Influences of Chemical Fertilizers and a Nitrification Inhibitor on Greenhouse Gas Fluxes in a Corn (*Zea mays* L.) Field in Indonesia

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The influences of chemical fertilizers and a nitrification inhibitor on greenhouse gas fluxes (N_2O and CH_4) in a corn field in Indonesia were investigated using a closed chamber. Plots received 45+45 kg-N ha^{-1} of nitrogen fertilizer by split applications of urea, a single application of controlled-release fertilizer (CRF-LP30) or urea+dicyandiamide (DCD; a nitrification inhibitor), and no nitrogen application (control). Cumulative amounts of N_2O emitted from the field were 1.87, 1.70, 1.06, and 0.42 kg $\text{N}_2\text{O-N ha}^{-1}$ season⁻¹ for the urea, CRF-LP30, urea+DCD, and control plots, respectively. The application of urea+DCD reduced the emission of N_2O by 55.8% compared with urea. On the other hand, the soil acted as a sink for CH_4 in the CRF-LP30, control, and urea+DCD plots with value of –0.09, –0.06 and –0.06 kg $\text{CH}_4\text{-C ha}^{-1}$ season⁻¹, respectively. When the viability of AOB (ammonia-oxidizing bacteria) and NOB (nitrite-oxidizing bacteria) were monitored, AOB numbers were correlated with the N_2O emission. These results suggest that 1) there is a potential for reducing emissions of N_2O by applying DCD, and 2) corn fields treated with CRF or urea+DCD can act as a sink for CH_4 in a tropical humid climate.

Key words: Indonesia upland soil, nitrification inhibitor, nitrifier population

The use of nitrogen (N) fertilizers increases the amount of available N in the soil and improves the productivity of agricultural crops. However, up to about 60% of N fertilizers can be lost through leaching of mobile N compounds such as nitrate (NO_3^-) and evolution as nitrous oxide (N_2O) and dinitrogen (N_2). N_2O is a greenhouse gas (GHG), and also an ozone layer disrupting gas^{28,30}.

Nitrogen is a key limiting factor for the growth of crops as well as soil microorganisms, and the addition of N can affect microbial biomass, activity, and species composition^{4,19,31}. Most of the nitrogen fertilizers applied to soils are in the form of ammonium (NH_4^+) or ammonium-producing compounds such as urea and organic manure. Nitrification, the oxidation of NH_4^+ to NO_3^- by nitrifiers, affects the fate of N fertilizers. Nitrification in the soil is considered to be one of the main processes for increasing N_2O levels in the atmosphere and ammonia-oxidizing bacteria (AOB) appear to be important in this process.

About 90% of N_2O produced was derived from autotrophic nitrification in aerobic soil at 60–80% water holding capacity¹⁵. Meanwhile, AOB may also be involved in the oxidation of atmospheric methane in aerobic soils, since methane is an alternative substrate for ammonia monooxygenase^{3,18,23,33}. Therefore, upland soil is thought to contribute to the oxidation of CH_4 ²³. However, upland soils with low CH_4 production also exist, particularly when the soil becomes wet or there is ammonia at the surface rather than in the subsoil which may cause inhibition of CH_4

oxidation²³. Therefore, the application of nitrification inhibitors may have some influence on the oxidation of methane in soil.

Most AOB fall within one taxonomic group in the β -subclass of the Proteobacteria and seven clusters based on 16S rRNA gene sequences are currently recognized in this group²². The AOB utilized ammonium as a substrate to produce nitrite via the intermediate hydroxylamine. Two gases, NO and N_2O , are produced in smaller amounts relative to the amount of ammonia oxidized, but their release into the atmosphere has significant consequences³.

A potentially effective strategy for reducing emissions of N_2O would be the inhibition of nitrification and denitrification. The rates at which these processes emit N_2O depend on the amount of NH_4^+ and NO_3^- in the soil as well as other factors including soil water content, organic matter, temperature and pH^{17,19,24,28}. Therefore, it might be expected that, in the worst-case scenario, agricultural land should be managed intensively in a climate under both warm and wet soil conditions with frequent fluctuations between wet and dry.

Nitrification inhibitors have been used in the field to improve the efficiency of fertilizers and to reduce both nitrate leaching and denitrification by maintaining the N in the soil as NH_4^+ ^{9,34}. Dicyandiamide (DCD) acts as a nitrification inhibitor by inhibiting the first stage of the nitrification process, the oxidation of NH_4^+ to NO_2^- , and by rendering bacterial enzymes ineffective⁶. Therefore, DCD can be regarded as a slow release N fertilizer (containing about 65% N). In addition, DCD is non-toxic, non-hygroscopic, and non-volatile and therefore has been considered a suitable combination with solid fertilizers. Moreover, DCD is not a

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bactericide, and does not affect other heterotrophs that are responsible for much of the biological activity in soil¹¹).

Another possible way to improve efficiency is to use controlled release fertilizers (CRFs). In temperate regions, CRF can reduce emission of N₂O and NO shortly after fertilization. However, no significant differences in the total amounts of N₂O and NO emitted over 77 days was reported between CRF and urea¹³.

Little investigation, however, has been carried out of the relationship between emissions of GHGs and populations of nitrifiers in upland fields in tropical regions such as Indonesia. To address these issues, we conducted field studies to determine the influences of different types of N chemical fertilizers or nitrification inhibitors on the flux of N₂O and CH₄ and the population of nitrifiers during a cropping season in a corn field in Makassar, Indonesia.

Materials and Methods

Field site and soil analysis

Corn (*Zea mays* L.) seeds were sown in a well drained field of Makassar State University in Makassar, South Sulawesi, Indonesia (5 m asl., 05°11.221'S 119°25.750'E) on October 15, 2005 and harvested on January 8, 2006 or 83 days after 1st fertilization (DAF).

Three replicates of four treatments were laid out in a completely randomized design. The field has an area of around 48 m² (6 m wide×8 m long) and each microplot measures 1.8 m² (1.2 m wide×1.5 m long). The arrangement of the plots in the field is shown in Fig. 1.

The soil was classified as Haplusterts, which was described as an Alluvial soil type (Center for Soil and Agroclimate Research, Indonesia, 2000). Before this experiment started, the soil had a pH (H₂O) of 5.7, EC of 5.0 mS m⁻¹, total carbon content of 15.4 g-C kg⁻¹, total N content of 2.1 g-N kg⁻¹ dry soil, and C/N ratio of 7.32, measured by electrode methods and a C/N analyzer (MT 700 Yanaco, Kyoto, Japan), respectively. Soil moisture was determined by the oven drying method⁹. The average annual amount of rainfall and annual air temperature were around 2364 mm and 21.5–34.2°C, respectively. The wet season is generally October to March and the dry season is from April to September. The data on daily temperature and precipitation were collected from the bureau of Meteorological and Geophysical of Makassar, Indonesia. Average temperature during the experiments was 22–32°C.

The nitrogen fertilizer types applied in the field were urea, a controlled-release fertilizer (CRF-LP30; designed to release 80% of applied nitrogen at 20°C over at least 30 days, Chissoasahi Fertilizer Co., Ltd., Kumamoto, Japan), and Urea+dicyanodiamide [DCD; a nitrification inhibitor was mixed with urea at a rate of 10% of urea N (w/w)]. The rate of N applied in each treatment was 45+45 kg-N ha⁻¹ applied in two splits on October 15, and November 30, 2005. Plots without N application were also set up as a control.

Soil samples were taken from triplicate plots at a depth of 0–10 cm and sieved through 2.00 mm mesh to analyze the NH₄⁺ and NO₃⁻ contents of the soil. Five grams, on an oven dried basis, of fresh soil was weighed into a 50-ml plastic bottle with a screw cap. Soils were immediately extracted with 25 ml of 0.5 M K₂SO₄ (1:5) solution by shaking for 30 min on reciprocal shaker and filtered with filter paper (Advantec No. 6, Tokyo, Japan). The amounts of NH₄⁺ and NO₃⁻ were determined by the nitroprusside and hydrazine reduction methods, respectively^{2,12}.

The viable populations of nitrifiers; i.e. ammonium oxidizers (AOB) and nitrite oxidizers (NOB), were estimated by the most probable number (MPN) method³². The inoculants were prepared as follows; 10 grams of fresh soil and 90 ml of sterile phosphate buffer were placed in a sterile bottle (for each composite soil sam-

Fig. 1. Arrangement of experimental plots and position of the chamber in each plot. Corn was harvested on January 8, 2006 or 83 days after the first fertilization. C=Control, U=Urea, CRF=Control release fertilizer and U+DCD=Urea+DCD.

ple) and shaken vigorously on a wrist action shaker for 30 min. For dilutions of 10³–10⁷, five replicates each of the culture tubes were employed.

Means and standard deviations of the data were calculated. Each mean was compared with others using the least significant differences (LSD=0.05) value by SPSS software (Ver. 11.0 for windows, SPSS Inc., Chicago, USA).

Nitrous oxide and methane flux measurements

Gaseous fluxes were measured at two-day intervals throughout the cropping season using a closed chamber (inner dia. 21 cm; height 14 cm) (Fig. 1)¹⁶. The gas samples were taken from the chamber at 0, 15, and 30 minutes using disposable syringes and then immediately transferred to evacuated air glass vials with butyl rubber stoppers. The gaseous samples were taken between 10:00 and 12:00 a.m. The concentrations of N₂O and CH₄ in the samples were quantified using gas chromatographs (Shimadzu, GC 14B, Kyoto, Japan) equipped with an electron capture detector (ECD) and a flame ionization detector (FID), respectively¹⁶. N₂O and CH₄ fluxes were calculated from the change in N₂O and CH₄ concentrations over time, after accounting for diffusion constraints.

Cumulative N₂O and CH₄ fluxes per season from the field were obtained by integration of N₂O and CH₄ fluxes during the cropping season, respectively. Analyses were done in triplicate and means and standard deviations were calculated.

The percentage of N₂O-N lost per amount of N applied as fertilizer to the field (EF=emission factor) was calculated using the following equation⁷.

$$EF (\%) = (T_F - T_{UF}) / N \times 100$$

where: T_F and T_{UF} =Cumulative N₂O flux emitted from the nitrogen-applied plot and the non-nitrogen applied plot (control), respectively (kg N₂O-N ha⁻¹ season⁻¹) and N =Total nitrogen applied in the field (kg N ha⁻¹ season⁻¹).

The percent reduction of N₂O emitted from the fields with a nitrification inhibitor or CRF was calculated using the following

equation²⁶⁾.

$$\text{N}_2\text{O reduction (\%)} = (A - C) / (A - B) \times 100$$

where *A* is the cumulative N₂O emission in the urea plot, *B* is the cumulative N₂O emission in the control plot, and *C* is the cumulative N₂O emission with a nitrification inhibitor or in the CRF plots.

Results and Discussion

Nitrous oxide emissions

N₂O emissions were highest during the first 7 days after the fertilizer was applied and slowly declined thereafter (Fig. 2). The application of nitrogen fertilizers in the field increased the emission of N₂O. The maximum flux of N₂O increased to 0.9 mg N₂O-N m⁻² h⁻¹ two days after the first application of N fertilizer and then gradually decreased to around 0.05 mg N₂O-N m⁻² h⁻¹. However, on the second application, a maximum flux of around 0.19 mg N₂O-N m⁻² h⁻¹ appeared after 18 days in the urea plot. Meanwhile, the highest peak of N₂O flux was 0.14 mg N₂O-N m⁻² h⁻¹ at 14 days in the CRF plot and 0.17 mg N₂O-N m⁻² h⁻¹ at 22 days after the second application in the urea+DCD plot (Fig. 2).

Fig. 2 shows the substantial repression of N₂O flux from soils with the combination of nitrogen fertilizer and DCD under the experimental conditions. Based on the equation of Majumdar *et al.*²⁶⁾, urea+DCD reduced N₂O flux from the soil by 55.8% compared to the urea plot and by 11.7% compared to the CRF plot. The results suggest a potential for reducing N₂O emissions even from nitrogen-fertilized corn fields under tropical conditions where the annual temperature is in the range of 20–35°C.

Dobbie and Smith⁷⁾ confirmed urea+DCD plots effectively depressed the nitrification reaction in warmer periods (soil temperature of around 15°C) and suggested the use of DCD with urea to be an effective way to reduce emissions from intensive grassland even under cooler conditions (soil temperature of around 4–6°C).

Akiyama and Tsuruta¹⁾ reported that CRF reduced N₂O emissions compared to urea, when there was heavy rainfall just after the fertilization. Therefore, seasonal changes of N₂O emission are highly dependent on precipitation and soil moisture (Fig. 2).

Many studies^{1,7,13–16)} show that different types of nitrogen fertilizers and soil moisture conditions influence temporal

Fig. 2. Change of precipitation, soil moisture, N₂O, and CH₄ flux from the corn field affected by different nitrogen fertilizers during the cropping season (October 2005 to January 2006). Vertical bars indicate $\pm$ standard deviation. Corn was harvested on January 8, 2006 or 83 days after the first fertilization. See Fig. 1 for abbreviations of symbols.

patterns of N_2O emissions. In our study, the flux of N_2O from a corn field was affected by the nitrogen fertilizers applied and also precipitation which directly changed the soil moisture content (Fig. 2). The emission of N_2O was greater just after the first fertilization when much precipitation and a high content of soil moisture were observed. Also plant N uptake might be weak just after 1st fertilization to compare with 2nd fertilization. Therefore, the N_2O flux peaked immediately after the first fertilization. Meanwhile, in the second fertilization, the precipitation and soil moisture were lower and the N_2O flux increased gradually after the rain started about 50–55 DAF. N_2O flux from the urea+DCD plot started to increase later than from other fertilized plots, indicating that inhibition by DCD of N_2O production became less effective in that period. Akiyama and Tsuruta¹⁾ also reported that the reduction of N_2O emissions by DCD was effective only in aerobic conditions, partly due to the greater contribution of denitrification to N_2O production under wet conditions¹⁵⁾.

Soil water content before and after the rainfall presumably influenced the porosity and consequently increased the possibility of denitrification and also the diffusion of N_2O out of the soil¹⁵⁾. Inubushi *et al.*¹⁵⁾ reported that at a water-holding capacity of 100%, an enormous amount of N_2O was produced rapidly in Andosols, mostly by denitrification. Khalil *et al.*²¹⁾ also suggested that rainfall and nitrogen input mainly regulated the amount of N_2O emitted from an upland cropping system in the humid tropics of Malaysia. In addition, several reports^{24,28,35)} indicate nitrogen fertilizers to be one of the dominant sources of N_2O emitted from agricultural soils as shown by our previous results¹⁹⁾.

The cumulative N_2O emission per cropping season (October 17, 2005 to January 5, 2006) was highest in the urea plot at 1.87 kg N_2O -N ha^{-1} season⁻¹, followed by the CRF-LP30 plot at 1.70 kg N_2O -N ha^{-1} season⁻¹. Urea+DCD and control plots emitted 1.06 and 0.42 kg N_2O -N ha^{-1} season⁻¹, respectively. The urea plot had an emission factor (EF) of 1.61%, compared to 1.42% in the CRF-LP30 plot and 0.71% in the urea+DCD plot.

The emission factor in soil from an Andisol amended with chemical fertilizers in a temperate region in Japan ranged from 0.05 to 0.32%¹⁾. These emission factors were slightly low compared with our results. In contrast, in acid oxisol in Puerto Rico, where a chemical fertilizer was also applied, emission factors ranged from 0.5 to 5%²⁹⁾. However, Watanabe *et al.*³⁵⁾ reported that the application of N fertilizers in corn fields at four sites in Thailand at a rate of around 47–75 kg N ha^{-1} emitted N_2O gas at 0.1 to 0.4%. Mahmood *et al.*²⁵⁾ suggested that the emission factors from an irrigated sandy-clay loam soil in a maize-wheat cropping system in subtropical Pakistan were around 30 to 45%. Therefore, these emission factor values are mostly dependent on management practices, fertilizer types, and climatic and soil conditions^{1,5,7,29,30)}.

Methane flux

Initial measurements of CH_4 (0–10 days) flux ranged from 0.05 to 0.13 mg m^{-2} h^{-1} across all treatments (Fig. 2). However after 10 days, no detectable amounts of CH_4 were observed with any of treatments except urea in which the cumulative emission was 0.06 kg CH_4 -C ha^{-1} season⁻¹. The

corn field acted as a sink for CH_4 in the CRL, Control, and urea+DCD plots with value of -0.09 , -0.06 and -0.06 kg CH_4 -C ha^{-1} season⁻¹, respectively.

Slight CH_4 production seemed to temporarily appear as positive peaks in the corn field soil, particularly in fertilized soil plots (Fig. 2). Le Mer and Roger²³⁾ suggested that urea and ammonium usually inhibit atmospheric CH_4 oxidation due to competition at the level of nitrification, a transfer of CH_4 -oxidizing activity towards nitrification, and the toxicity of N_2O produced. However, cumulative CH_4 fluxes throughout seasonal measurements in the corn field were also negative, indicating the soil acted mostly as a sink for CH_4 .

The uptake of atmospheric CH_4 in upland soil is affected by a number of field management practices such as tillage and N-application. In this study, chemical fertilizer reduced CH_4 uptake in the urea plot where a positive CH_4 flux was observed. Delmas *et al.*⁵⁾ also reported a reduced CH_4 sink strength in agricultural fields compared to savanna soil in the Congo. Flux rates in the Savanna averaged -0.46 μg CH_4 -C m^{-2} h^{-1} while in agricultural soils they averaged around -0.33 μg CH_4 -C m^{-2} h^{-1} . Mosier *et al.*²⁹⁾ reported a sink of CH_4 flux averaging -1.2 μg CH_4 -C m^{-2} h^{-1} in a sorghum field in a Oxisol soil near Isabela, Puerto Rico.

Our results indicated that the corn field could be a sink for CH_4 gas. However, more study is needed to investigate the interactive effects of nitrification inhibitors on N_2O and CH_4 flux in different crops and land types in the tropics.

Population of nitrifiers and NH_4^+ and NO_3^- contents of soil

AOB (ammonia-oxidizing bacteria) and NOB (nitrite-oxidizing bacteria) populations were counted 4 times during the first half of the crop season (Fig. 3). The number of nitrifiers in all plots at first measurement 16 days after the first application (31/10/05) was around 1×10^3 cells g^{-1} DS (dry soil) and slightly increased afterward particularly in the plots amended with fertilizer. However, they were still not significantly different from each other until 30 days after the fertilizers were applied.

The AOB counts peaked at the third measurement (50 days after fertilization) in the urea and CRF plots, while NOB tended to increase gradually in all fertilized plots. At 50 and 64 days, the number of NOB was significantly higher in soil amended with fertilizer. However, at the same time, the number of AOB did not differ between the control and urea+DCD plots, while it was significantly higher in the urea and CRF plots.

During corn cropping, nitrifiers seemed to be stimulated in the fertilizer-treated soil possibly as a result of the amount of inorganic nitrogen in the soils since fertilizer-treated soil had relatively high NH_4^+ and NO_3^- contents compared to unfertilized soil (Fig. 4).

The 1st application of N fertilizers increased the concentrations of NH_4^+ and NO_3^- in the soil (Fig. 4). The level of NO_3^- 4 and 20 days after first fertilization was significantly higher in the urea plot than the other plots, but no differences in NH_4^+ content were found during that time. However, in the last measurements before the second fertilization (28 and 36 days after first fertilization), the level of NH_4^+ was significantly higher in the CRF-LP30 plot than in the other plots, while the NO_3^- concentration did not differ significantly

Fig. 3. Changes of the viable population of nitrifiers during the cropping season. Vertical bars indicate $\pm$ standard deviation. Same letters are not significantly different at 5% LSD. AOB (ammonia-oxidizing bacteria) and NOB (nitrite-oxidizing bacteria). See Fig. 1 for other abbreviations of symbols.

among plots.

The decrease in the NH_4^+ content of the soil was accompanied by an increased in NO_3^- content. The NO_3^- in the soils appeared within 4 days after urea was applied and this result was supported by Venterea and Rolston³⁴) who found NO_3^- accumulated within 2 days in clay soil and the level of NO_3^- increased over the first 4 days of incubation.

The concentration of NH_4^+ remained higher in the CRF plot on 28 and 36 DAF compared to the other soils. Hou and Tsuruta¹⁴) reported that CRF increased the amount of NH_4^+ in an Andosol following fertilization and the level decreased afterwards. This delay could be partly explained by the hypothesis that a certain amount of time is needed for this fertilizer to release NH_4^+ and diffuse into the surrounding soil.

Urea+DCD seemed to have no impact on the NH_4^+ content of the soil before the second fertilization while it reduced the NO_3^- content compared to urea alone 4 and 20 days after the first fertilization (Fig. 4). Francis *et al.*⁹) found that the application of DCD resulted in a greater amount of NH_4^+ at a depth of 1–2 cm and a lesser amount of NO_3^- at 1–4 cm in wheat leguminous pasture. The reason for the discrepancy in our experimental results is not clear, but DCD might need longer than 40 days to increase the NH_4^+ content under these experimental conditions.

Positive correlations were observed between the cumulative emission of N_2O and AOB counts at 64 DAF ($R^2=0.854$, $P<0.05$) (Fig. 5). However, early on, the number of AOB was not significantly different probably due to delayed growth and also AOB have slower growth characteristic, therefore at that time the number was similar to the indigenous number

Fig. 4. Change of NH_4^+ and NO_3^- in the corn field affected by different nitrogen fertilizers during the cropping season. Vertical bars indicate $\pm$ standard deviation. At each sampling time for each soil, means followed by the same letter are not significantly different at 5% LSD. See Fig. 1 for abbreviations of symbols.

of AOB in the field. The results were supported by Martikainen²⁷) who reported that the numbers of AOB and NOB in pine forest soil increased on amendment with urea alone or together with apatite+biotite and with apatite+biotite+micronutrients. In addition, he also found that there was a correlation between the number of AOB and $\text{NH}_4^+\text{-N}$ content in these pine forest soils.

The population of NOB was significantly larger in the fertilizer-treated plots than control plot 50 and 64 DAF, nearly at the end of cropping. NOB have been known to have slower growth rates in response to nitrogen amendment compared with the AOB population^{10,20}). As Venterea and Rolston³⁴) reported, during nitrification, residual NO_2^- appeared and could induce the nitrifier population particularly NOB which preferentially grew where NO_2^- was adsorbed as primary substrate.

Fukumoto *et al.*¹⁰) reported a correlation of AOB and NOB numbers on the production of N_2O and NO_2 during pig manure composting, and suggested that the addition of NOB after the thermophilic phase of composting reduced N_2O production and also the number of AOB compared to no addition of NOB. Moreover, the continuous culture of AOB and NOB together strongly reduced N_2O production as an effect of the lower NO_2^{20}).

The results of the present study showed that the type of nitrogen fertilizer influenced the emissions of N_2O and CH_4 from the corn field and affected the population of nitrifiers in the soil. Therefore, further detailed studies are needed to assess the structural communities of nitrifiers as affected by the application of different types of fertilizers in tropical field soils.

Fig. 5. Relationship between cumulative N₂O flux and AOB (ammonia-oxidizing bacteria) counts 64 days after the first fertilization.

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References

- Akiyama, H., and H. Tsuruta. 2002. Effect of chemical fertilizer form on N₂O, NO and NO₂ flux from an Andisol field. *Nutr. Cycl. Agroecosyst.* **63**:219–230.
- Anderson, J.M., and J.S.I. Ingram. 1989. *Tropical Soil Biology and Fertility*, pp. 42–43. Colometric determination of ammonium. ISSS, CAB International, Wallingford.
- Arp, J.D., and L.Y. Stein. 2003. Metabolism of inorganic N compounds by ammonium-oxidizing bacteria. *Critic. Rev. Biochem. Mol. Biol.* **38**:471–495.
- Crecchio, C., M. Curci, R. Mininni, P. Ricciuti, and P. Ruggiero. 2001. Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. *Biol. Fertil. Soils* **34**:311–318.
- Delmas, R., D. Serca, and C. Jambert. 1997. Global inventory of NO_x sources. *Nutr. Cycl. Agroecosyst.* **48**:51–60.
- Di, H.J., and K.C. Cameron. 2002. The use of nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. *Soil Use Manage.* **18**:395–403.
- Dobbie, K.E., and K.A. Smith. 2003. Impact of different form of N fertilizer on N₂O emissions from intensive grassland. *Nutr. Cycl. Agroecosyst.* **67**:37–46.
- Foster, J.C. 1995. Soil physical analysis, pp. 105–106. In K. Alef and Nannipieri (ed.), *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London.
- Francis, G.S., R.J. Haynes, T.W. Speir, and P.H. Williams. 1995. The effect of a nitrification inhibitor on leaching losses and recovery on mineralized nitrogen by a wheat crop after ploughing-in temporary leguminous pasture. *Fert. Res.* **41**:33–39.
- Fukumoto, Y., K. Suzuki, T. Osada, K. Kuroda, D. Hanajima, T. Yasuda, and K. Haga. 2006. Reduction of nitrous oxide emission from pig manure composting by addition of nitrite-oxidizing bacteria. *Environ. Sci. Technol.* **40**:6787–6791.
- German-Bauer, M.P., and A. Amberger. 1989. Degradation of the nitrification inhibitor 1-amidino-2-thiourea in soils, and its action in *Nitrosomonas* pure culture and soil incubation experiments. *Fert. Res.* **19**:13–19.
- Hayashi, A., K. Sakamoto, and T. Yoshida. 1997. A rapid method for determination of nitrate in soil by hydrazine reduction procedure. *Jpn. J. Soil Sci. Plant Nutr.* **68**:322–326 (In Japanese).
- Hou, A.X., H. Akiyama, Y. Nakajima, S. Sudo, and H. Tsuruta. 2000. Effects of urea form and soil moisture on N₂O and NO emissions from Japanese Andosols. *Chemosphere: Global Sci. Change.* **2**:321–327.
- Hou, A.X., and H. Tsuruta. 2003. Nitrous oxide and nitric oxide fluxes from an upland field in Japan: effect of urea, placement, and crop residues. *Nutr. Cycl. Agroecosyst.* **65**:191–200.
- Inubushi, K., H. Naganuma, and S. Kitahara. 1996. Contribution of denitrification and autotrophic and heterotrophic nitrification to nitrous oxide production in andosols. *Biol. Fertil. Soils* **23**:292–298.
- Inubushi, K., Y. Furukawa, A. Hadi, E. Purnomo, and H. Tsuruta. 2003. Seasonal change of CO₂, CH₄ and N₂O fluxes in relation to land-use change in tropical peatlands located in coastal area of South Kalimantan. *Chemosphere* **52**:603–608.
- Inubushi, K., and S. Acquaye. 2004. Role of microbial biomass in biogeochemical processes in paddy soil environments. *Soil Sci. Plant Nutr.* **50**:793–805.
- Jiang, Q.Q., and L.R. Bakken. 1999. Nitrous oxide and methane oxidation by different ammonium-oxidizing bacteria. *Appl. Environ. Microbiol.* **65**:2679–2684.
- Jumadi, O., Y. Hala, and K. Inubushi. 2005. Production and emission of nitrous oxide and responsible microorganisms in upland soil in Indonesia. *Soil Sci. Plant Nutr.* **51**:693–696.
- Kester, R.A., W.D. Boer, and H.J. Laanbroek. 1997. Production of NO and N₂O by pure culture of nitrifying and denitrifying bacteria during change in aeration. *Appl. Environ. Microbiol.* **63**:3872–3877.
- Khalil, M.I., O.V. Cleemput, A.B. Rosenani, and U. Schmidhalter. 2007. Daytime, temporal, and seasonal variations of N₂O emissions in an upland cropping system of the humid tropics. *Comm. Soil Sci. Plant Anal.* **38**:189–205.
- Kowalchuk, G.A., and J.R. Stephen. 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annu. Rev. Microbiol.* **55**:485–529.
- Le Mer, J., and P. Roger. 2001. Production, oxidation, emission and consumption of methane by soil: A review. *Eur. J. Soil Biol.* **37**:25–50.
- Li, X., K. Inubushi, and K. Sakamoto. 2002. Nitrous oxide concentration in an Andisol profile and emissions to the atmosphere as influenced by the application of nitrogen fertilizers and manure. *Biol. Fertil. Soils* **35**:108–113.
- Mahmood, T., K.A. Malik, S.R.A. Shamsi, and M.I. Sajjad. 1998. Denitrification and total N losses from an irrigated sandy-clay loam under maize-wheat cropping system. *Plant Soil* **199**:239–250.
- Majumdar, D., H. Pathak, S. Kumar, and M.C. Jain. 2002. Nitrous oxide emission from a sandy loam inceptisol under irrigated wheat in India as influenced by different nitrification inhibitors. *Agri. Ecosyst. Environ.* **91**:283–293.
- Martikainen, P.J. 1985. Number of autotrophic nitrifiers and nitrification in fertilized forest soil. *Soil Biol. Biochem.* **17**:245–248.
- Mosier, A.R., and C. Kroeze. 2000. Potential impact on the global atmospheric N₂O budget of the increased nitrogen input required to meet future global food demands. *Chemosphere-Global Change Sci.* **2**:465–473.
- Mosier, A.R., J.A. Delgado, and M. Keller. 1998. Methane and nitrous oxide fluxes in an acid Oxisol in western Puerto Rico: Effect of tillage, liming and fertilization. *Soil Biol. Biochem.* **30**:2087–2098.
- Robertson, G.P., and J.M. Tiedje. 1987. Nitrous oxide source in aerobic soils: Nitrification, denitrification and other biological processes. *Soil Biol. Biochem.* **19**:187–193.
- Sarathchandra, S.U., A. Ghani, G.W. Yeates, G. Burch, and N.R. Cox. 2001. Effect of nitrogen and phosphate fertilizers on microbial and nematode diversity in pasture soils. *Soil Biol. Biochem.* **33**:953–964.
- Schmidt, E.L., and L.W. Belser. 1982. Nitrifying bacteria, pp. 1027–1042. In D.R. Keeney (ed.), *Method of Soil Analysis, part 2. Soil Science of American*. Madison, WI, U.S.A.
- Schimel, J.P., and J. Gullledge. 1998. Microbial community structure and global trace gases. *Global Change Biol.* **4**:745–758.
- Venterea, R.T., and D.E. Rolston. 2000. Mechanisms and kinetic of nitric and nitrous oxide production during nitrification in agricultural soil. *Global Change Biol.* **6**:303–316.
- Watanabe, T., P. Chairaj, H. Tsuruta, W. Masarngsan, C.W. Wiwatchai, S. Wonprasaid, W. Cholitkul, and K. Minami. 2000. Nitrous oxide emission from fertilized upland field in Thailand. *Nutr. Cycl. Agroecosyst.* **57**:55–65.