

EFFECT OF DICYANDIAMIDE ON MICROBIAL ACTIVITY IN THE RHIZOSPHERE AND BULK SOILS UNDER COTTON

REHMAT ALI¹, JAVED IQBAL², UM-E-ROBAB¹ AND TARIQ MAHMOOD^{1*}

¹Nuclear Institute for Agriculture & Biology, PO Box 128, Jhang Road, Faisalabad-38000, Pakistan.

²School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.

Abstract

Study was conducted under greenhouse conditions to elucidate the effects of dicyandiamide (DCD, a nitrification inhibitor) on microbial activity in the root-zone and bulk soils under cotton fertilized with two levels (60 and 120 mg kg⁻¹) of urea-N. Dicyandiamide applied @ 15–30 mg kg⁻¹ effectively inhibited nitrification at relatively high soil temperatures (19.5–33°C) prevailing during the four-week experiment period by the end of which 25–46% of the mineral N was found in NH₄⁺ form in DCD-treated pots. Application of DCD caused a minor leaf tip necrosis but without negative effects on the biomass and N yields. Averaged across treatments, microbial activity (aerobic and anaerobic soil respiration, denitrification potential, microbial biomass carrying capacity and dehydrogenase activity) was highest in the root-zone soil followed by planted-bulk and unplanted soils. Averaged across soil types, microbial activity parameters generally showed higher values in the DCD-treated soil. The stimulatory effect of DCD was more pronounced at lower N application rate, and was consistently observed in the root-zone, planted bulk and unplanted soils. At higher N application rate, however, DCD had no effect on the microbial activity of the unplanted soil. Results suggested that relatively high concentrations of DCD required to inhibit nitrification under warm climates may not be phytotoxic but may have significant implications in soil microbial processes.

Introduction

Dicyandiamide (DCD) is an effective nitrification inhibitor and widely used in agriculture for improving fertilizer N efficiency (Vilsmeier, 1991; Aulakh *et al.*, 2001; Gioacchini *et al.*, 2002; Di & Cameron, 2004; Chaves *et al.*, 2006). Besides, DCD may serve as N source for plants as it contains 67% N that becomes plant-available following its mineralization (Reider & Michaud, 1980). The mineralization of DCD in soil is strongly influenced by soil temperature (Rajbanshi *et al.*, 1992), availability of organic carbon (Reddy, 1964) and Fe³⁺ hydroxides (Amberger & Vilsmeier, 1979). Although DCD can improve fertilizer N efficiency, it is well known to have phytotoxic effects including leaf tip and margin chlorosis and necrosis, and a reduction in the biomass yield (Sommer & Rossig 1978; Maftoun & Sheibany, 1979; Reeves & Toughton, 1986; Macadam *et al.*, 2003). However, severity of these symptoms varies with plant species and with the DCD application rate (Reeves & Toughton, 1986). Dicyandiamide applied at 67-72 mg kg⁻¹ is reported to reduce root and shoot growth of cotton (Reeves & Toughton, 1986).

Studies pertaining to DCD have been mostly conducted at relatively moderate soil temperature, whereas only few deal with the behaviour of DCD under soil temperatures $\geq 30^{\circ}\text{C}$ (Rajbanshi *et al.*, 1992; Aulakh *et al.*, 2001) that are common during the summer season in semiarid subtropics. Besides, most of the literature on the use of DCD and other

*Corresponding author E.mail: tmahmood114@yahoo.com

nitrification inhibitors deals with their effectiveness in keeping the applied N fertilizer in NH_4^+ form and relatively less attention has been paid to their effects on the overall microbial activity in soil, particularly in the rhizosphere. Priming effect of DCD leading to increased mineralization of soil organic matter is well documented (Prasad & Power, 1995; Gioacchini *et al.*, 2002; Chaves *et al.*, 2006) and is attributed to NH_4^+ , which being the predominant N source (e.g. in DCD-treated soils) is utilized preferably by microbes as compared to NO_3^- (Recous *et al.*, 1990; Rangle-Castro & Taylor, 2002). Only limited information exists on the effect of N source on the belowground carbon partitioning in plants, particularly root exudation and subsequent effects on the rhizosphere microbial activity. Ammonium compared to NO_3^- nutrition of plants is known to stimulate root sugar exudation in hydroponics (Mahmood *et al.*, 2002); the phenomenon proposed as an important mechanism through which plants feed the root-associated N_2 fixing soil microbes. In the soil-grown wheat and maize plants, which were supplied with either NH_4^+ or NO_3^- as N source, microbial activity in the rhizosphere was significantly higher under NH_4^+ compared to NO_3^- nutrition (Mahmood *et al.*, 2005); the stimulatory effect was attributed mainly to the increased root sugar exudation under NH_4^+ nutrition. In that study, however, the effect of DCD *per se* was not discernable since the inhibitor was applied to both NH_4^+ - and NO_3^- -treated soils in order to keep the applied N in NH_4^+ form (in the NH_4^+ -treated soil) and to balance the side effects of DCD (in NO_3^- -treated soil). Since increased C availability is also known to stimulate mineralization of DCD (Reddy, 1964), persistence and activity of DCD in the rhizosphere may be different than that observed in bulk soils. However, comparative effects of DCD on microbial activity in the rhizosphere and bulk soils have not been reported earlier. The present study was conducted to examine the effects of DCD on microbial activity in the root-zone, planted-bulk and unplanted soils under cotton plants grown in pots with two urea-N levels.

Materials and Methods

The soil used in this study was a sandy-clay loam (Hafizabad Series; Haplic Yermosol; Anonymous, 1966) collected from the plough layer of a cotton field. It contained: total organic C, 0.54%; total nitrogen, 0.06%; pH (saturation paste), 7.9; EC (saturation extract), 0.06 S m^{-1} ; maximum water-holding capacity (WHC), 35.5%; bulk density, 1.5 g cm^{-3} ; porosity, 44.4%; sand, 57.8%; silt, 22.7% and clay, 19.5%. Five kg soil in plastic pots ($20 \times 20 \text{ cm}$, inner diameter \times depth) received a basal dose of K (as K_2SO_4) and P (as single superphosphate), each applied at 50 mg kg^{-1} . The pots were irrigated with tap water (60% WHC) and after 24-h equilibration the soil was mixed and sown with cotton (*Gossypium hirsutum* L. var CM-499; 10 seeds pot^{-1}). Fifteen days after germination, the plant population was reduced to four pot^{-1} and treatments applied. There were eight treatments (replicated four times) comprising two urea-N levels (60 and 120 mg kg^{-1}), each with four levels of DCD (0, 7.5, 15 and 30 mg kg^{-1}). In a preliminary laboratory study employing various nitrification inhibitors, only DCD applied at high concentrations ($15\text{--}30 \text{ mg kg}^{-1}$) was found effective in retarding nitrification at high soil temperatures (33°C) that prevail during the cotton-growing season in this region (unpublished data). To evaluate the efficacy of DCD in retarding nitrification of the applied urea-N, an unplanted set of pots with similar treatments was also kept under same conditions as the planted pots. The soil moisture was maintained at 60% WHC throughout the experiment.

Four weeks after treatment application, plants were harvested by cutting shoots 2-cm above the soil surface. The root-zone soil was sampled by inserting a core sampler (10×150 mm, inner diameter×depth) around the tap root. In this way, four root-zone soil cores were extracted from each pot and were pooled together to represent one replicate. Rest of the soil of the planted pots represented as the planted-bulk soil. Visible roots were manually removed, and all soils (root-zone, planted-bulk and unplanted) were sieved (<2 mm) and stored overnight at 4°C before measuring soil microbial activity parameters viz., aerobic and anaerobic respiration, denitrification capacity, microbial biomass carrying capacity and dehydrogenase activity. Roots and shoots were dried at 60°C for 48 h, weighed and ground (<0.5 mm) before analyzing for the total N.

For measuring aerobic soil respiration, 10-g portions of the moist soil were taken in 100-ml serum vials, vials sealed with silicone rubber septa and incubated at 30°C. After 12, 24, 36 and 48 h, the headspace was analyzed for CO₂-C by gas chromatography using a thermal-conductivity detector (TCD). For denitrification potential and anaerobic soil respiration, 10-gram portions of the moist soil were taken in 100-ml serum vials and treated with 10 ml of KNO₃ solution to provide NO₃⁻ level of approximately 200 mg N kg⁻¹. After sealing with silicone rubber septa, the vials were evacuated and flushed with O₂-free N₂ three times. The headspace was then replaced with 5% acid-washed C₂H₂ and the vials incubated at 30°C. After 12, 24, 36 and 48 h, the contents in the vials were vigorously shaken by hand and the headspace analyzed for N₂O (denitrification potential) and CO₂ (anaerobic soil respiration) by gas chromatography using a TCD. The N₂O data were corrected for that dissolved in the solution phase using Bunsen absorption coefficients (Moraghan & Buresh, 1977). Microbial biomass carrying capacity of the soil (Groffman & Tiedje, 1989) was determined by chloroform-fumigation incubation technique (Jenkinson & Powelson, 1976). Ten-g portions of the moist soil in 100-ml serum vials were fumigated with ethanol-free CHCl₃ for 24 h at 25°C. The CHCl₃ vapours were removed by repeated evacuation, the soils reinoculated with 0.1 g fresh soil, vials sealed with rubber septa and incubated at 30°C. After 10 days, the headspace was analyzed for CO₂-C by gas chromatography employing TCD. Microbial biomass carrying capacity was calculated by dividing the amount of CO₂-C evolved from the fumigated soil by a *k_c* factor of 0.45 (Jenkinson & Ladd, 1981). Soil dehydrogenase activity was determined by triphenyl formazan method as described by Casida *et al.*, (1964). Total N content of plant samples was determined by a micro-Kjeldahl method (Bremner & Mulvaney, 1982), whereas the soil mineral-N was measured by a micro-Kjeldahl method after extracting 10-g soil with 50 ml of 2N KCl (Keeney & Nelson, 1982).

Data were subjected to an analysis of variance followed by DMRT (Duncan's multiple range test) as described by Gomez & Gomez (1984). Results are reported on the basis of soil/plant dry weight and as mean of four replicates.

Results

The soil temperature in pots of 5 cm depth during the four-week treatment period was relatively high and varied from 19.5–33°C (average 26.8°C). In unplanted pots without DCD, nitrification of the applied urea-N was almost complete within four weeks (Table 1). The inhibitory effect of DCD on nitrification was more pronounced in treatments receiving higher urea-N level where after four weeks, 9, 23 and 46% of the mineral N was still present as NH₄⁺ in treatments receiving DCD at 7.5, 15 and 30 mg kg⁻¹, respectively (*p*<0.05; Table 1). At the lower N application rate, however, the inhibitory effect of DCD persisted only in treatment receiving highest DCD concentration (30 mg kg⁻¹) where 25% of the mineral N was found in the NH₄⁺ fraction after four weeks.

Table 1. Effect of dicyandiamide (DCD) on nitrification, and dry matter and N yields of cotton.

Parameter	Urea-N applied (mg kg ⁻¹)							
	60			120				
	DCD applied (mg kg ⁻¹)							
	0	7.5	15	30	0	7.5	15	30
Soil mineral-N (mg kg ⁻¹) ^a								
NH ₄ ⁺ -N	6.0 d ^b	7.2 d	6.5 d	20.4 c	5.3 d	12.7 c	29.2 b	56.9 a
NO ₂ ⁻ +NO ₃ ⁻ -N	87.6 c	81.0 cd	76.5 d	62.2 e	130.1 a	125.2 a	99.7 b	67.4 e
Dry matter yield (g pot ⁻¹)								
Root	1.05 abc	1.12 ab	1.27 a	1.01 bc	0.86 c	0.93 bc	1.00 bc	1.14 ab
Shoot	6.82 ab	7.02 a	6.99 a	6.50 ab	6.15 b	6.63 ab	7.06 a	7.06 a
N concentration (%)								
Root	1.28 a (13.4) ^c	1.09 a (12.2)	1.02 a (13.0)	1.12 a (11.3)	1.24 a (10.7)	1.25 a (11.6)	1.24 a (12.4)	1.08 a (12.3)
Shoot	2.23 c (152.1)	2.04 c (143.2)	2.01 c (140.5)	2.26 c (146.9)	2.56 b (157.4)	2.66 ab (176.4)	2.86 a (201.9)	2.62 ab (185.0)

^aSoil mineral-N of unplanted pots four weeks after application of urea and DCD; at the time of treatment application, the soil already contained 8 and 34 mg N kg⁻¹ of NH₄⁺ and NO₃⁻, respectively.

^bFigures within a row followed by different letter are significantly different at $p < 0.05$ (Duncan's multiple range test).

^cFigures in parentheses indicate nitrogen uptake (mg pot⁻¹).

At the lower N application rate, DCD did not affect the root and shoot dry matter yields, nor did it show significant effects on N concentration or uptake (Table 1). However, at the higher N application rate, root biomass was significantly higher with DCD applied at 30 mg kg⁻¹, whereas shoot biomass also increased due to DCD applied at 15–30 mg kg⁻¹ ($p < 0.05$; Table 1). At the higher N application rate, DCD applied at 15 mg kg⁻¹ significantly increased the shoot N concentration and uptake ($p < 0.05$; Table 1).

In the absence of DCD, increasing the N application rate from 60 to 120 mg kg⁻¹ generally had no effect on the soil microbial activity except that it increased the anaerobic soil respiration of the planted-bulk and unplanted soils, and the dehydrogenase activity of the unplanted soil. The effect of DCD varied with its concentration, N application rate and the soil type. Averaged across treatments, aerobic and anaerobic soil respiration, denitrification potential, microbial biomass carrying capacity and dehydrogenase activity showed highest values for the root-zone soil, followed by the planted-bulk and unplanted soils ($p < 0.05$; Table 2, soil type means). Averaged across soil types (treatment means, Table 2), microbial activity parameters showed higher values in the DCD-treated soil; the stimulatory effect being more pronounced and consistent in treatments receiving 60 mg kg⁻¹ of urea-N.

At the lower N application rate, DCD applied at 15–30 mg kg⁻¹ increased aerobic respiration (26–31% increase), denitrification potential (19–30% increase), microbial biomass carrying capacity (28–29% increase) and dehydrogenase activity (8–17% increase) of the root-zone soil ($p < 0.05$), whereas anaerobic soil respiration also increased (12% increase) with DCD applied at 7.5 mg kg⁻¹. The stimulatory effect of DCD was also recorded in the planted-bulk and unplanted soils ($p < 0.05$), except that microbial biomass carrying capacity of the planted-bulk and denitrification potential of the unplanted soils were not affected. In the unplanted soil, the stimulatory effect of DCD (where observed) was generally of the same magnitude (for anaerobic soil respiration and microbial biomass carrying capacity) or even higher (for aerobic soil respiration and dehydrogenase activity) than that observed for the root-zone or the planted-bulk soils.

At higher N application rate also, at least one concentration of DCD (mostly 15 mg kg⁻¹) significantly increased the microbial activity in the root-zone soil, except that denitrification potential was not affected. Increasing the N application rate from 60 to 120 mg kg⁻¹ greatly modified the effect of DCD in the planted-bulk and the unplanted soils. For the planted-bulk soil, the stimulatory effect of DCD was only observed on aerobic respiration (33% increase), whereas dehydrogenase activity was even suppressed ($p < 0.05$). For the unplanted soil, DCD applied at 30 mg kg⁻¹ even decreased the anaerobic soil respiration (29% decrease; $p < 0.05$), whereas other parameters were not affected.

Discussion

Dicyandiamide effectively inhibited nitrification during the four-week treatment period, and in unplanted pots treated with DCD at 30 mg kg⁻¹ as much as 25% (in low-N treatment) to 46% (high-N treatment) of the mineral N existed in NH₄⁺ form. We may not rule out the contribution of DCD-N itself toward the soil NH₄⁺ since the high soil temperatures during the experimental period could have caused substantial mineralization of DCD. Present results indicate that at high soil temperatures, such as those prevailing during the summer season in semiarid subtropics, relatively higher concentrations of DCD would be required for effective inhibition of nitrification. Despite the high DCD concentrations used in the present study, the only visible phytotoxicity symptom was a minor leaf-tip necrosis in all DCD-treated cotton plants but without showing negative effects on the biomass and N yields. In some treatments, DCD even stimulated the cotton

Table 2. Effect of dicyandiamide (DCD) on different indices of microbial activity in soil under cotton.

Soil type	Urea-N applied (mg kg ⁻¹)							Soil type mean
	60			120				
	0	7.5	15	30	0	7.5	15	
	DCD applied (mg kg ⁻¹)							
	60			120				
	0	7.5	15	30	0	7.5	15	30
	----- Aerobic respiration (ng C g ⁻¹ h ⁻¹) -----							
Root-zone	602 b ^a	622 b	756 a	787 a	600 b	651 b	777 a	641 b
Planted-bulk	216 ef	365 c	340 cd	356 c	262 de	350 c	351 c	348 c
Unplanted	87 h	119 gh	198 efg	172 efg	127 fgh	199 efg	197 efg	159 fgh
Treatment mean	301 D ^b	369 BC	431 A	438 A	330 CD	400 AB	441 A	383 B
	----- Anaerobic respiration (ng C g ⁻¹ h ⁻¹) -----							
Root-zone	162 bc	181 a	168 abc	151 c	151 c	174 ab	177 ab	168 abc
Planted-bulk	77 ij	132 d	104 fgh	105 fgh	108 efg	125 de	119 def	115 def
Unplanted	69 j	111 efg	95 gh	95 gh	93 ghi	106 fgh	89 hi	66 j
Treatment mean	103 E	141 A	122 CD	117 D	117 D	135 AB	128 BC	116 D
	----- Denitrification potential (ng N g ⁻¹ h ⁻¹) -----							
Root-zone	554 cd	546 cd	721 a	657 ab	584 bcd	575 bcd	642 abc	512 d
Planted-bulk	233 fg	363 e	298 ef	310 ef	224 fgh	298 ef	302 ef	315 ef
Unplanted	103 i	119 i	129 hi	130 hi	116 i	125 hi	159 ghi	152 ghi
Treatment mean	296 B	343 AB	383 A	366 A	308 B	333 AB	368 A	326 AB
	----- Microbial biomass carrying capacity (µg C g ⁻¹) -----							
Root-zone	121 def	137 cd	155 b	156 b	128 cde	145 bc	175 a	134 cd
Planted-bulk	97 ghi	112 efg	101 fgh	100 ghi	94 ghij	106 fgh	107 fgh	113 efg
Unplanted	61 l	77 jkl	82 ijk	88 hijk	70 kl	83 ijk	82 ijk	82 ijk
Treatment mean	93 C	109 B	115 AB	115 AB	97 C	111 AB	121 A	110 B
	----- Dehydrogenase activity (µg triphenyl formazan g ⁻¹ h ⁻²⁴) -----							
Root-zone	173 cd	171 cd	186 b	203 a	166 d	171 cd	177 c	175 c
Planted-bulk	89 hi	107 f	95 gh	111 ef	116 e	116 e	103 fg	97 gh
Unplanted	84 i	103 fg	110 ef	107 f	85 i	92 i	93 hi	93 hi
Treatment mean	115 E	127 BC	130 B	141 A	122 CD	126 BCD	124 CD	122 D

^aFor each parameter, figures followed by different letter (*lower case*) are significantly different at $P < 0.05$ (Duncan's multiple range test).

^bTreatment or soil type means followed by different letter (*upper case*) are significantly different at $P < 0.05$ (Duncan's multiple range test).

^cDetermined under denitrifying conditions in the presence of nitrate.

plant growth as well as the N uptake. The lack of the injurious effects of DCD on cotton plant growth in the present study may be due to relatively short period of exposure to DCD because of the high soil temperature that was conducive for its mineralization, which probably started soon after its application. These results signify that under hot summer climates such as those prevailing in the Indo-Pakistan subcontinent, the high DCD application rates (e.g., 30 mg kg⁻¹ or ~ 67 kg ha⁻¹ in the present study) required to achieve effective inhibition of nitrification in cotton fields may not be phytotoxic as compared to relatively mild or cool climates which prolong the half-life of DCD (Di & Cameron, 2004). Besides, keeping in view the increased root sugar exudation by plant roots under NH₄⁺ nutrition (Mahmood *et al.*, 2002) and the increased mineralization of DCD in the presence of available C (Reddy, 1964), it is envisaged that conditions in the rhizosphere of DCD-treated plants particularly favoured the mineralization of DCD thus alleviating its injurious effects on the cotton plant growth.

Much higher microbial activity observed in the root-zone and the planted-bulk than unplanted soils is a common phenomenon and attributable to the root-derived C (Priha *et al.*, 1999; Mahmood *et al.*, 1997; Mahmood *et al.*, 2005). In the present study, almost all microbial activity parameters generally showed higher values in treatments receiving DCD, particularly at the lower N application rate. At the lower urea application rate, the stimulatory effect of DCD was generally consistent in the root-zone, planted-bulk and unplanted soils, whereas at higher N application rate, it was totally absent in the unplanted soil. The increased microbial activity in the DCD-treated unplanted soil is attributable to the abundance of NH₄⁺-N and the preferential use of NH₄⁺ by soil microbes. However, the stimulated microbial activity in the unplanted soil only at the lower urea-N application (i.e. N-limiting conditions) indicates the role of DCD-N that was made available to soil microbes following its mineralization. In the root-zone and the planted-bulk soils, the stimulatory effect of DCD may also be attributed to the increased root exudation due to NH₄⁺ (Mahmood *et al.*, 2002; 2005). In the present study, such indirect stimulatory effect of DCD on the microbial activity in the root-zone soil was obvious in treatments receiving higher N level. At the lower N application rate, the magnitude of the stimulatory effect in the unplanted soil that was at least as much as that observed in the root-zone soil, suggests that the role of NH₄⁺ and DCD-N *per se* was also important besides the indirect stimulatory effects of NH₄⁺ through increased root sugar exudation.

In the present study, the lower urea-N application rate (60 mg kg⁻¹) is close to that recommended for cotton crop in this region (~140–160 kg ha⁻¹). Therefore, the observed stimulatory effects of DCD may also be expected under field conditions. Moreover, considering the relatively high concentrations of DCD required for inhibiting nitrification under warm climates, and the role of DCD *per se* in stimulating the microbial activity in soil, the use of DCD under semiarid subtropics may have significant implication in nutrient transformations in soil besides nitrification.

Acknowledgements

The authors thank Muhammad Asad Mehmood for technical assistance and Ghulam Rasul Tahir for help in statistical analysis. Equipment grant by the Alexander-von-Humboldt-Stiftung to carry out this work is also sincerely acknowledged.

References

- Amberger, A. and K. Vilsmeier. 1979. Dicyandiamideabbau in Quarzsand und Boden. *Z. Pflanzenernaehr. Bodendkd.*, 142: 778-785.

- Anonymous. 1966. *Guidelines for soil profile description*. Soil Survey and Fertility Branch, Land and Water Development Division, Food and Agricultural Organization of the United Nations, Rome
- Aulakh, M.S., Kuldip-Singh and J. Doran. 2001. Effects of 4-amino 1,2,4-triazole, dicyandiamide and encapsulated calcium carbide on nitrification inhibition in a subtropical soil under upland and flooded conditions. *Biol. Fertil. Soils*, 33: 258-263.
- Bremner, J.M. and C.S. Mulvaney. 1982. Nitrogen-total. In: *Methods of soil analysis*. (Eds.): A.L. Page, R.H. Miller and D.R. Keeney. Agron. Monogr. No. 9, Vol. 2, 2nd edn., American Society of Agronomy, Madison, pp. 595-662.
- Casida, L.E. Jr., D.A. Klein and T. Santoro. 1964. Soil dehydrogenase activity. *Soil. Sci.*, 98: 371-376.
- Chaves, B., A. Opoku, S. De Neve, P. Boeckx, O.V. Cleemput and G. Hofman. 2006. Influence of DCD and DMPP on soil N dynamics after incorporation of vegetable crop residues. *Biol. Fertil. Soils*, 43: 62-68.
- Di, H.J. and K.C. Cameron. 2004. Effect of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial biomass in a grazed pasture soil. *Aust. J. Soil Res.*, 42: 927-932.
- Gioacchini, P., A. Natri, C. Marzadori, C. Giovannini, L.V. Antisari and C. Gessa. 2002. Influence of urease and nitrification inhibitors on N losses from soil fertilized with urea. *Biol. Fertil. Soils*, 36: 129-135.
- Gomez, K.A. and A.A. Gomez. 1984. *Statistical procedures for agricultural research*. Wiley, Singapore.
- Groffman, P.M. and J.M. Tiedje. 1989. Denitrification in north temperature forest soils: relationships between denitrification and environmental parameters at the landscape scale. *Soil Biol. Biochem.*, 21: 621-626.
- Jenkinson, D.S. and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biol. Biochem.*, 8: 209-213.
- Jenkinson, D.S. and J.N. Ladd. 1981. Microbial biomass in soil: Measurement and turnover. In: *Soil Biochemistry*. (Eds.) E.A. Paul and J.N. Ladd, Vol. 5, Marcel Dekker, New York, pp. 415-471.
- Keeney, D.E. and D.W. Nelson. 1982. Nitrogen - inorganic forms. In: *Methods of soil analysis*. (Eds.) A.L. Page, R.H. Miller and D.R. Keeney. Agron. Monogr. No. 9, Vol. 2, 2nd Edn, Soil Science Society of Agronomy, Madison, pp. 643-698.
- Macadam, X.M.B., A. Prado, P. Merino, J.M. Estavillo, M. Pinto and C. Gonzalez-Murua. 2003. Dicyandiamide and 3,4-dimethyl pyrazole phosphate decrease N₂O emissions from grassland but dicyandiamide produces deleterious effects in clover. *J. Pl. Physiol.*, 160: 1517-1523.
- Maftoun, M. and B. Sheibany. 1979. Comparative phytotoxicity of several nitrification inhibitors to soybean *Glycine max* plants. *J. Agric. Food Chem.*, 27: 1365-1368.
- Mahmood, T., M. Woitke, H. Gimmler and W.M. Kaiser. 2002. Sugar exudation by roots of kallar grass [*Leptochloa fusca* (L.) Kunth] is strongly affected by the nitrogen source. *Planta*, 214: 887-894.
- Mahmood, T., R. Ali, K.A. Malik and S.R.A. Shamsi. 1997. Denitrification with and without maize (*Zea mays* L.) plants under irrigated field conditions. *Biol. Fertil. Soil*, 24: 323-328.
- Mahmood, T., W.M. Kaiser, R. Ali, M. Ashraf, A. Gulnaz and Z Iqbal. 2005. Ammonium versus nitrate nutrition of plants stimulates microbial activity in the rhizosphere. *Plant Soil*, 277: 233-243.
- Moraghan, J.T. and R. Buresh. 1977. Correction for dissolved N₂O in nitrogen studies. *Soil Sci. Soc. Am. J.*, 41: 1201-1202.
- Prasad, R. and J. Power. 1995. Nitrification inhibitors for agriculture, health and the environment. *Adv. Agron.*, 54: 233-282.
- Priha, O., T. Hallanttic and A. Smolander. 1999. Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizosphere of *Pinus sylvestris*, *Picea abies*, and *Betula pendula* seedlings by microscale methods. *Biol. Fertil. Soils*, 30: 14-19.

- Rajbanshi, S.S., G. Benckiser and J.C.G. Ottow. 1992. Effects of concentration, incubation temperature, and repeated applications on degradation kinetics of dicyandiamide (DCD) in model experiments with a silt loam soil. *Biol. Fertil. Soils*, 13: 61-64.
- Rangel-Castro, I.J. and D.E. Taylor. 2002. Use of different nitrogen sources by the edible ectomycorrhizal mushroom *Cantharellus cibarius*. *Mycorrhiza*, 12: 131-137.
- Recous, S., B. Mary and G. Faurie. 1990. Microbial immobilization of ammonium and nitrate in cultivated soils. *Soil Biol. Biochem.*, 22: 913-922.
- Reddy, G.R. 1964. Effect of mixing varying quantities of dicyandiamide with ammonium fertilizers on nitrification of ammonia in soil. *Can. J. Soil Sci.*, 44: 254-259.
- Reeves, D.W. and J.T. Touchton. 1986. Relative phytotoxicity of dicyandiamide and availability of its nitrogen to cotton, corn, and grain sorghum. *Soil Sci. Soc. Am. J.*, 50: 1353-1357.
- Reider, G. and H. Michaud. 1980. Improving fertilizer efficiency. The use of a dicyandiamide nitrification inhibitor. *Nitrogen*, 124: 31-35.
- Sommer, K. and K. Rossig. 1978. Einfluss der Art Nitrifikation-shemmung auf der Ertrag bei verschiedenartiger N-Dungung und Vorschlag für eine Klassifizierung. *Landwirtsch. Forsch.*, 831: 291-299.
- Vilsmeier, K. 1991. Turnover of ^{15}N ammonium sulfate with dicyandiamide under aerobic and anaerobic soil conditions. *Nutr. Cycl. Agroecosyst.*, 29: 191-196.

(Received for publication 2 December 2007)