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Effect of dicyandiamide and hydroquinone on the transformation of urea-nitrogen-15 in soil cropped to wheat

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Abstract A pot experiment with a loam soil and spring wheat as test crop showed that an application of dicyandiamide (DCD), and especially its combination with hydroquinone (HQ), gave a much larger recovery of soil urea- ^{15}N than treatments based on the application of urea alone or urea plus HQ. Most of the urea- ^{15}N applied to soil was present as organic plus chemically fixed ^{15}N in the DCD and DCD plus HQ treatments. These two treatments showed the smallest accumulation of urea-derived ($\text{NO}_3^- + \text{NO}_2^-$)- ^{15}N . Under well-drained conditions, there was a synergistic effect of the nitrification inhibitor DCD and the urease inhibitor HQ on urea- ^{15}N transformations and the recovery of fertilizer ^{15}N in soil after the application of urea.

Keywords Urea-nitrogen-15 transformation · Hydroquinone · Dicyandiamide · Synergistic effect

Introduction

Inhibitors have been used to regulate the transformation processes of fertilizer N in soil so as to improve ferti-

zer efficiency and decrease environmental pollution (Bielek and Kudeyarov 1991; Zhao et al. 1992, 1993; Zhu et al. 1997; Chen et al. 1998; Xu et al. 1999, 2000). In the presence of urease inhibitors such as hydroquinone (HQ), phenyl phosphorodiamidate and N-(*n*-butyl) phosphorothioic triamide, the urea- ^{15}N recovery in an alkaline soil was increased by 5–30% of the amount applied as fertilizer ^{15}N , and the effect depended on the inhibitor and soil type (Wang et al. 1991). In some incubation experiments, the application of nitrification inhibitors such as 4-amino-1,2,4-triazole (Juma and Paul 1983), dicyandiamide (DCD) (Guiraud et al. 1989; Vilsmeier et al. 1991; Xu et al. 1999), Didin (Guiraud et al. 1992) and nitrapyrin (Chalk et al. 1990) resulted in an increased N immobilization of the labelled fertilizer N in soil. The mineralization of the newly immobilized N in the soil was slow, occurring over periods measured in months (Shen et al. 1989). Of course, the concentration of mineral N as well as fertilizer-N efficiency depended on the competition between microbial immobilization and plant uptake (Machet et al. 1987). As far as we know, less attention has been paid to the synergistic effect of urease and nitrification inhibitors on the immobilization-mineralization turnover of fertilizer ^{15}N in soil.

Our previous work with unlabelled urea (Chen et al. 1998; Xu et al. 1999) showed that a combined application of the urease inhibitor HQ and the nitrification inhibitor DCD retarded the hydrolysis of soil urea and nitrification for a long period and decreased the accumulation of soil NO_3^- plus NO_2^- . Unfortunately, the application of unlabelled urea did not allow us to show the immobilization of fertilizer N in soil and the mineralization of the newly immobilized N fraction.

This paper mainly reports the effects of HQ and DCD on the transformation of urea- ^{15}N in soil cropped to wheat, with particular attention to N mineralization and immobilization. This investigation complemented that of Xu et al. (2000) who studied N losses and fertilizer-N distribution in wheat by using ^{15}N -enriched urea under the same experimental conditions.

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Materials and methods

Pot experiment and sampling

A pot experiment with five urease/nitrification inhibitor treatments was carried out: without urea, ^{15}N -labelled urea ($^{15}\text{N-U}$), ^{15}N -labelled urea plus HQ ($^{15}\text{N-U+HQ}$), ^{15}N -labelled urea plus DCD ($^{15}\text{N-U+DCD}$), and ^{15}N -labelled urea plus HQ plus DCD ($^{15}\text{N-U+HQ+DCD}$). Each treatment was replicated 9 times. The crop tested was spring wheat. The soil tested was a loamy meadow brown soil from the northern district of China and was classified, according to FAO taxonomy (FAO 1982), as a cambisol. Main physical and chemical properties of the soil, determined as described by Keeney and Nelson (1982), are: total N 0.11%; total P 0.06%; total C 1.77%; available N 126.6 mg kg⁻¹; available P 26.5 mg kg⁻¹; pH 6.7 (soil:water=1:5); CEC 17.9 cmol kg⁻¹; sand 34%; silt 40%; clay 36% and soil bulk density 1.36 Mg m⁻³. A composite surface soil sample (0–20 cm) was air-dried, crushed to <5 mm and thoroughly mixed. Air-dried soil (6.0 kg) was thoroughly mixed with the corresponding amount of fertilizers and inhibitors, if necessary, and placed into each experimental pot. Fertilizers of P and K as K₂HPO₄ (1.0 g) were applied as basal dressing (88.6 mg kg⁻¹ K and 75.3 mg kg⁻¹ P as K₂HPO₄). The application rate of labelled urea- ^{15}N (5.36 atom % excess, supplied by Shanghai Chemical Institute, China) was 333.3 mg kg⁻¹ air-dried soil (equivalent to 345 kg N ha⁻¹), and those of HQ and DCD were 0.3% and 5% (w/w) of the labelled urea, respectively (Zhao et al. 1992; Xu et al. 2000). Shanghai Chemical Institute supplied HQ, and its purity was 99.5%. Each experimental pot contained 12 plants.

In accordance with Kim (1995), soil and plant samples were collected at stem elongation, milky grain and mature stages of wheat growth (equivalent to 49, 62 and 94 days after fertilization), respectively. The grain, straw and root of wheat plant were separated at the mature stage. Three replicates were used for each sampling.

^{15}N analysis

Fresh soil samples were sieved (<5 mm) and extracted (20 g moist soil) with 2.0 M KCl (100 ml) in 250-ml Erlenmeyer flasks under shaking on a rotary shaker (175 r.p.m.) at 25°C for 30 min; then the extracts were filtered, and the filtrates were kept in a freezer (4°C) until analysis. Total NH₄⁺-N and (NO₃⁻+NO₂⁻)-N concentrations were determined by steam distillation with MgO-Devarda's alloy method (Keeney and Nelson 1982). The NH₃ liberated was absorbed in 2% H₃BO₃ and determined by titration with 10 mM H₂SO₄. For the measurement of ^{15}N enrichment, the liberated NH₃ was absorbed in 10 mM H₂SO₄ instead of H₃BO₃. The (NH₄)₂SO₄ solution was concentrated up to 0.250 mg N ml⁻¹ and the $^{15}\text{N}/^{14}\text{N}$ ratio measured (Axmann 1990) using a JAS CO-150 ^{15}N analyser.

Total N in plants and in soil was determined by Kjeldahl digestion (Keeney and Nelson 1982). An aliquot of digest containing 1 mg N was distilled into 10 mM H₂SO₄ and $^{15}\text{N}/^{14}\text{N}$ measured as before. The double-distillation procedure of Pruden et al. (1985) was used to minimize ^{15}N memory effects.

Calculation and statistical analysis

All data were transformed to an oven-dried-weight basis. Urea- ^{15}N recovery in plant N and in soil N (total N, exchangeable NH₄⁺-N and NO₃⁻+NO₂⁻-N) was calculated after considering the natural ^{15}N enrichment of relative samples from the unfertilized N treatment. Because soil total N was determined by Kjeldahl procedure with HF modification, the difference between total ^{15}N and mineral ^{15}N (exchangeable NH₄⁺- ^{15}N and NO₃⁻- ^{15}N plus NO₂⁻- ^{15}N) represented the organic plus chemically fixed ^{15}N pool. Means and SEs of urea- ^{15}N recovery were calculated for each fertilized N treatment. Significant differences between means were analysed by Duncan's multiple range test using STATISTICA software for Windows (release 4.5), with a confidence interval of 95%.

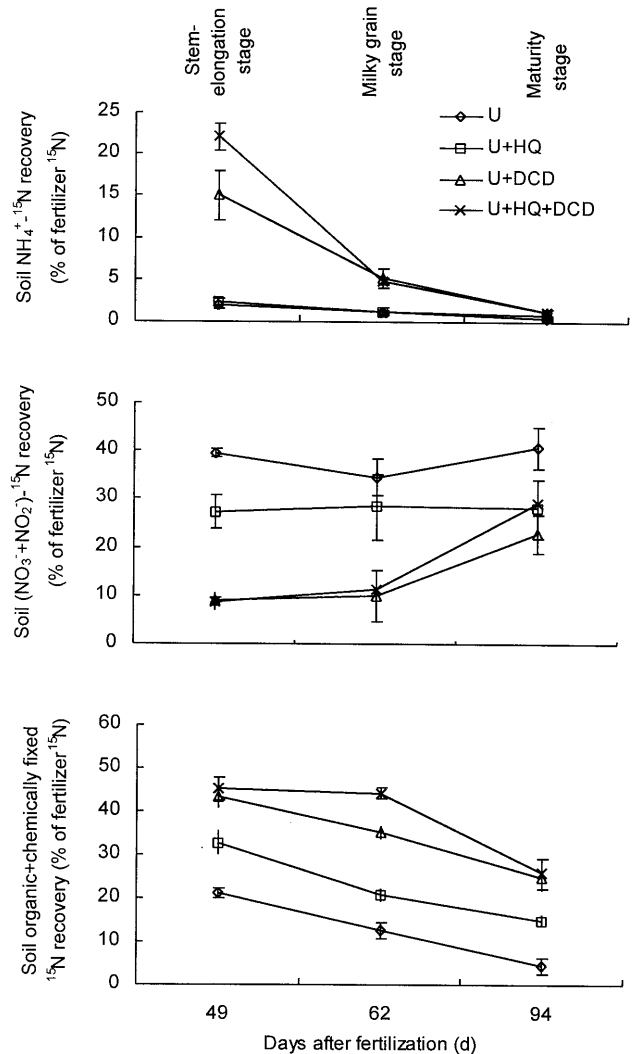


Fig. 1 Recoveries of exchangeable NH₄⁺- ^{15}N , (NO₃⁻+NO₂⁻)- ^{15}N and organic plus chemically fixed ^{15}N throughout the wheat growth period; means±SE ($n=3$). U ^{15}N -labelled urea, U+HQ U plus hydroquinone, U+DCD U plus dicyandiamide

Results and discussion

Figure 1 shows that the DCD and DCD+HQ treatments had a much larger concentration of NH₄⁺- ^{15}N in soil during the wheat growth period. The NH₄⁺- ^{15}N concentration in any treatment decreased during the wheat growth period, and this reduction in the DCD-treated soil was larger than in the urea-treated soil. Under the well-drained conditions, nitrification of soil NH₄⁺- ^{15}N was very rapid in the absence of DCD, with <5% fertilizer ^{15}N present as exchangeable NH₄⁺ in soil at the stem-elongation stage of wheat growth (49 days after fertilization) (Fig. 1). The inhibition of nitrification by DCD was short-lived because the NH₄⁺- ^{15}N concentration decreased markedly after 49 days in the DCD-treated soil (Rodgers et al. 1985; Guiraud et al. 1989; Hauser and Haselwandter 1990). Under the well-drained conditions, the influence of HQ on the hydrolysis of the applied urea

was also short-lived (Zhao et al. 1992), and for this reason there was not any difference in soil $\text{NH}_4^{+}\text{-}^{15}\text{N}$ concentrations between the $^{15}\text{N-U}$ and $^{15}\text{N-U+HQ}$ treatments after 49 days. However, the combination of HQ with DCD gave the largest $\text{NH}_4^{+}\text{-}^{15}\text{N}$ concentration in soil at the stem-elongation stage of wheat growth. Indeed, about $22.1\pm 1.6\%$ of fertilizer ^{15}N was present as exchangeable NH_4^{+} in the $^{15}\text{N-U+HQ+DCD}$ treatment, a value significantly larger than the $15\pm 3\%$ recovered in the $^{15}\text{N-U+DCD}$ treatment (Fig. 1). Therefore, under these experimental conditions, there was a synergistic effect of HQ and DCD on the retention of exchangeable $\text{NH}_4^{+}\text{-}^{15}\text{N}$ in soil after fertilization. Different experimental conditions might influence this synergistic effect, which is worthy of further investigation.

In comparison with the $^{15}\text{N-U}$ treatment, the soil treated with inhibitor(s) had a smaller concentration of $(\text{NO}_3^{-}+\text{NO}_2^{-})\text{-}^{15}\text{N}$ during the wheat growth period, with the smallest values in the $^{15}\text{N-U+DCD}$ and $^{15}\text{N-U+HQ+DCD}$ treatments prior to the milky stage (62 days after fertilization) (Fig. 1). In the absence of inhibitor(s), approximately 40% of the applied urea- ^{15}N in the soil was oxidized to $(\text{NO}_3^{-}+\text{NO}_2^{-})\text{-}^{15}\text{N}$ at the stem-elongation stage of wheat growth.

The increased $(\text{NO}_3^{-}+\text{NO}_2^{-})\text{-}^{15}\text{N}$ concentration in the DCD-treated soil after the milky stage of wheat growth (Fig. 1) was probably related to the decrease in the uptake of fertilizer ^{15}N during the wheat active phase (Xu et al. 2000) and to the decreased inhibition of nitrification prior to the milky stage. Usually, under well-drained conditions, the effective inhibitory period of added DCD on soil nitrification is 60–90 days (Rodgers et al. 1985; Hauser and Haselwandter 1990). Our results were in good agreement with those reported by Chen et al. (1998) and by Zhu et al. (1997).

In comparison with the $^{15}\text{N-U}$ treatment, the treatments with inhibitor(s) significantly increased the concentration of organic plus chemically fixed ^{15}N (Fig. 1). At the stem-elongation and heading stages, the concentration of this pool in the $^{15}\text{N-U+HQ+DCD}$ treatment was 2–3.5 times larger than in the $^{15}\text{N-U}$ treatment, whereas at the mature stage, the concentration was 6 times larger. Wang et al. (1991) also reported that urease inhibitors enhanced the N immobilization of fertilizer ^{15}N by 5–30% in an alkaline soil following the application of urea. In the presence of the nitrification inhibitor 4-amino-1,2,4-triazole, 5–8% of fertilizer N was recovered in the non-exchangeable NH_4^{+} fraction of the A horizon soil (Juma and Paul 1983). Under laboratory conditions, application of DCD has been reported to increase the immobilization of labelled N (including non-exchangeable NH_4^{+}) in soil (Guiraud et al. 1989; Vilsmeier 1991; Xu et al. 1999).

The amount of organic plus chemically fixed ^{15}N in the soil of any treatment decreased after the stem-elongation stage (Fig. 1), indicating the mineralization of the newly immobilized ^{15}N and that the release of fixed ^{15}N occurred. The mineralization of the newly immobilized ^{15}N in the $^{15}\text{N-U+HQ+DCD}$ treatment was slow before

Table 1 Balance of soil urea- ^{15}N in the various treatments (% of fertilizer ^{15}N). Values shown in the table are means of three replicates with SEs in parentheses. Within a column/row, means followed by different small/capital letters are significantly different (Duncan's multiple range test, $P<0.05$). $^{15}\text{N-U}$ ^{15}N -labelled urea, $^{15}\text{N-U+HQ}$ U plus hydroquinone, $^{15}\text{N-U+DCD}$ U plus dicyandiamide

Treatments	Stem-elongation stage			Milky grain stage			Maturity stage			
	Total	NH_4^{+}	$(\text{NO}_3^{-}+\text{NO}_2^{-})$ Organic+ chemically fixed N	Total	NH_4^{+}	$(\text{NO}_3^{-}+\text{NO}_2^{-})$ Organic+ chemically fixed N	Total	NH_4^{+}	$(\text{NO}_3^{-}+\text{NO}_2^{-})$ Organic+ chemically fixed N	
$^{15}\text{N-U}$	62.5 (0.8) aA	1.9 (0.4) aA	39.5 (0.8) cA	48.3 (5.3) aB	1.2 (0.5) aA	34.6 (3.9) bA	45.5 (2.7) aB	0.3 (0.1) aC	40.7 (4.4) bA	4.5 (1.8) aC
$^{15}\text{N-U+HQ}$	62.3 (4.5) aA	2.3 (0.4) aA	27.3 (3.5) bA	50.3 (4.0) aB	1.2 (0.2) aB	28.4 (6.8) bA	43.7 (3.1) aB	0.7 (0.1) bC	28.0 (3.2) aA	15.0 (1.4) bC
$^{15}\text{N-U+DCD}$	67.5 (2.4) aA	15.0 (3.0) bA	9.1 (0.6) aA	50.4 (3.2) aB	5.2 (1.1) bB	10.0 (5.2) aA	48.8 (1.3) aB	1.2 (0.4) bC	22.9 (3.9) aB	24.7 (2.7) cC
$^{15}\text{N-U+HQ+DCD}$	76.0 (0.5) bA	22.1 (1.6) cA	8.7 (1.8) aA	60.0 (3.5) bB	4.7 (0.4) bB	11.1 (2.8) aA	56.3 (1.3) bB	1.2 (0.4) bC	29.3 (2.0) aB	25.8 (3.4) cB

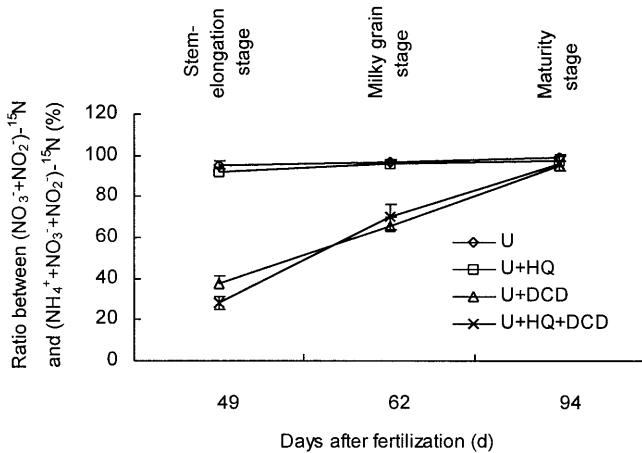


Fig. 2 Nitrification rate of $\text{NH}_4^{+}\text{-}^{15}\text{N}$ in the soil throughout the wheat growth period; means \pm SE ($n=3$). For abbreviations, see Fig. 1

the milky stage of wheat growth. Accordingly, DCD and DCD plus HQ can markedly regulate the immobilization-mineralization turnover of fertilizer ^{15}N in soil. Wickramasinghe et al. (1985) and Okereke and Meints (1985) observed the partial immobilization of fertilizer N during the growing season, without mineralization of the newly immobilized fraction. Shen et al. (1989) showed that the re-mineralization of immobilized labelled N was slow, occurring over periods of months. Under our experimental conditions, the mineralization of the newly immobilized ^{15}N in soil was probably related to N uptake by wheat plants (Xu et al. 2000) and to the high atmospheric temperature ($>25^{\circ}\text{C}$).

The balance of soil urea- ^{15}N in the various treatments is shown in Table 1. From the stem-elongation stage to the mature stage, the ^{15}N -U+HQ+DCD treatment had the largest soil ^{15}N recovery whereas the other three treatments showed no significant difference. Urea is hydrolyzed into NH_4^{+} by urease, and the NH_4^{+} can be nitrified or immobilized. The ratio of $(\text{NO}_3^{-}+\text{NO}_2^{-})\text{-N}/(\text{NO}_3^{-}+\text{NO}_2^{-}+\text{NH}_4^{+})\text{-N}$ can give an idea of the nitrification rate. The ^{15}N -U treatment presented the larger ratio values throughout the wheat growth period, and these values did not differ from those in the ^{15}N -U+HQ treatment (Fig. 2). However, the ratio values were much smaller in the ^{15}N -U+DCD and ^{15}N -U+HQ+DCD treatments, especially at the stem-elongation and milky grain stages (Fig. 2). Accordingly, under the well-drained conditions, DCD and DCD plus HQ could markedly inhibit nitrification of urea-released NH_4^{+} , with beneficial effects on the conservation and re-utilization of soil urea ^{15}N .

In conclusion, DCD, especially in combination with HQ, gave a much larger recovery of soil urea ^{15}N , with the largest concentration of organic plus chemically fixed ^{15}N and with the smallest $(\text{NO}_3^{-}+\text{NO}_2^{-})\text{-}^{15}\text{N}$ concentration. Under the well-drained conditions, there was a synergistic effect of the nitrification inhibitor DCD and the urease inhibitor HQ on the transformations of applied urea- ^{15}N and ^{15}N recovery in soil after fertilization. In

our experiment, fertilizer and inhibitors were mixed thoroughly with soil, and this procedure cannot be applied in the field. Therefore, further studies under field conditions are required to assess the effects of these inhibitors in situ.

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References

- Axmann H (1990) Method for ^{15}N determination in use of nuclear techniques in studies of soil-plant relationship. IAEA training course, series no. 2. IAEA, Vienna
- Bielek P, Kydeyarov VN (1991) Nitrogen cycles in present agriculture. Priroda, Brastilava
- Chalk PM, Victoria RL, Muraoka T (1990) Effect of a nitrification inhibitor on immobilization and mineralization of soil and fertilizer nitrogen. *Soil Biol Biochem* 22:533–538
- Chen LJ, Boeckx P, Zhou LK, Van Cleemput O, Li RH (1998) Effect of hydroquinone, dicyandiamide and encapsulated calcium carbide on urea-N uptake by spring wheat, soil mineral N content and N_2O emission. *Soil Use Manage* 14:230–233
- FAO (1982) UNESCO document D: draft definitions of soil units at high levels. FAO, Rome
- Guiraud G, Marol C, Thibaud MC (1989) Mineralization of nitrogen in the presence of a nitrification inhibitor. *Soil Biol Biochem* 21:29–34
- Guiraud G, Marol C, Fardeau JC (1992) Balance and immobilization of $(^{15}\text{NH}_4)_2\text{SO}_4$ in a soil after the addition of Didin as a nitrification inhibitor. *Biol Fertil Soils* 14:23–29
- Hauser M, Haselwandter K (1990) Degradation of dicyandiamide by soil bacteria. *Soil Biol Biochem* 22:113–114
- Juma NG, Paul EA (1983) Effect of a nitrification inhibitor on N immobilization and release of ^{15}N from nonexchangeable NH_4^{+} and microbial biomass. *Can J Soil Sci* 63:167–175
- Keeney DR, Nelson DW (1982) Nitrogen – inorganic forms. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, part 2. Chemical and microbiological properties*, 2nd edn. American Society of Agronomy, Madison, Wis., pp 643–687
- Kim HT (1995) *Soil sampling, preparation and analysis*. Dekker, New York
- Machet J-M, Pierre D, Recous S, Rémy J-C (1987) Real utilization coefficient significance and consequences for nitrogenous fertilization of crops. *C R Acad Agric Fr* 73:39–55
- Okereke GU, Meints VW (1985) Immediate immobilization of labeled ammonium sulfate and urea nitrogen in soil. *Soil Sci* 140:105–109
- Pruden G, Powlson DS, Jenkinson DS (1985) The measurement of ^{15}N in soil and plant material. *Fert Res* 6:205–218
- Rodgers GA, Wickramasinghe KN, Jenkinson DS (1985) Mineralization of dicyandiamide, labeled with ^{15}N , in soils. *Soil Biol Biochem* 17:253–254
- Shen SM, Hart PBS, Powlson DS, Jenkinson DS (1989) The nitrogen cycle in the Broadbalk wheat experiment: ^{15}N -labelled fertilizer residue in the soil microbial biomass. *Soil Biol Biochem* 21:529–533
- Vilsmeier K (1991) Turnover of ^{15}N ammonium sulfate with dicyandiamide under aerobic and anaerobic soil conditions. *Fert Res* 29:191–196

- Wang ZP, Van Cleemput O, Li LT, Baert L (1991) Effect of organic matter and urease inhibitors on urea hydrolysis and immobilization of urea nitrogen in an alkaline soil. *Biol Fertil Soils* 11:101–104
- Wickramasinghe KN, Rodgers GA, Jenkinson DS (1985) Transformations of nitrogen fertilizers in soil. *Soil Biol Biochem* 17:625–630
- Xu XK, Wang ZJ, Zhou LK, Liu Y (1999) Research advances for fate of fertilizer-N in soil-plant system. *Adv Environ Sci* 7:83–88
- Xu XK, Zhou LK, Van Cleemput O, Wang ZJ (2000) Fate of urea-¹⁵N in a soil-wheat system as influenced by urease inhibitor hydroquinone and nitrification inhibitor dicyandiamide. *Plant Soil* 220:261–270
- Zhao XY, Zhou LK, Wu GY (1992) Urea hydrolysis in a brown soil: effect of hydroquinone. *Soil Biol Biochem* 24:165–170
- Zhao XY, Zhou LK, Li RH, An GR, Zhang B (1993) Effect of hydroquinone on maize yield and urea efficiency. *Soil Biol Biochem* 25:147–148
- Zhu ZL, Wen QX, Freney JR (1997) *Nitrogen in soils of China*. Kluwer, Dordrecht