil decreased with the application of chemical fertilizer. The result of PCR to identify the genus of methanotrophic (methane oxidizing) bacteria based on the system classification by 16S rRNA using specific primar, identified the strain isolated from all experiments plots to be genus *Methylococcus*.

INTRODUCTION

Methane (CH_4) and nitrous oxide (N_2O) attract attention as important greenhouse gases, and arable soil is considered as CH_4 sink and N_2O source

(IPCC, 1996). However, it is thought that various factors contribute to CH_4 uptake and N_2O emission. Fertilizer application is known to affect the behavior of these gases. In order to clarify the mechanisms and factors affecting the dynamics of these gases in soil, field experiments applied with different fertilizer forms were performed using Andosol which is dominant upland soil in Japan (Ministry of Agriculture, Fishery and Forestry, 1991). We also tried to isolate and identify the species of methanotrophic bacteria in the soil.

MATERIALS AND METHOD

FIELD SITE

The experiment was performed in an arable field of the Faculty of Horticulture, Chiba University, Matsudo, Japan. The soil is classified as a light-coloured Andosol. The experimental treatments consisted of N fertilizers and cow manure compost as follows: ammonium sulphate at 150 kg N ha^{-1} yr^{-1} (CF); cow manure compost at 150 kg N ha^{-1} yr^{-1} (CM); and no fertilizer (Con). These treatments were applied yearly to the same plots in May or June. In 2002, the treatments were applied on 14 May. The result presented in this report was from October 2001 to September 2002. Total organic C, N and CEC of the soils were 497, 38.2, 22.2 (Con), 502, 38.4, 24.7 (CF) and 511, 39.9, 25.7 (CM) mgC kg^{-1}, mgN kg^{-1} and cmol(+) kg^{-1} respectively.

CH_4 AND N_2O FLUX

CH_4 and N_2O flux were measured by the closed chamber method. Gas sample in the chamber was collected by 30 mL plastic syringe at 0, 15, 30 minutes, after chamber closed. Amount of CH_4 and N_2O concentration were measured by gas chromatograph (Shimadzu, GC-14B) with flame ionization detector (FID) and electron capture detector (ECD), respectively (Inubushi et al., 1996).

SOIL WATER POTENTIAL

Measurement of soil water potential was performed using tensiometer. This was laid to a soil depth of 20 and 40cm in the chemical fertilizer plot, and soil moisture potential was measured at the time of gas sampling.

NH_4^+-N AND NO_3^--N CONTENTS IN THE EACH EXPERIMENT PLOTS

Soil samples for analyzing NH_4^+-N and NO_3^--N were taken from the cultivation layer (0-15cm) of each plot at the same time as gas sampling. All the soil samples were sieved (<2mm) in the laboratory and sub-samples extracted with

1M KCl solutions for 30 min. NH_4^+-N and NO_3^--N contents in soil were determined colorimetrically by the nitroprusside and hydrazine-reduction methods, respectively (Anderson et al. 1989, Hayashi et al. 1997).

METHANOTROPHIC POPULATION, ISOLATION AND IDENTIFICATION OF METHANOTROPHIC BACTERIA

Two layers (0-10, 10-20cm) of soils of each treatment plot were collected, and the number of methanotrophic bacteria present was measured by the most probable number method using culture medium with methane as the only source of carbon. Sequential agar plate incubations of methanotrophic bacteria in culture solution of the highest order of dilution in which CH_4 oxidation was recognized was performed. The methanotrophic bacteria was finally isolated and PCR was performed to identify the genus of methanotrophic bacteria based on the system classification by 16S rRNA using specific a primer (MacDonald et al. 1996).

RESULTS AND DISCUSSION

Figure 1 shows the time-courses of CH_4 and N_2O fluxes from Andosol applied with different fertilizer forms. Since CH_4 flux was always negative during the experiment, it was suggested that CH_4 is taken by soil from the atmosphere. The average methane uptake rates of Con, CM, and CF were -0.47, -0.62 and -0.68 mg C $m^{-2}d^{-1}$, respectively. Both the chemical fertilizer and cow manure compost were able to influence methane flux, but to different extents. Methane uptake rates decreased over three weeks after fertilization, as compared to the rates before applications. On the other hand, N_2O emission increased immediately after fertilizer application. This was mostly in agreement with the period of nitrification as can be seen from the amount of reduction in the amount of the NH_4^+-N in the soil (Figure 2). The CH_4 uptake and N_2O emission corresponded with changes in the soil water potential, soil temperature (10cm) (Figure 3). The total amount of N_2O emissions from Con, CM and CF were 34.92, 80.17 and 93.58 mg N m^{-2}, respectively, and corresponded to 0.62, 0.53% of applied N in CM and CF, respectively.

Suppression of methane uptake by chemical fertilizer has been reported in Europe for non-Andosol by Hürsch et al. (1996) and Hürsch (1996). The reports discussed possible reasons as the methanotrophic (methane oxidizing) bacteria may be suppressed by nitrifying bacteria directly or indirectly. In our experiment, methane flux in CF plot can be expressed as;

$$\text{Methane flux} = (0.04 \times \log(NH_4^+\text{-N})) + (0.003 \times \text{nitrification activity}) + (0.007 \times \text{liquid phase\%}) - 0.86 \quad (R=0.76,\ p<0.01)$$

where nitrification activity was the rate of ammonium decrease (mgN kg^{-1} d^{-1}).

It is well-documented that application of chemical fertilizer enhances nitrous oxide emission (Bouwman, 1990; Thornton and Valente, 1996) which is produce mostly by nitrification from applied ammonium in aerobic arable soil. In our experiment, not only chemical fertilizer but also cow manure compost enhanced nitrous oxide emission. When largest peak was observed, ammonium content in soil was very low in CM plot, indicating that denitrification took place after heavy rain to produce nitrous oxide in anaerobic microsite of Andosol. In our experiment, nitrous oxide emission in CF plot can be expressed as;

$$N_2O \text{ flux} = (0.04 \times WFPS) + (0.04 \times \text{air temp}) + (0.12 \times \log(NO_3^- \text{-N})) - 2.9$$

(R=0.64, <0.05)

where WFPS as water filled pore space (%), indicating that soil moisture, temperature and nitrate concentration in soil were determining factors of nitrous oxide emission from this plot.

Figure 4 shows the methanotrophic population in Andosol applied with the different treatments. In the CM and Con, methanotrophic population level was 10^3 g^{-1}, but in the CF, it was 10^2 g^{-1}. It was therefore suggested that methanotrophic bacteria in soil decreased with the application of chemical fertilizer. The result of PCR to identify the genus of methanotrophic bacteria based on the system classification by 16S rRNA using specific primar, identified the strain isolated from all experiments plots to be probably genus *Methylococcus* (Figure 5).

Methane oxidizing bacteria has two types; Type I classifies gamma proteobacteria with *Methylomonas, Methylobacter, Methyloccocus* and *Methylomicrobium*, while type II as alpha proteobacteria with *Methylosinus, Methylocystis*, and additionally type X is thermophilic *Methylococcus capsulatus*-like bacterium (Hanson et al., 1996). Type I adapt to low methane and high oxygen condition, while type II to high methane and low oxygen conditions. Fukunaga (2000) isolated *Methylococcus* from various ecosystems like forest soil, temperate and tropical paddy soils and peat wetland. Our results agreed this observation and suggest that fertilization had no significant influence on microflora of methanotrophs in Andosol.

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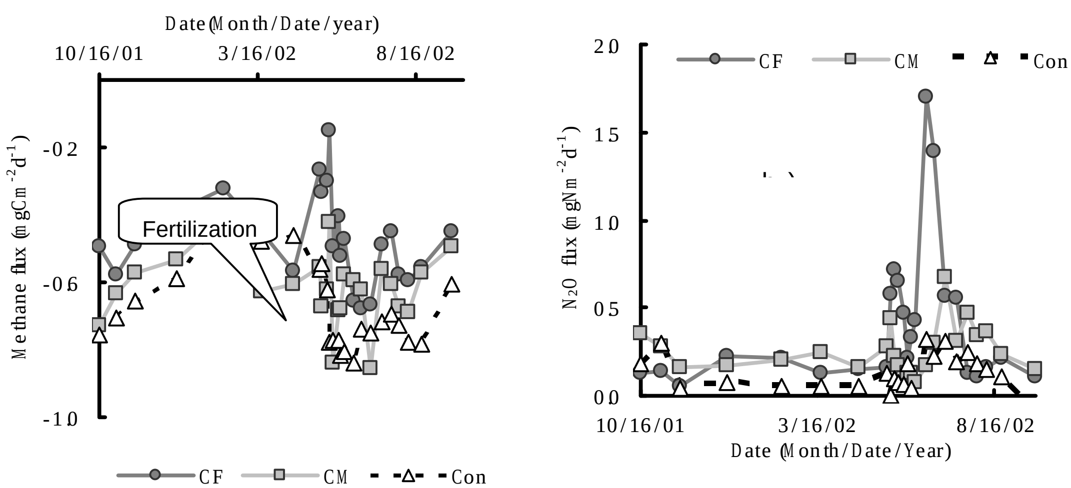
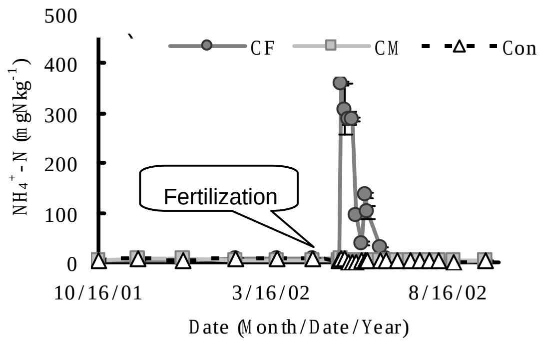


Fig 1. Time-courses of (a) CH_4 and (b) N_2O fluxes from Andosol applied with different forms of fertilizers



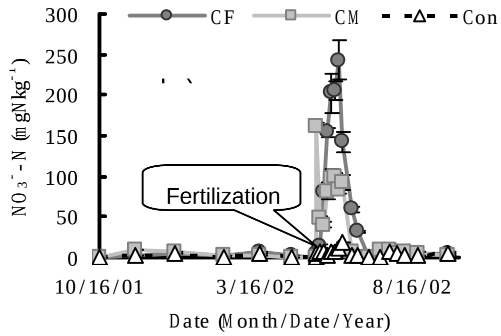


Fig 2. Time-courses of (a) NH_4^+-N and (b) NO_3^--N contents in surface soil (0-15cm)

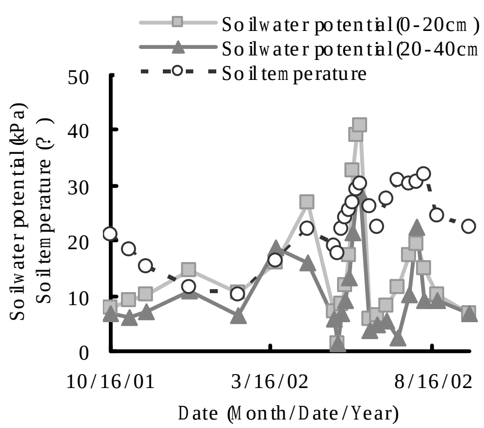


Fig 3. Time-courses of soil water potential and soil temperature

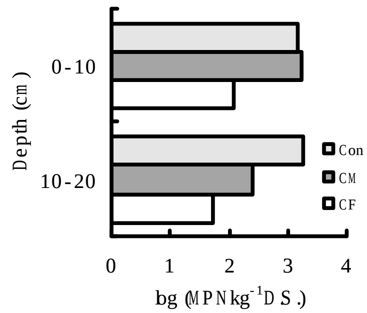


Fig 4. Methanotrophic population in Andosol applied with different forms of fertilizers

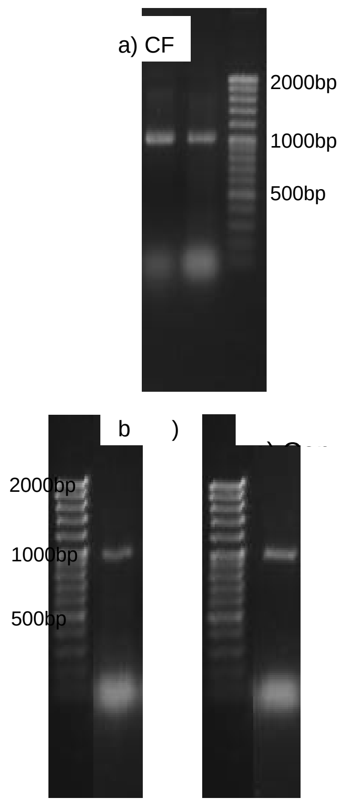


Fig 5. PCR amplification of DNA obtained from methanotrophic bacteria isolated from Andosol applied with different fertilizer forms. The primer was used Mc1005r.