

# Impact of nitrification inhibitor with organic manure and urea on nitrogen dynamics and N<sub>2</sub>O emission in acid sulphate soil

Shordar Mohamed Shamsuzzaman<sup>1</sup>, Mohamed Musa Hanafi<sup>2,3\*</sup>, Abd Wahid Samsuri<sup>3</sup>, Soud Mohd Halimi<sup>2,4</sup>, Masuda Begum<sup>5</sup>, Jantan Nur Maisarah<sup>6</sup>

1. Universiti Putra Malaysia - Institute of Tropical Agriculture - Laboratory of Food Crops - Serdang (Selangor), Malaysia.
2. Universiti Putra Malaysia - Institute of Tropical Agriculture - Laboratory of Plantation Crops - Serdang (Selangor), Malaysia.
3. Universiti Putra Malaysia - Faculty of Agriculture - Department of Land Management - Serdang (Selangor), Malaysia.
4. Universiti Putra Malaysia - Faculty of Agriculture - Department of Agriculture Technology - Serdang (Selangor), Malaysia.
5. Soil Resource Development Institute - Dakka, Bangladesh.
6. Malaysian Palm Oil Board - Biological Research Division - Kajang (Selangor), Malaysia.

**ABSTRACT:** The accurate prediction of N transformation is an important requisite for optimizing N use efficiency in cropping systems. An incubation study was conducted to verify the impacts of nitrification inhibitor (NI) with organic manure (OM) and urea on N dynamics and N<sub>2</sub>O emission in acid sulphate soil. The conducted experiment was two-level factorial with 4 N sources ( $N_1 = 100\%$  of N from urea,  $N_2 = 75\%$  of N from urea + 25% N from rice straw,  $N_3 = 75\%$  of N from urea + 25% of N from cow dung and  $N_4 = 75\%$  of N from urea + 25% of N from poultry dung) and two levels of NI (with and without DCD). The NI (Dicyandiamide — DCD) with OM + urea enhanced mineral N contents and it was the highest (255.07 µg·g<sup>-1</sup>) for urea with DCD applications. The highest net N-mineralization (213.07 µg·g<sup>-1</sup>) was

recorded for the application of urea with DCD and net nitrification (16.26 µg·g<sup>-1</sup>) was recorded for the application of urea alone, but the highest cumulative N<sub>2</sub>O emission (5.46 µg·g<sup>-1</sup>) was in urea + poultry dung (PD). In addition, DCD most effectively inhibited net nitrification (28.78%) and N<sub>2</sub>O emission (32.40%) from cow dung (CD) and urea in the tested soils. The combination of DCD with CD and urea was more effective in reducing N<sub>2</sub>O emissions (43.69%). These results suggest that the DCD with CD and urea may be the most potential combination to reduce nitrification and N<sub>2</sub>O emission as well as N loss from acid sulphate soil.

**Key words:** DCD, OM, urea, mineral N, net N-mineralization, net nitrification, N<sub>2</sub>O emission.

## INTRODUCTION

The available N content of soils is produced through N-mineralization, which governs the supply rate of N to plants. Fertilizer N management practices have a cumulative effect on N cycling and availability over time. Mineralized N or NH<sub>4</sub><sup>+</sup> released under anaerobic incubation are significantly correlated with soil organic matter; however, both quality and quantity of organic matter clearly affect N-mineralization in wetland rice soils (Sahrawat 2006). The greater part of N in paddy soil exists in soil organic matter. This tends to be conserved more in paddy soils than in upland soils, because of the anaerobic conditions. Microbial decomposition of the organic matter gradually releases ammonium N (NH<sub>4</sub><sup>+</sup>-N).

As NH<sub>4</sub><sup>+</sup>-N is stable under anaerobic conditions, it is retained as a cation on negatively charged soil mineral and organic particles, until the time when rice roots take it up. Rice plant acquires half to two-thirds of its N requirement from the soil mineralizable N pool even in a well-fertilized paddy (International Atomic Energy Agency 1978). Thus, accurate prediction of N-mineralization is important to avoid substantial N losses either to water bodies or to the atmosphere with related potential environmental risks (Fan et al. 2005; Su et al. 2005).

Nitrification converts the relatively immobile mineral N form ammonium (NH<sub>4</sub><sup>+</sup>-N) into highly mobile nitrate (NO<sub>3</sub><sup>-</sup>-N) after the application of NH<sub>4</sub><sup>+</sup>-based fertilizers. The net production of NO<sub>3</sub><sup>-</sup> is a key ecological process that can →

\*Corresponding author: mmhanafi@agri.upm.edu.my

Received: Apr. 17, 2015 – Accepted: Sept. 12, 2015

affect the chemistry and nutrient capital of soils. Moreover, nitrification is considered to be an indirect driver of N loss during the flooded rice growing season, because the rate of denitrification in flooded soils is controlled by the rate of nitrification (Zhou et al. 2012). Thus, suppressing nitrification can play a key role to improve fertilizer N use efficiency (NUE) and to mitigate N<sub>2</sub>O emissions from irrigated rice-upland crop rotation systems. Nitrification inhibitor, such as Dicyandiamide (DCD), deactivates the enzyme ammonia monooxygenase of Nitrosomonas and/or Nitrosospira, the genus of nitrifying bacteria responsible for the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>. This helps to retain N in the NH<sub>4</sub><sup>+</sup> form longer in soil, providing more opportunity for plants to uptake NH<sub>4</sub><sup>+</sup> (Di et al. 2009). Thus, nitrification inhibitor (NI) can reduce N<sub>2</sub>O emissions both from nitrification and denitrification of NO<sub>3</sub><sup>-</sup>. An insight into the N dynamics and its related processes (N-mineralization and nitrification) provides knowledge for improving crop management to optimum nutrient use efficiency. Hence, the objectives of this study were to: (i) determine the effect of DCD with organic manure (OM) and urea on mineral-N availability, N-mineralization and net nitrification, and (ii) evaluate the N<sub>2</sub>O emission potential of OM used with urea and DCD from acid sulphate soil.

## MATERIALS AND METHODS

An incubation study was conducted at Analytical Laboratory-2, Department of Land Management, Universiti Putra Malaysia. Soils used this application were collected from Kampung Golok, Semerak, Kelantan, Malaysia (06°00'N, 102°23'E). Soil texture analysis was done by hydrometer method (Bouyoucos 1962). Soil pH was determined using a 1:2.5 (soil:H<sub>2</sub>O) diluted soil solution using a pH meter (Accumet 910, Fisher Scientific Ltd., Pittsburgh, PA, USA). The organic C in soil was measured by combustion in a LECO FP-2000 CNS (LECO Corp., MI, USA). Total C and N in the soil were measured by combustion in a TRU MAC CNS (LECO Corp., MI, USA). Available P was determined by the Bray's P1 test, using 0.03 M of NH<sub>4</sub>F in 0.02 M of HCl as extractant and measuring the extracted P colorimetrically at 660 nm by the molybdenum blue method (Bray and Kurtz 1945). Exchangeable K was determined by extraction with neutral normal NH<sub>4</sub>OAC at soil: solution ratio, 1:10. Zinc was extracted by double acid method using 0.05 M HCl in

0.0125 M H<sub>2</sub>SO<sub>4</sub>, determining by AAS. The soil physical and chemical characteristics are given in Table 1.

Rice straw (RS), cow dung (CD) and poultry dung (PD) were used as OM. The subsample of each OM was dried in an oven at 65 °C for 48 h and then grounded by a grinding machine to pass through a 1-mesh sieve. The organic C was measured by combustion in a LECO FP-2000 CNS (LECO Corp., MI, USA). Total C, N and S were measured by combustion in a TRU MAC CNS (LECO Corp., MI, USA). Nutrient content in each OM was determined by H<sub>2</sub>O<sub>2</sub>-H<sub>2</sub>SO<sub>4</sub> digestion (Ohyama et al. 1991) using a Kel Plus auto N analyzer for N and P spectrophotometer for K, Ca and Mg. Concentrations of Fe, Mn, Cu and Zn were measured with an ICP-MS (Agilent 7500a). Some chemical characteristics of organic materials are given in Table 2.

The experimental design was a two-level factorial with 4 N sources (100% N of recommended dose from urea, 75% N from urea + 25% N from RS, 75% N from urea + 25% N from CD and 75% N from urea + 25% N from PD) and two levels of NI (with and without DCD). The treatments were arranged in randomized complete block design (RCBD) with three replicates. Air-dried soil samples (75 g) were placed into 250-mL plastic containers for each input treatment. The amount of N applied through urea or urea + OM was 48 mg·kg<sup>-1</sup> (120 kg N·ha<sup>-1</sup>), and DCD was added at 15% of applied N. OM (powder of RS, CD and PD), fertilizer grade triple super phosphate (11.6 mg P·kg<sup>-1</sup> of soil or 30 kg P·ha<sup>-1</sup>), and muriate of potash (22 mg K·kg<sup>-1</sup> of soil

**Table 1.** Physical and chemical properties of initial soil.

Characteristics	Value
Textural class	Clay loam
Sand (%)	2.85
Silt (%)	49.22
Clay (%)	47.88
Soil pH (soil and water, 1:2.5)	3.9
Organic carbon (%)	2.78
Total nitrogen (%)	0.238
Carbon/nitrogen ratio (C/N)	11.68
Phosphorus (mg·kg <sup>-1</sup> )	32.50
Potassium (cmol <sub>+</sub> ·kg <sup>-1</sup> )	0.22
Calcium (cmol <sub>+</sub> ·kg <sup>-1</sup> )	1.03
Magnesium (cmol <sub>+</sub> ·kg <sup>-1</sup> )	0.75
Sulphur (%)	0.17
Zinc (mg·kg <sup>-1</sup> )	1.28

**Table 2.** Chemical composition of the used organic manures.

Characteristics	Rice straw	Cow dung	Poultr dung
Organic carbon (%)	36.85	35.6	27.64
Organic matter (%)	63.30	61.4	47.65
Total carbon (%)	43.28	44.0	35.91
Total nitrogen (%)	0.78	1.24	3.14
Carbon/nitrogen ratio (C/N)	55.49	35.5	11.43
Phosphorus (%)	0.02	0.31	1.37
Potassium (%)	0.77	0.33	0.93
Calcium (%)	0.61	0.29	3.01
Magnesium (%)	0.58	0.65	0.86
Zinc ( $\text{mg}\cdot\text{kg}^{-1}$ )	400	420	560

or 60 kg K·ha<sup>-1</sup>) were added before 1 day of incubation. Urea and DCD were applied just before the incubation. Water was added to maintain flooded condition (5 cm depth) and kept in a dark room at 20 ± 2 °C with a relative humidity of 83 ± 3% for 30 days.

The soils were destructively sampled on 10<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup> day of incubation. For mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) contents, wet soil was extracted in the next day after sampling using 1 M of KCl shaken for 1 h in a reciprocating shaker at 170 rpm, placed in plastic containers of 250 mL, and then filtered through Whatman® No.1 filter paper. Mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was determined in these extracts by steam distillation in the presence of MgO and Devarda's alloy (Keeney and Nelson 1982). A part of the sample was oven-dried (105 °C) for adjustment of the moisture content, and results were calculated in an oven-dry soil weight basis. The rate of N-mineralization was calculated as the difference in the concentration of mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) ions in the incubated and initial sample (Hart et al. 1994). Net nitrification was calculated as the difference in the  $\text{NO}_3^-$  N concentration in the incubated and initial sample (Hart et al. 1994). The rates of N-mineralization and nitrification are expressed in units of microgram of N per gram of dry soil per 30 days.

To measure  $\text{N}_2\text{O}$  emission, each bottle was sealed using an airtight butyl rubber stopper perforated by centered Perspex tubes for sampling, including a gas inlet and outlet equipped with a glass piston at times 0, 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup> day after the beginning of incubation. Samplings were done in the morning between 09:00 and 12:00 in order to minimize diurnal variation in flux patterns. Each time, three samples of chamber air were manually pulled

into 10-mL syringes at 0, 30, and 60 min after closure. Then, the air samples were transferred to 4-mL pre-evacuated vials and fitted with butyl rubber stoppers. The  $\text{N}_2\text{O}$  was quantified with Agilent Technology 4890D gas chromatograph equipped with an electron capture detector (ECD). The gas emission flux was calculated from the difference in gas concentration (Ahmad et al. 2009). Average fluctuations and standard deviations of  $\text{N}_2\text{O}$  were calculated from three replicated plots. Cumulative  $\text{N}_2\text{O}$  emissions were calculated from the emissions between two adjacent measurement intervals following the equation by Li-me et al. (2011).

$$\text{Cumulative } \text{N}_2\text{O} \text{ emission} =$$

$$\Sigma \frac{(\text{F}_{i+1} + \text{F}_i)}{2} \times (t_{i+1} - t_i) \times 24$$

where:

F represents  $\text{N}_2\text{O}$  ( $\text{kg N}_2\text{O-N ha}^{-1}\cdot\text{h}^{-1}$ ) flux; i is the sampling frequency; and t is the day after incubation.

All data were subjected to a two-way analysis of variance (ANOVA) (N source and NI) using the PROC GLM function of the SAS statistical programme (SAS Institute 1996). When there was a significant treatment effect, means were compared using Duncan Multiple Range Test (DMRT). Treatment comparisons were deemed significant at p < 0.05.

## RESULTS AND DISCUSSION

The concentrations of extractable  $\text{NH}_4^+$ -N was, in general, higher in the DCD applied soils than in control,

with pronounced differences among the N source (Table 3). Average values for NH<sub>4</sub><sup>+</sup>-N after the incubation ranged from a minimum of 175.01 ± 6.75 µg·g<sup>-1</sup> of dry soil (urea + RS) to a maximum of 226.72 ± 7.09 µg·g<sup>-1</sup> of dry soil (urea alone) with DCD application. Without DCD, the average values ranged from 159.35 ± 6.77 µg·g<sup>-1</sup> of dry soil (urea + RS) to 216.91 ± 6.16 µg·g<sup>-1</sup> of dry soil (urea alone). DCD did not have any effect on urea hydrolysis and exhibited higher accumulation of soil NH<sub>4</sub><sup>+</sup> compared to other N inhibitors treatments, probably due to strong inhibit of NH<sub>4</sub><sup>+</sup> oxidation and retention of NH<sub>4</sub><sup>+</sup> (Zaman et al. 2009). In this study, the NH<sub>4</sub><sup>+</sup>-N concentration in soil was the lowest for urea + RS and the highest for urea only, whereas intermediate values were observed for urea + PD and urea + CD application irrespective of DCD. Addition of urea led to a clear increase in NH<sub>4</sub><sup>+</sup> soil concentration due to quick mineralization (Noguera et al. 2010). The concentration of NH<sub>4</sub><sup>+</sup> also increased soon after application of urine compared to the control treatment, because the majority of urine-N consists of urea (80%) and easily mineralizable amino acids which undergo quick hydrolysis and ammonification to produce NH<sub>4</sub><sup>+</sup> (Zaman et al. 2009).

Soil NO<sub>3</sub><sup>-</sup>-N concentrations differed significantly among the N sources with or without DCD (Table 3). The concentration of NO<sub>3</sub><sup>-</sup>-N was higher in soils without DCD applied than DCD-applied soils, and the range of values were 19.77 ± 1.32 — 30.26 ± 2.02 µg·g<sup>-1</sup> of dry soil and 17.86 ± 1.28 — 28.34 ± 2.13 µg·g<sup>-1</sup> of dry soil, respectively. The DCD slowed down nitrification from any day and therefore exhibited comparatively lower amounts of NO<sub>3</sub><sup>-</sup>-N in surface soil. Such reduction in nitrification is related to the partial inhibition of the nitrifying bacteria activity by DCD (Zaman et al. 2009). A similar pattern of NO<sub>3</sub><sup>-</sup> production

from urea fertilizer coated with Agrotain, and DCD was also observed (Zaman et al. 2008). The NO<sub>3</sub><sup>-</sup>-N was found in highest concentration (30.26 ± 2.02 µg·g<sup>-1</sup> of dry soil) for urea alone than the other N source, which was statistically similar to urea + PD application (29.55 ± 2.69 µg·g<sup>-1</sup> of dry soil). In general, the NO<sub>3</sub><sup>-</sup>-N concentration in soil was the lowest (17.86 ± 1.28 — 19.77 ± 1.32 µg·g<sup>-1</sup> of dry soil) in urea + RS and the highest (28.34 ± 2.13 — 30.26 ± 2.02 µg·g<sup>-1</sup> of dry soil) in urea alone, whereas intermediate value (20.19 ± 1.35 — 23.73 ± 1.36 µg·g<sup>-1</sup> of dry soil) was observed for urea + CD. The fertilization treatment involved the addition of urea and led to a clear increase (about fivefold) in nitrate soil concentration due to quick mineralization (Noguera et al. 2010). Similarly, Malhi et al. (2006) reported that the NO<sub>3</sub><sup>-</sup>-N concentration increased considerably with increasing N rate to ≥ 80 kg N·ha<sup>-1</sup> in 0 — 15 cm and to 120 kg N·ha<sup>-1</sup> in 15 — 30 and 30 — 60 cm depths. Other researchers also reported the accumulation of nitrate-N in the soil profile when an excessive rate of N fertilizer was applied (Guillard et al. 1995). The soil NO<sub>3</sub><sup>-</sup>-N level in annual cropping system in northern Great Plains was observed to increase with N rate, and the greatest increase was at the highest rate (101 kg N·ha<sup>-1</sup>) (Halvorson et al. 1999).

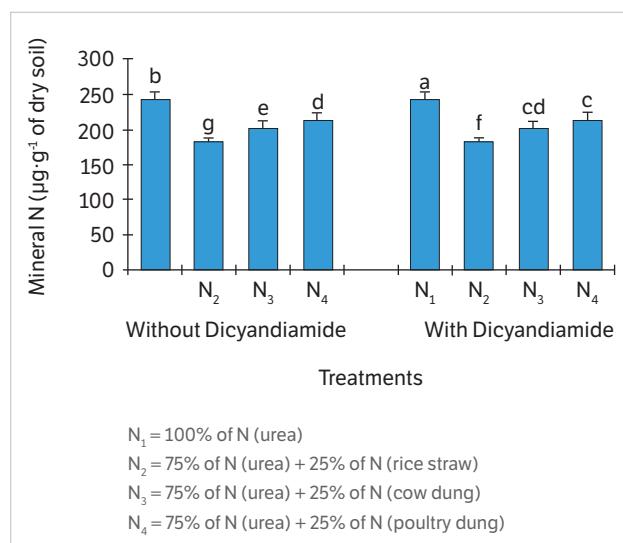
After incubation period, significant differences in retaining total mineral-N between the levels of DCD were observed for all N sources (Figure 1). Average values for mineral-N ranged from a minimum of 179.26 ± 5.10 µg·g<sup>-1</sup> of dry soil (urea + RS) to a maximum of 242.16 ± 5.78 µg·g<sup>-1</sup> of dry soil (urea alone) without DCD application. Under DCD application, the average value ranged from 192.88 ± 4.41 µg·g<sup>-1</sup> of dry soil to 255.07 ± 4.96 µg·g<sup>-1</sup> of dry soil for the N sources. However, the DCD with N source exhibited the maximum concentrations of mineral N in soil. The application of urea with

**Table 3.** Effect of DCD with organic manure and urea on NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N after 30 days of incubation.

N source	NH <sub>4</sub> <sup>+</sup> -N		NO <sub>3</sub> <sup>-</sup> -N	
	Without DCD	With DCD	Without DCD	With DCD
			µg·g <sup>-1</sup> of dry soil	µg·g <sup>-1</sup> of dry soil
N <sub>1</sub>	216.91 ± 6.16b	226.72 ± 7.09a	30.26 ± 2.02a	28.34 ± 2.13b
N <sub>2</sub>	159.35 ± 6.77f	175.01 ± 6.75e	19.77 ± 1.32e	17.86 ± 1.28f
N <sub>3</sub>	178.96 ± 6.34e	191.31 ± 6.60cd	22.20 ± 1.90d	19.79 ± 1.32e
N <sub>4</sub>	183.11 ± 6.49de	195.00 ± 6.89c	29.55 ± 2.69a	25.28 ± 0.90c
CV (%)	<b>2.79</b>		<b>2.50</b>	

Means followed by the same letter within the same parameter are not significantly different ( $p > 0.05$ ) using DMRT. N<sub>1</sub> = 100% of N (urea), N<sub>2</sub> = 75% of N (urea) + 25% of N (RS), N<sub>3</sub> = 75% of N (urea) + 25% of N (CD), and N<sub>4</sub> = 75% of N (urea) + 25% of N (PD). DCD = Dicyandiamide; CV = coefficient of variation; RS = rice straw; CD = cow dung; PD = poultry dung.

DCD resulted in the highest mineral N ( $255.07 \pm 4.96 \mu\text{g}\cdot\text{g}^{-1}$  of dry soil), and the lowest value ( $178.22 \pm 5.01 \mu\text{g}\cdot\text{g}^{-1}$  of dry soil) was observed for urea + RS application. The input treatments in order of descending mineral N contents were: urea alone > urea + PD = urea + CD > urea + RS. Due to the significant immobilization of fertilizer-derived N in the fertilizer + OM treatment, there was a negative interactive effect on the amount of mineral N with combining fertilizer and OM inputs. Urea is an ammoniacal N fertilizer which mineralizes quickly. Hence, fertilization with only urea led to a clear increase of mineral N in soil (Noguera et al. 2010). Other researchers also recorded the increase in inorganic N value of 88% due to 67 kg N ha<sup>-1</sup> of application (El-Haris et al. 1983). An increase in net formation of mineral- N after N-fertilization has also been reported by Priha and Smolander (1995).



**Figure 1.** Effect of DCD with organic manure and urea on mineral N after 30 days of incubation. Vertical bars represent  $\pm$  standard error of mean.

Variation in the net soil N-mineralization was significant due to the N source, DCD, and DCD and N source interaction (Table 4). The DCD had significant effect on N- mineralization. The N-mineralization was substantially lower in the absence of DCD than in its presence. The net N-mineralization ranged from  $137.26 \pm 5.10 \mu\text{g}\cdot\text{g}^{-1}$  of dry soil per month (urea + RS) to  $205.16 \pm 5.75 \mu\text{g}\cdot\text{g}^{-1}$  of dry soil per month (urea alone) in the absence of DCD. In the presence of DCD, the net N-mineralization ranged from  $150.88 \pm 4.42 \mu\text{g}\cdot\text{g}^{-1}$  of dry soil per month (urea + RS) to  $213.07 \pm 4.96 \mu\text{g}\cdot\text{g}^{-1}$  per month (urea alone). Previous studies also reported that the DCD significantly increased total mineralization (recalcitrant organic N + labile organic N) following cattle slurry (CS) application (McGeough et al. 2014). The N source increased the net soil N-mineralization; the highest ( $213.07 \pm 4.96 \mu\text{g}\cdot\text{g}^{-1}$ ) was in the urea with DCD and the lowest ( $137.26 \pm 5.10 \mu\text{g}\cdot\text{g}^{-1}$ ) was in the urea + RS. For the present study, the ranking of N source was urea alone > urea + PD > urea + CD > urea + RS. Urea mineralized quickly (Noguera et al. 2010) due to the form of N as NH<sub>4</sub><sup>+</sup> in urea. Other researchers also found that net N-mineralization was higher in the soils that had been fertilized with urea than in the fertilized ones with urea + OM (Arnio and Martikainen 1992).

Net nitrification was strongly influenced by N source, DCD and an interaction of N source and DCD (Table 4). The net nitrification ranged from  $5.84 \pm 0.42 \mu\text{g}\cdot\text{g}^{-1}$  per month to  $16.26 \pm 1.02 \mu\text{g}\cdot\text{g}^{-1}$  per month in the absence of DCD. In the presence of DCD, it ranged from  $3.86 \pm 0.28 \mu\text{g}\cdot\text{g}^{-1}$  per month to  $14.34 \pm 0.73 \mu\text{g}\cdot\text{g}^{-1}$  per month. The DCD slowed down nitrification from any day of application. Such reduction in nitrification is related to the partial inhibition of the nitrifying bacteria activity by DCD. For example, application of dairy urine with DCD showed slow nitrification to pasture

**Table 4.** Effect of DCD with organic manure and urea on net N-mineralization and net nitrification after 30 days of incubation.

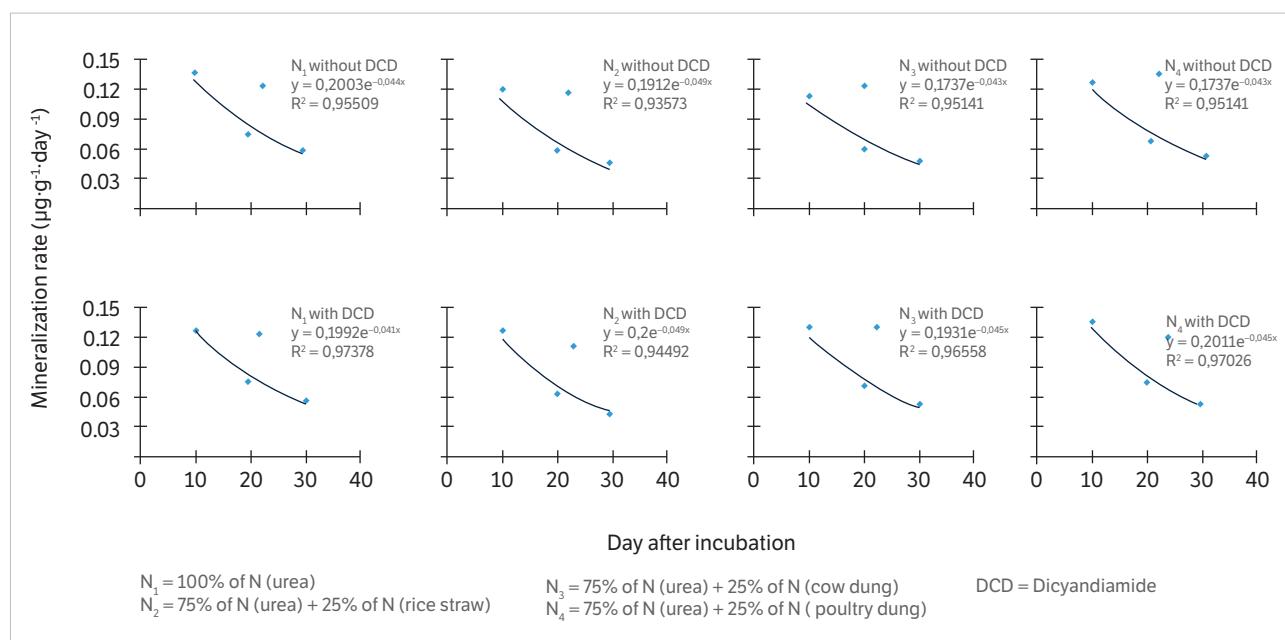
N source	Mineralization		Nitrification	
	Without DCD	With DCD	Without DCD	With DCD
	$\mu\text{g}\cdot\text{g}^{-1}$ of dry soil			
$N_1$	$205.16 \pm 5.75\text{b}$	$213.07 \pm 4.96\text{a}$	$16.26 \pm 1.02\text{a}$	$14.34 \pm 0.73\text{c}$
$N_2$	$137.26 \pm 5.10\text{g}$	$150.88 \pm 4.42\text{f}$	$5.84 \pm 0.42\text{f}$	$3.86 \pm 0.28\text{g}$
$N_3$	$159.16 \pm 5.25\text{e}$	$169.10 \pm 4.76\text{d}$	$8.13 \pm 0.91\text{e}$	$5.79 \pm 0.32\text{f}$
$N_4$	$170.66 \pm 5.18\text{d}$	$176.28 \pm 5.75\text{c}$	$15.55 \pm 0.69\text{b}$	$11.28 \pm 0.90\text{d}$
CV (%)	2.60		3.83	

Means followed by the same letter within the same parameter are not significantly different ( $p > 0.05$ ) using DMRT.  $N_1 = 100\% \text{ of N (urea)}$ ,  $N_2 = 75\% \text{ of N (urea)} + 25\% \text{ of N (RS)}$ ,  $N_3 = 75\% \text{ of N (urea)} + 25\% \text{ of N (CD)}$ , and  $N_4 = 75\% \text{ of N (urea)} + 25\% \text{ of N (PD)}$ . DCD = Dicyandiamide; CV = coefficient of variation; RS = rice straw; CD = cow dung; PD = poultry dung.

soil (Di et al. 2007). The N source increased the net nitrification with the highest value ( $16.26 \pm 1.02 \mu\text{g}\cdot\text{g}^{-1}$  per month) in the urea alone, and the lowest value ( $5.84 \pm 0.42 \mu\text{g}\cdot\text{g}^{-1}$  per month) was in the urea + RS treatment. It has been shown that a decrease of nitrification by DCD is accompanied by an increase in the immobilization of added ammonium (Clay et al. 1990). Across the DCD, the net nitrification rates were lower for the present DCD and higher for the absent DCD in all N sources. This result was at par with McCarty and Bremner (1989), who found the effective inhibition of nitrification in 3 soils at 21 days using 10 mg of DCD kg<sup>-1</sup> of soil.

Variations in the rate of the soil N-mineralization were significant due to the N source and DCD. The highest maximum mineralization rate ( $K_{\max}$ ) occurred at the 10<sup>th</sup> day of incubation in both control and treated soils (Figure 2). The mineralization rate increased due to N source and it was the highest in urea alone and the lowest in urea + RS. DCD had no significant effect on the N-mineralization rate. These results are consistent with those of De-Zhi et al. (2006), who observed that the mineralization rate increased rapidly in the 1<sup>st</sup> week, then declined, and became negative after the 7<sup>th</sup> week. They also observed that the soils treated with N fertilizer mineralized more N than the unfertilized soils, and N-mineralization increased with increasing N application dosage. Other researchers have also reported that N-mineralization was greater in fertilized

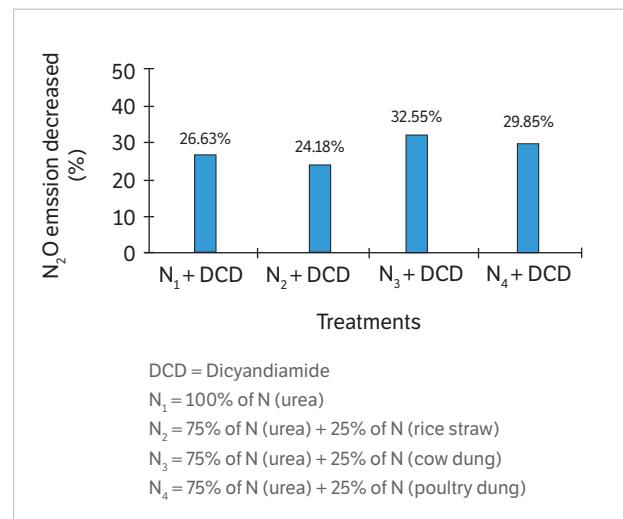
soils than unfertilized ones (Forge and Simard 2001). The N-mineralization rate during the incubation period exhibited a similar pattern for the N sources (Figure 2). The relationship between the N-mineralization rate and time was best described by an exponential function (Figure 2). The mineralization rate (k) for different times during the incubation period was determined by using first-order kinetics. By regression analysis, it was found that the kinetics of N-mineralization best fitted in power model (Figure 2). The steep of the curve (k) obtained by N source followed power model, and most of the N mineralized within ten days of incubation. This indicates that rapid N was mineralized at the initial stage of incubation period, which is associated with readily available C and N in the soil for microorganisms. As microbes decompose carbon, they use the liberated energy to grow and reproduce. Nitrogen is also needed for microbial growth, and, to supply this need, they will convert organic N into inorganic N. The labile C and N contents in agroecosystems can be increased by long-term fertilizer application, particularly by application of OM and chemical fertilizer (Zhang et al. 2009), and hence contribute to more mineralization. Management of mineral N fertilization during the cultivation of wetland soils might have changed the composition of soil microflora and ammonia-oxidizing bacteria (AOB) population size, thus influencing mineralization (Jin et al. 2012).



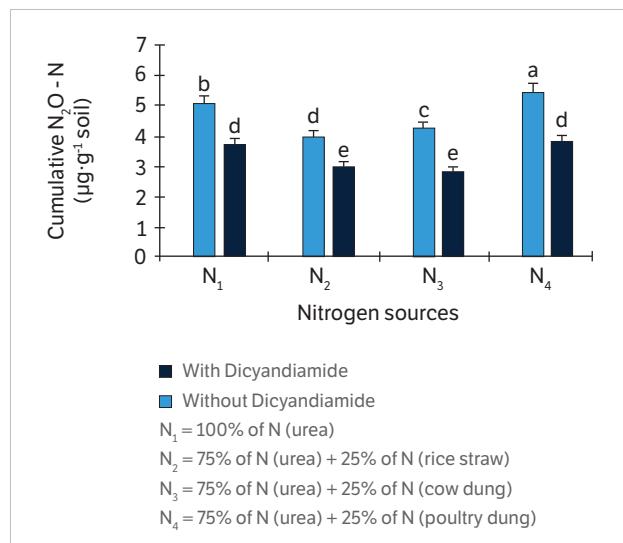
**Figure 2.** Effect of DCD with organic manure and urea on kinetics of N mineralization in acid sulphate soil.

There was an apparent interaction on  $\text{N}_2\text{O}$  emission between N source and DCD. For all N sources, cumulative  $\text{N}_2\text{O}$  emission in the absence of DCD was always higher than in the presence of DCD (Figure 3). The highest cumulative  $\text{N}_2\text{O}$  emission occurred in urea + PD amounted to as much as  $5.07 \pm 0.25 \mu\text{g}\cdot\text{g}^{-1}$  of soils and the lowest value of  $3.97 \pm 0.13 \mu\text{g}\cdot\text{g}^{-1}$  was recorded in urea + RS without DCD application. Such an effect of residue application on  $\text{N}_2\text{O}$  emission is in close accordance with previous reported results (Baggs et al. 2003) and was attributed to a more rapid release of N from the PD treatment resulting in the availability of N for nitrification and denitrification. When DCD was applied with N source, cumulative  $\text{N}_2\text{O}$  emission was decreased by 24.18 — 32.55% (Figure 4). Based on cumulative  $\text{N}_2\text{O}$  emission with/without DCD, N sources showed the following order: urea + PD > urea alone > urea + CD > urea + RS, because soil C/N ratio is an important parameter affecting  $\text{N}_2\text{O}$  emission (DeDatta 1995). Among urea + OM treatments,  $\text{N}_2\text{O}$  emission was low in urea + RS, probably due to a high C/N ratio (97.59:1) and consequential immobilization of available N. Higher  $\text{N}_2\text{O}$  emissions were recorded following incorporation of low C/N ratio (11.43:1) of PM treatment than the incorporation of high C/N ratio (97.59:1) of RS treatment (Das and Adhya 2014). Generally,  $\text{N}_2\text{O}$  emissions are negatively correlated with C/N ratio of the incorporated residues (Huang et al. 2004). In this study, application of DCD suppresses nitrification as well as  $\text{N}_2\text{O}$  for denitrification, and thus less  $\text{N}_2\text{O}$  was emitted. These findings are in agreement with Merino et al. (2002),

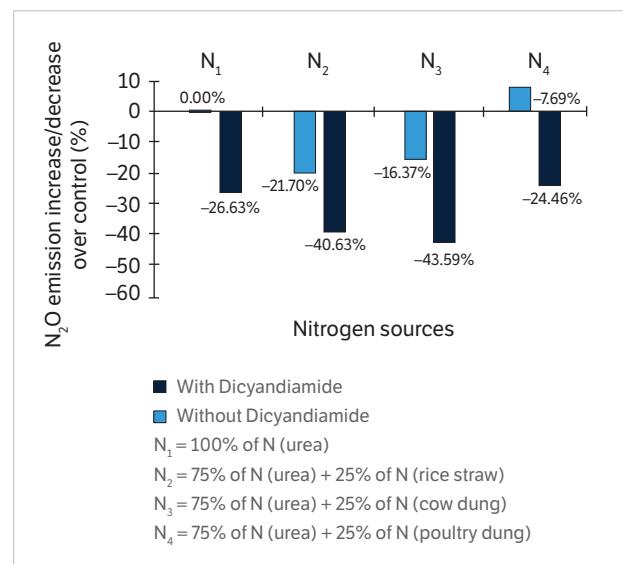
who found that DCD reduced  $\text{N}_2\text{O}$  emissions when added to cattle urine and cattle slurry, respectively. Among the OM, PD along with urea increased  $\text{N}_2\text{O}$  emission (7.75%), but other combination of OM with urea decreased  $\text{N}_2\text{O}$  emission (16.37 — 21.70%) over urea alone. On the other hand, DCD with OM decrease 24.46 — 43.59% of  $\text{N}_2\text{O}$  emission and the highest reduction (43.56%) was observed for the combined application of DCD with urea + CD (Figure 5). Incorporation of crop residues provides a source of readily available C and N in the soil and subsequently influences  $\text{N}_2\text{O}$  emissions (Huang et al. 2004). The increase in  $\text{N}_2\text{O}$  emissions following different types of OM application observed in our study is →



**Figure 4.** Influence of DCD on reduction of cumulative  $\text{N}_2\text{O}$  emission.



**Figure 3.** Influence of DCD with organic manure and urea on cumulative  $\text{N}_2\text{O}$  gas emission from acid sulphate soil. Vertical bars represent  $\pm$  standard error of mean.



**Figure 5.** Influence of DCD with organic manure and urea on the change of cumulative  $\text{N}_2\text{O}$  emission compared to urea alone.

consistent with the study of Zou et al. (2005), who reported that the incorporation of rapeseed cake increased N<sub>2</sub>O by 17% and wheat straw incorporation decreased seasonal N<sub>2</sub>O emissions by 8 – 19%.

## CONCLUSION

NI with OM and urea influence N transformations and N<sub>2</sub>O emission. DCD with OM and urea enhance mineral N contents, and the highest amount (255.07 µg·g<sup>-1</sup>) was for DCD with urea application. Throughout the incubation period, the highest net N mineralization (213.07 µg·g<sup>-1</sup>) was recorded for the application of DCD with urea,

and net nitrification (16.26 µg·g<sup>-1</sup>) was recorded for the application of urea alone, but the highest cumulative N<sub>2</sub>O emission (5.46 µg·g<sup>-1</sup>) was in urea + PD. DCD alone decreased N<sub>2</sub>O emission in 24.18 — 32.55%, and DCD with OM decreased N<sub>2</sub>O emission in 24.56 — 43.59%; the combination of DCD with CD and urea was more effective in reducing nitrification and N<sub>2</sub>O emissions. These results suggest that the combination of DCD with urea + CD may be the most potential combination to reduce nitrification and N<sub>2</sub>O emission as well as N loss from acid sulphate soil. Future studies under field condition are needed to measure the effect of DCD with OM and urea on mineral N, net N-mineralization, and net nitrification to better understand the underlying mechanisms of the effects of this combination on N cycle.

## REFERENCES

- Ahmad, S., Dai, G., Zhan, M., Wang, J., Pan, S. and Cao, C. (2009). Greenhouse gas emission from direct seeding paddy field under different rice tillage systems in central China. *Soil and Tillage Research*, 106, 54-61. <http://dx.doi.org/10.1016/j.still.2009.09.005>.
- Arnio, T. and Martikainen, J. (1992). Nitrification in forest soil after refertilization with urea or urea and dicyandiamide. *Soil Biology and Biochemistry*, 24, 951-954. [http://dx.doi.org/10.1016/0038-0717\(92\)90022-P](http://dx.doi.org/10.1016/0038-0717(92)90022-P).
- Baggs, E. M., Stevenson, M., Pihlatie, M., Roger, A., Cook, H. and Cadisch, G. (2003). Nitrous oxide emissions following application of residues and fertiliser under zero and conventional tillage. *Plant and Soil*, 254, 361-370. <http://dx.doi.org/10.1023/A:1025593121839>.
- Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal*, 54, 464-465. <http://dx.doi.org/10.2134/agronj1962.00021962005400050028x>.
- Bray, R. M. and Kurtz, L.T. (1945). Determination of total organic P and available forms of phosphorus in soils. *Soil Science*, 59, 39-45. <http://dx.doi.org/10.1097/00010694-194501000-00006>.
- Clay, D. E., Malzer, G. L. and Anderson, J. L. (1990). Ammonia volatilisation from urea as influenced by soil temperature, soil water content, and nitrification and hydrolysis inhibitors. *Soil Science Society of America Journal*, 54, 263-266. <http://dx.doi.org/10.2136/sssaj1990.03615995005400010042x>.
- Das, S. and Adhya, T. K. (2014). Effect of combine application of organic manure and inorganic fertilizer on methane and nitrous oxide emissions from a tropical flooded soil planted to rice. *Geoderma*, 213, 185-192. <http://dx.doi.org/10.1016/j.geoderma.2013.08.011>.
- De-Zhi, Y., De-Jian, W., Rui-Juan, S. and Jing-Hui, L. (2006). N mineralization as affected by long-term N fertilization and its relationship with crop N uptake. *Pedosphere*, 16, 125-130. [http://dx.doi.org/10.1016/S1002-0160\(06\)60034-9](http://dx.doi.org/10.1016/S1002-0160(06)60034-9).
- DeDatta, S. K. (1995). Nitrogen transformations in wetland rice ecosystems. *Plant and Soil*, 42, 193-203. [http://dx.doi.org/10.1007/978-94-009-1706-4\\_20](http://dx.doi.org/10.1007/978-94-009-1706-4_20).
- Di, H. J., Cameron, K. C., Shen J. P., Winefield, C. S., O'Callaghan, M., Bowatte, S. and He, J. Z. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience*, 2, 621-624. <http://dx.doi.org/10.1038/ngeo613>.
- Di, H. J., Cameron, K. C. and Sherlock, R. R. (2007). Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. *Soil Use and Management*, 23, 1-9. <http://dx.doi.org/10.1111/j.1475-2743.2006.00057.x>.
- El-Haris, M. K., Cochran, V. L., Elliot, L. F. and Bezdecik, D. F. (1983). Effect of tillage, cropping and fertilizer management on soil nitrogen mineralization potential. *Soil Science Society*

- of America Journal, 47, 1157-1161. <http://dx.doi.org/10.2136/sssaj1983.03615995004700060020x>.
- Fan, X. H., Y. S., Song, D. X. Lin, L. Z. Yang, J. M. and Zhou, Y. (2005). Ammonia volatilization losses from urea applied to wheat on a paddy soil in Taihu Region, China. *Pedosphere*, 15, 59-65.
- Forge, T. A. and Simard, S. W. (2001). Short-term effects of nitrogen and phosphorus fertilizers on nitrogen mineralization and trophic structure of the soil ecosystem in forest clearcuts in the southern interior of British Columbia. *Canadian Journal of Soil Science*, 81, 11-20. <http://dx.doi.org/10.4141/S00-018>.
- Guillard, K., Griffin, G. F., Allinson, D. W., Yamartino, W. R., Rafey, M. M. and Pietryzk, S. W. (1995). Nitrogen utilization of selected cropping systems in the U.S. northeast. II. Soil profile nitrate distribution and accumulation. *Agronomy Journal*, 87, 199-207. <http://dx.doi.org/10.2134/agronj1995.00021962008700020011x>.
- Halvorson, A. D., Curtis A. R. and Follett, R. F. (1999). Nitrogen fertilization effects on soil carbon and nitrogen in a dryland cropping system. *Soil Science Society of America Journal*, 63, 912-917. <http://dx.doi.org/10.2136/sssaj1999.634912x>.
- Hart, S. C., Stark, J. M., Davidson, E. A. and Firestone, M. K. (1994). Nitrogen mineralization, immobilization, and nitrification. In S. C. Hart, J. M. Stark, E. A. Davidson, M. K. Firestone (Eds.), *Methods of soil analysis part 2: microbiological and biochemical properties* (p. 985-1018). Madison: American Society of Agronomy.
- Huang, Y., Zou, J., Zheng X., Wang, Y. and Xu, X. (2004). Nitrous oxide emissions as influenced by amendment of plant residues with different C/N ratios. *Soil Biology and Biochemistry*, 36, 973-981. <http://dx.doi.org/10.1016/j.soilbio.2004.02.009>.
- International Atomic Energy Agency (1978). Isotope studies on rice fertilization: results of a five-year co-ordinated research programme of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture Using Nitrogen-15-labelled Fertilizers. Vienna: IAEA.
- Jin, X., Huang, J. and Zhou, Y. (2012). Impact of coastal wetland cultivation on microbial biomass, ammonia-oxidizing bacteria, gross N transformation and N<sub>2</sub>O and NO potential production. *Biology and Fertility of Soils*, 48, 363-369. <http://dx.doi.org/10.1007/s00374-011-0631-8>.
- Keeney, D. R. and Nelson, D. W. (1982). Nitrogen-inorganic forms. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), *Methods of soil analysis, part 2* (p. 643-698). Madison: American Society of Agronomy.
- Li-Mei, Z., Hong-Bin, L., Ji-Zong, Z., Jing, H. and Bo-Ren, W. (2011). Long-term application of organic manure and mineral fertilizer on N<sub>2</sub>O and CO<sub>2</sub> emissions in a red soil from cultivated maize-wheat rotation in China. *Agricultural Sciences in China*, 10, 1748-1757. [http://dx.doi.org/10.1016/S1671-2927\(11\)60174-0](http://dx.doi.org/10.1016/S1671-2927(11)60174-0).
- Malhi, S. S., Lemke, R., Wang, Z., Baldev, H. and Chhabra, S. (2006). Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. *Soil and Tillage Research*, 90, 171-183. <http://dx.doi.org/10.1016/j.still.2005.09.001>.
- McCarty, G. W. and Bremner, J. M. (1989). Laboratory evaluation of dicyandiamide as a soil nitrification inhibitor. *Communications in Soil Science and Plant Analysis*, 20, 2049-2065. <http://dx.doi.org/10.1080/00103628909368200>.
- McGeough, K. L., Müller, C., Laughlin, J., Watson, C. J., Ernfors, M., Cahalan, E. and Richards, G. (2014). The effect of dicyandiamide addition to cattle slurry on soil gross nitrogen transformations at a grassland site in Northern Ireland. *The Journal of Agricultural Science*, 152, 125-136. <http://dx.doi.org/10.1017/S0021859613000762>.
- Merino, P., Estavillo, J. M., Graciolli, L. A., Pinto, M., Lacuesta, M., Munozrueda, A. and Gonzalez-Murua, C. (2002). Mitigation of N<sub>2</sub>O emissions from grassland by nitrification inhibitor and Actilith F<sub>2</sub> applied with fertiliser and cattle slurry. *Soil Use and Management*, 18, 135-141. <http://dx.doi.org/10.1111/j.1475-2743.2002.tb00231.x>.
- Noguera, D., Kam-Rigne, L. M., Hoyos, V., Lavelle, P., Decarvalho, M. H. C. and Barot, S. (2010). Contrasted effect of biochar and earth wormson rice growth and resource allocation in different soils. *Soil Biology and Biochemistry*, 42, 1017-1027.
- Ohyama, T., Ito, M., Kobayashi, K., Araki, S., Yasuyoshi, S., Sasaki, O., Yamazaki, T., Soyama, K., Tanemura, R., Mizuno, Y. and Ikarashi, T. (1991). Analytical procedures of N, P, K contents in plant and manure materials using H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> Kjeldahl digestion method. *Bulletin of the Faculty of Agriculture, Niigata University*; [accessed 2015 Oct 19]. <http://www.researchgate.net/publication/224903946>
- Priha, O. and Smolander A. (1995). Nitrification, denitrification and microbial biomass N in soil from two N-fertilized and limed Norway spruce forests. *Soil Biology and Biochemistry*, 27, 305-310. [http://dx.doi.org/10.1016/0038-0717\(94\)00181-Y](http://dx.doi.org/10.1016/0038-0717(94)00181-Y).
- Sahrawat, K. L. (2006). Organic matter and mineralizable nitrogen relationships in wetland rice soils. *Communication in Soil Science and Plant Analysis*, 37, 787-796.

- SAS Institute. (1996). SAS User's Guide. Cary: SAS Institute Inc..
- Su, C. G., Yin, B., Zhu, Z. L. and Shen, Q. R. (2005). Gaseous loss of nitrogen from fields and wet deposition of atmospheric nitrogen and their environmental effects. *Soils* (in Chinese), 37, 113-120.
- Zaman, M., Nguyen, M. L., Blennerhassett, J. D. and Quin, B. F. (2008). Reducing NH<sub>3</sub>, N<sub>2</sub>O and NO<sub>3</sub><sup>-</sup>-N losses from a pasture soil with urease or nitrification inhibitors and elemental S amended nitrogenous fertilizers. *Biology and Fertility of Soils*, 44, 693-705.
- Zaman, M., Saggar, S., Blennerhassett, J. D. and Singh J. (2009). Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. *Soil Biology and Biochemistry*, 41, 1270-1280. <http://dx.doi.org/10.1016/j.soilbio.2009.03.011>.
- Zhang, H. M., Wang, B. R., Xu, M. G. and Fant. L. (2009). Crop yield and soil responses to long-term fertilization on a Red Soil in Southern China. *Pedosphere*, 19, 199-207. [http://dx.doi.org/10.1016/S1002-0160\(09\)60109-0](http://dx.doi.org/10.1016/S1002-0160(09)60109-0).
- Zhou, S., Sakiyama, Y., Riya, S., Song, X. F., Terada, A. and Hosomi, M. (2012). Assessing nitrification and denitrification in a paddy soil with different water dynamics and applied liquid cattle waste using the N-15 isotopic technique. *Science of the Total Environment*, 430, 93-100. <http://dx.doi.org/10.1016/j.scitotenv.2012.04.056>.
- Zou, J. W., Huang, Y., Lu, Y. Y., Zheng, X. H. and Wang, Y. S. (2005). Direct emission factor for N<sub>2</sub>O from rice winter wheat rotation systems in southeast China. *Atmospheric Environment*, 39, 4755-4765. <http://dx.doi.org/10.1016/j.atmosenv.2005.04.028>.