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Effects of chlorothalonil and carbendazim on nitrification and denitrification in soils

LANG Man, CAI Zucong*

State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China. E-mail: mlang@issas.ac.cn

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Abstract

The effects of chlorothalonil and carbendazim on nitrification and denitrification in six soils in upland and rice paddy environments were investigated. Laboratory aerobic (60% water holding capacity) and anaerobic (flooded) conditions were studied at 25°C and fungicide addition rates of 5.5 mg/kg A. I. (field rate, FR), 20 times (20FR) and 40 times (40FR) field rate, respectively. The results indicated that chlorothalonil at the field rate had a slight inhibitory effect on one soil only, and that soil did not nitrify much in the first place. But chlorothalonil at higher rates inhibited nitrification significantly in all soils. For soils JXP and JXU with a pH of less than 5.0, chlorothalonil almost completely stopped their nitrification at 20FR and 40FR during the whole 14 d incubation period. For soils HNP and HNU with a pH of greater than 8.0, chlorothalonil also significantly inhibit nitrification at 20FR and 40FR ($p < 0.05$). However, NH_4^+ that was added to the soil was also almost completely nitrified by the end of the incubation period in these two soils. The effects of chlorothalonil at 20FR and 40FR on the nitrification of JSP and JSU soils, with a pH of 5.4 and 7.2, respectively, were intermediate between the other soil types. Chlorothalonil had no effect on denitrification at the field rate and had little effect at the higher rates of application in some soils. Carbendazim had essentially no effect on nitrification and denitrification in soils assessed.

Key words: fungicide; nitrification; denitrification; chlorothalonil; carbendazim

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Introduction

Fungicides are widely applied to control various plant diseases around the world. When using fungicides to prevent or reduce fungal pathogens on crop plants, a thin plant canopy, over-application, or application followed by irrigation or rainfall may cause the accumulation of fungicides in soils (Cisar and Snyder, 1996; Petrovic *et al*., 1996). Like other pesticides, fungicides are biotoxicants, which interfere not only with the biochemical and physiological reactions of the target plant pathogens, but may also influence populations or the activity of other non-target microorganisms in the soil (Tu, 1993). With pesticides, the toxic effects on microorganisms associated with steps in the N cycle are of particular interest (Lal, 1988).

Chlorothalonil and carbendazim are two popular fungicides used commonly to control a broad spectrum of plant diseases in a wide variety of crops (Sherrard *et al*., 2003; Regitano *et al*., 2001; Koolhaas *et al*., 2004). In many parts of China, chlorothalonil is used in the control of rice blast (*Pyricutaria oryzae* Cav) which is the most harmful disease induced by fungi and can lead to severe yield loss (Zhang *et al*., 2006). Carbendazim is the most widely used active ingredient in the benzimidazole carbamate class of fungicides. Moreover, carbendazim is the major product of the degradation of other benzimidazole fungicides (Buchenauer *et al*., 1973; Singh *et al*., 1990; Singh and Chiba, 1993).

Nitrification and denitrification are two important processes involved in N cycling which may be affected by the application of fungicides. However, previous studies have shown contradictory effects of fungicides on these processes. For example, Chen and Edwards (2001) indicated that chlorothalonil at a level of 54 mg/kg A. I. (Active ingredient) increased the net nitrification rate. Tu (1994) measured a 40% reduction in nitrification when chlorothalonil was added to soils at a rate of 10 mg/kg A. I. In support of the latter work, Martens and Bremner (1997) observed that chlorothalonil inhibited nitrification by up to 90% at 50 mg/kg A. I. Other studies have indicated that chlorothalonil has no effects on NO_3^--N production in forestry soil at 10 and 100 mg/kg (Nakos, 1980). In addition to chlorothalonil, carbendazim may also exert no appreciable effect (Ramakrishna *et al*., 1979) or a slight stimulatory (Wessén *et al*., 1978) or inhibitory effect (Burrows and Edwards, 2000) on the nitrification of soil. Since different dosages and incubation conditions were employed in the studies described above, it is difficult to compare results across experiments and to

* Corresponding author. E-mail: zccai@issas.ac.cn

identify the main reasons responsible for the contrasting findings. Little information is available about the effects of chlorothalonil and carbendazim on denitrification. Although, as a group, denitrifiers are regarded as being more tolerant to fungicides than nitrifiers (Nakos, 1980; Martínez-Toledo *et al*., 1998), the effects of pesticides on denitrification may be affected by the amount of available carbon present in the soil (Carlisle and Trevors, 1986). In addition, other factors such as soil texture, pH and organic matter content may impact the availability and toxicity of pesticides to microorganisms in the soil (Beulke and Malkomes, 2001; Kim *et al*., 2002; Monkiedje and Spiteller, 2002).

Our objectives were to investigate the effects of chlorothalonil and carbendazim on nitrification and denitrification in soils differing in pH and land use and to confirm previous conclusion that nitrification is more susceptible to be affected by fungicide than denitrification.

1 Materials and methods

1.1 Soil sample

To compare changes in nitrification and denitrification in response to fungicides in upland and rice paddy soils, samples were taken from upland soils and adjacent paddy fields from Jiangxi (JX), Jiangsu (JS) and Henan (HN) Provinces, China. Soil samples were collected from 0 to 20 cm depth and named as upland soils JXU, JSU and HNU and rice paddy soils JXP, JSP and HNP. According to Soil Taxonomy System of the USA, the red soils JXP and JXU are classified as Ultisols, HNP and HNU as Fluvo-aquic soils, JSP and JSU as Aquic Primosols and Udic Argosols, respectively. Before use, soil samples were air-dried, ground to pass a 2-mm sieve, and stored at 4°C.

1.2 Physical and chemical analysis

The characteristics of the soils are summarized in Table 1. The pH measurements were performed after shaking the soil with deionized water (1:2.5 mass ratio) for 10 min. The mixture was then left for 2 min and the pH recorded using a digital type DMP-2 mV/pH meter (Ronghua, Jintan, China). Total N and total organic C contents were determined using a macro elemental analyzer (Elementar Analysensysteme, Germany). For the calcareous soils HNP and HNU, the samples were treated with HCl before total N and organic C contents were determined. Soil texture was measured with a laser particle characterization analyzer (Beckman Coulter, Los Angeles, USA). Soil cation exchange capacity (CEC) was quantified following the procedure by Lu (2000). The NH_4^+-N and NO_3^--N components of the soils were extracted by shaking for 1 h on a rotary shaker with 75 mL of 2 mol/L KCl solution and the filtrates were stored at 4°C and analysed within 1 week. The concentrations of NH_4^+-N and NO_3^--N in the extracts were determined by a colorimetric method, using a continuous flow analyzer (Skalar, Breda, Holand).

1.3 Soil incubation

Technical-grade chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile, purity 99.2%) and carbendazim (methyl benzimidazole-2-ylcarbamate, purity 98.5%) from Dr. Ehrenstorfer GmbH Company (Augsburg, Germany) were used in the experiments. Fungicides were added at rates of 0 (CK), 5.5 mg/kg A. I. (field rate, FR), 110 mg/kg A. I. (20 times field rate, 20FR), and 220 mg/kg A. I. (40 times field rate, 40FR) to the soils and mixed thoroughly. Field rate was determined by calculating the single weight of the active ingredient when applied to the soil, assuming homogenous distribution of the pesticide to a depth of 2 cm (surface application) and a soil density of 1.2 g/cm^3.

Nitrification experiments were carried out in the laboratory as described by Zhao *et al*. (2007). For each soil treatment sample, a series of 250-mL conical flasks were prepared, each with 30 g (oven-dry basis) of soil pretreated with fungicide at rates of FR, 20FR and 40FR, respectively. Deionised water was added evenly over the soil surface with a pipette to bring the moisture content to 40% water-holding capacity (WHC). The flasks were sealed with rubber stoppers and preincubated at 25 ± 1°C for 3 d. Ammonium sulphate solution was then applied uniformly to the preincubated soils at a rate of 150 mg N/kg, and the final soil moisture content was adjusted to 60% WHC. All the flasks were covered and incubated at 25 ± 1°C in the dark for 14 d. During the incubation, the samples were aerated by removing the covers for 1 h each day. The moisture content of the incubated soil samples was maintained by adding water every 3 or 4 d to compensate for water lost through evaporation.

Denitrification was measured using an incubation procedure described by Drury *et al*. (1998). Thirty gram of soil treated with fungicide was transferred into a 250-mL conical flask and deionised water was added with a soil/water ratio 1:1 (*W/W*). The amount of water added was adjusted for the quantity of water already contained in the soil. The samples were pre-incubated at 25 ± 1°C for 3 d. Potassium nitrate (150 mg N/kg) solution (10 mL) was subsequently added to each flask. A multiport vacuum manifold allowed six flasks to be simultaneously flushed

Table 1 Characteristics of the soils studied

Soil code	Soil type	pH	Organic C (g/kg)	Total N (g/kg)	C/N	Sand (%)	Silt (%)	Clay (%)	CEC (cmol/kg)	NH_4^+-N (mg N/kg)	NO_3^--N (mg N/kg)
JXP	Ultisols	4.66	14.3	1.46	9.79	53.4	34.2	12.5	9.08	8.17	6.91
JXU	Ultisols	4.58	7.9	0.86	9.19	40.8	42.2	17.0	11.3	5.63	1.21
JSP	Aquic Primosols	5.41	19.4	1.81	10.7	41.7	46.9	11.5	10.3	5.81	3.34
JSU	Udic Argosols	7.20	20.5	1.78	11.5	41.9	38.8	19.3	13.4	5.74	9.22
HNP	Fluvo-aquic soils	8.24	4.7	0.45	10.5	67.5	24.4	8.08	7.08	4.25	3.39
HNU	Fluvo-aquic soils	8.51	7.5	0.71	10.5	68.3	22.9	8.82	7.73	3.71	3.28

with N_2 at 0.101 MPa pressure. The flasks were vacuumed and then filled with N_2 on three occasions and then sealed and incubated at 25 ± 1°C in the dark for 14 d.

At day 0, 1, 3, 5, 9, and 14, three flasks from each treatment were taken randomly as replicates to measure the changes of NO_3^--N and NH_4^+-N contents in soil by the 2 mol/L KCl extraction procedure as described above.

1.4 Data analysis

Taking the NO_3^--N and NH_4^+-N contents before the incubation as baselines, the net nitrification rate (N), net denitrification rate (D) and net mineralization rate (M) were calculated as follows:

$$N = (N_t - N_0)/t, \quad D = (N_0 - N_t)/t, \quad M = (N_{it} - N_{i0})/t \quad (1)$$

where, N_0 and N_t are the NO_3^--N (including NO_2^--N) contents in the soil at time 0 and t after incubation, respectively, and N_{i0} and N_{it} are the inorganic N (NO_3^--N + NH_4^+-N) contents in the soil at time 0 and t after incubation. The degree of inhibition of nitrification and denitrification following fungicide application was calculated by the following Eq. (2).

$$N_I = \frac{N_{CK} - N}{N_{CK}} \times 100\% \qquad D_I = \frac{D_{CK} - D}{D_{CK}} \times 100\% \quad (2)$$

where, N_I (%) is the degree of inhibition of nitrification by fungicide addition; D_I (%) is the degree of inhibition of denitrification by fungicide addition; N_{CK} (mg N/(kg·d)) is the net nitrification rate in soils without fungicide addition; N (mg N/(kg·d)) is the net nitrification rate in soils treated with fungicide; D_{CK} (mg N/(kg·d)) is the net denitrification rate in soils without fungicide addition; D (mg N/(kg·d)) is the net denitrification rate in soils treated with fungicide.

A one-way ANOVA analysis and Duncan's multiple range test (DMRT) at a significance of $p < 0.05$ were applied to examine differences in the net nitrification rate, net mineralization rate, net denitrification rate, and the degree of inhibition. The differences in degree of inhibition between nitrification and denitrification of each treatment were analyzed by t-tests. All statistical calculations were performed using SPSS procedures (SPSS 13.0).

2 Results

2.1 Nitrification and denitrification in soils without fungicide addition

The time courses of NO_3^- accumulation and changes in the nitrification ratio (percentage of NO_3^--N in inorganic N) differed markedly among the 6 soils (Fig. 1). Nitrification proceeded rapidly in the alkaline soils HNP, HNU and the neutral soil JSU, with the nitrification ratio 99% at day 5 and 9 after incubation, respectively (Fig. 1). The acid soil JSP showed evidence of a lower nitrification rate and obtained a nitrification ratio of only 52% by the end of the 14 d incubation period. Nitrification activity in the other two acid soils JXP and JXU was extremely weak, and the nitrification ratio of these two soils was below 10% at the end of the incubation (Fig. 1). There was a significant positive correlation between the net nitrification rate in the first 3 days of incubation and soil pH (R^2 = 0.990, $p < 0.001$). Denitrification of the soils followed a different pattern to that of nitrification (Fig. 2). The fastest denitrification rate was observed in JSP soil in which NO_3^- added to the samples disappeared completely within 5 d at the start of incubation, and the slowest rate was found in JXU soil where about 80% of this NO_3^- was retained at the end of the incubation. Statistical analysis showed that the denitrification rate in the first 3 days of incubation was positively and significantly correlated with the organic C content in the soil ($R^2 = 0.573$, $p < 0.001$).

2.2 Effect of fungicide addition on nitrification

The field application rate of chlorothalonil inhibited nitrification activity in the JXP and JXU soils to a less degree but had no effect on the other 4 soils (Fig. 3). Chlorothalonil at the levels of 20FR and 40FR almost completely inhibited nitrification in JXP and JXU soils throughout the entire incubation period. For JSP and JSU soils, nitrification was drastically reduced by chlorothalonil at 20FR and 40FR. Although a slow decrease in NH_4^+-N and slow increase in NO_3^--N was apparent, activity was inhibited more at the 40FR level than 20FR. The nitrification of NH_4^+ added to samples of

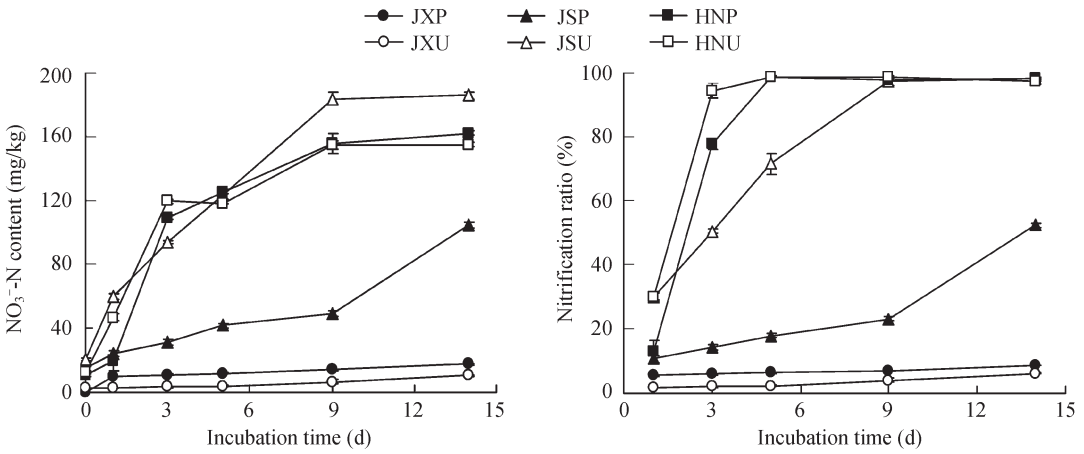

Fig. 1 Nitrification patterns in 150 mg N/kg $(NH_4)_2SO_4$-amended soils without fungicide addition during a 14-d incubation period at 25°C and a moisture content of 60% WHC.

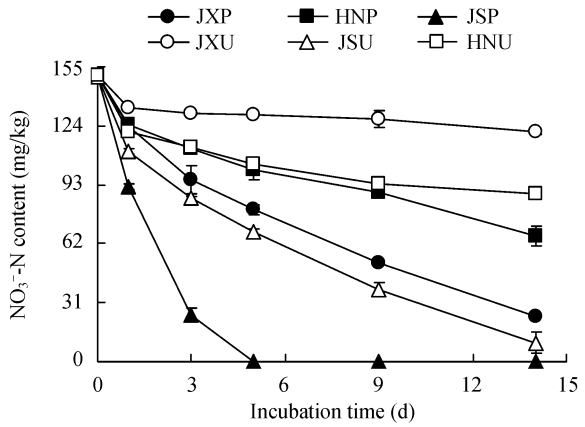

Fig. 2 Nitrate contents in 150 mg N/kg KNO_3 amended soils without fungicide addition during a 14-d anaerobic incubation period at 25°C.

HNP and HNU was complete within 5 d in the absence of chlorothalonil (Fig. 4). The addition of chlorothalonil at the 20FR and 40FR levels dramatically inhibited the oxidation of ammonium to nitrate corresponding to a slower increase in NO_3^- contents in these soils during the first 5 d of incubation (Fig. 3). The inhibitory effects of chlorothalonil at the 20FR and 40FR levels were more apparent in the time course of NH_4^+ disappearance than in the time course of NO_3^- accumulation (Figs. 3 and 4). However, at the end of the 14 d incubation, NH_4^+ added to the samples was almost completely nitrified in HNP and HNU soils with chlorothalonil at the rates of 20FR and 40FR.

In general, the inhibitory effect of carbendazim on nitrification was very weak or absent (Figs. 5 and 6). No appreciable inhibition of nitrification occurred in JXP or HNU soils treated with carbendazim, as evidenced by the overlap between treatments in the time courses of NO_3^- and NH_4^+ content changes in the soils during incubation. Very slight inhibition occurred in JSP and JSU soils because, during incubation, the NO_3^- content was smaller and the NH_4^+ content was larger after treatment with carbendazim at 40FR compared to control. However, the NO_3^- concentration was greater in JXU and HNP soils treated with carbendazim than in the controls, while the NH_4^+ content was similar at all fungicide concentration levels during the incubation period. Thus, nitrification in these two soils would be stimulated by application of carbendazim at any level studied.

2.3 Effect of fungicide addition on mineralization

Chlorothalonil at the FR level had no significant effects on the average net N mineralization rate over the 14 d incubation period in any soil. However, the 20FR and 40FR levels resulted in a significant increase in this rate in all soils except JXU (Fig. 7A). This stimulatory effect was more noticeable in JSP, HNP and HNU soils than in JXP and JSU. The effects of carbendazim on the net mineralization rate were weaker than those of chlorothalonil and also varied with soils (Fig. 7). No effect of carbendazim was observed in the soils JSP, JSU and HNU at any addition rate. Conversely, in JXP and JXU soils, the net mineralization rate was slightly, but significantly stimulated by the addition of carbendazim and, in the case of HNP, it increased significantly as the concentration of carbendazim rose.

2.4 Effect of fungicide addition on denitrification

The denitrification capacity of all soils treated with chlorothalonil at the field application rate was not significantly different from that of the controls throughout the

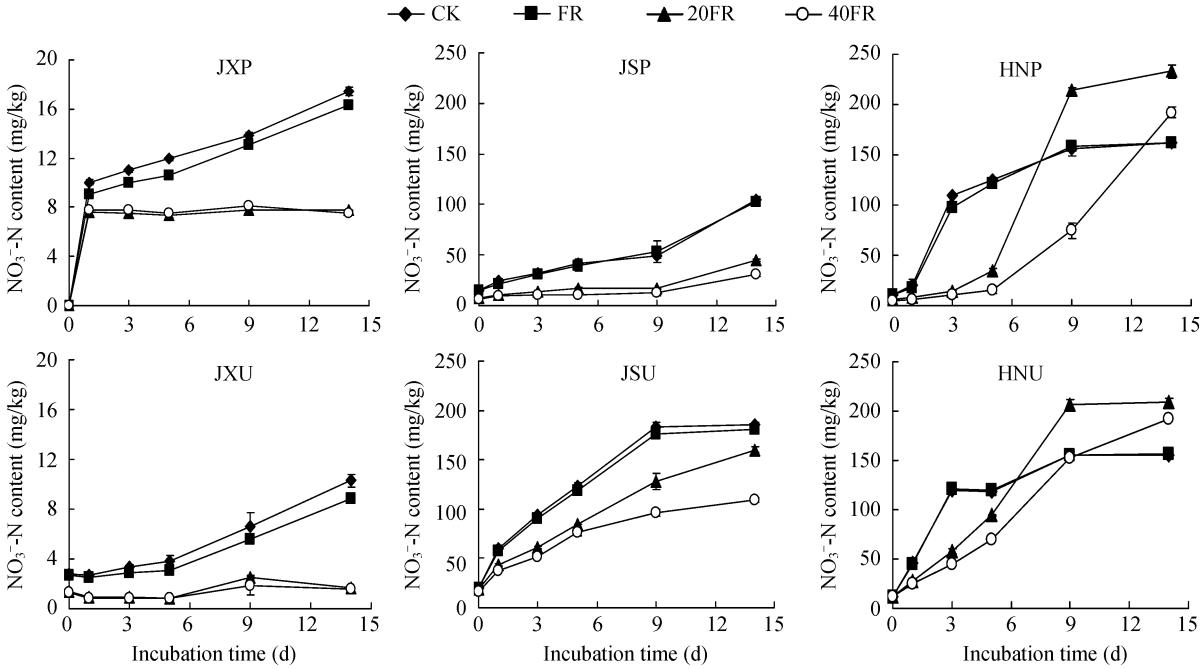

Fig. 3 Changes in nitrate concentration in 150 mg NH_4^+-N/kg amended soil samples with chlorothalonil added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d incubation period at 25°C and 60% WHC.

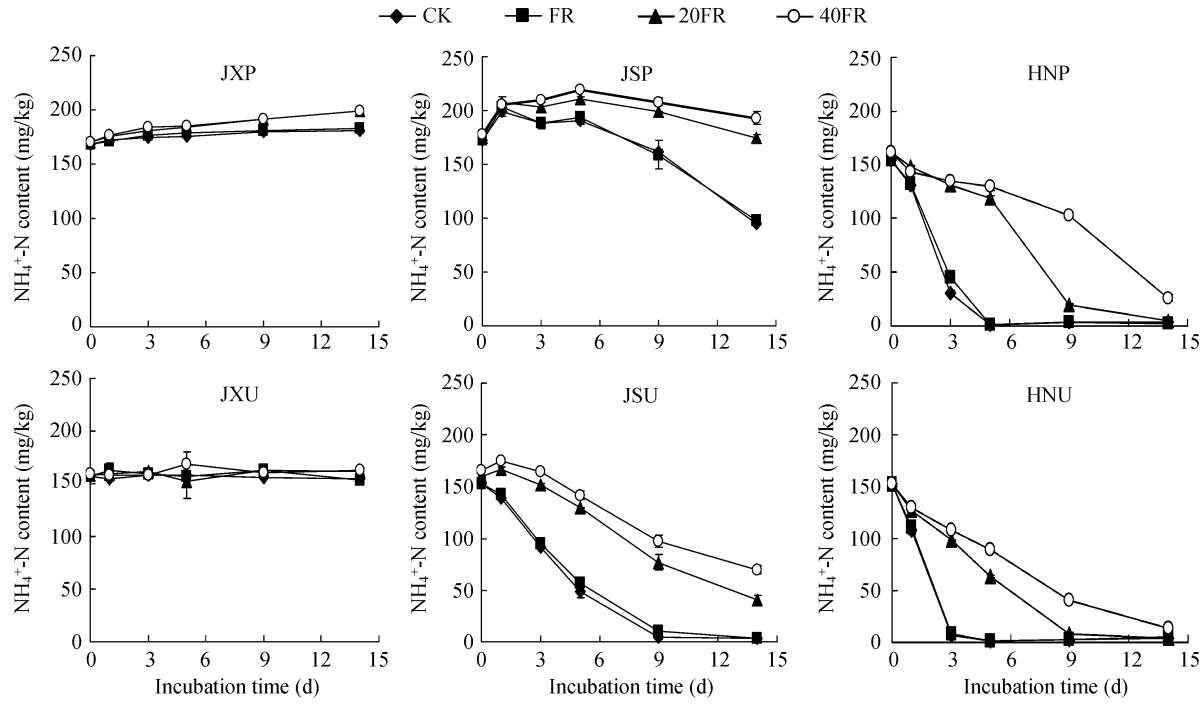

Fig. 4 Changes in ammonium concentrations in 150 mg NH_4^+-N/kg amended soil samples with chlorothalonil added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d incubation period at 25°C and 60% WHC.

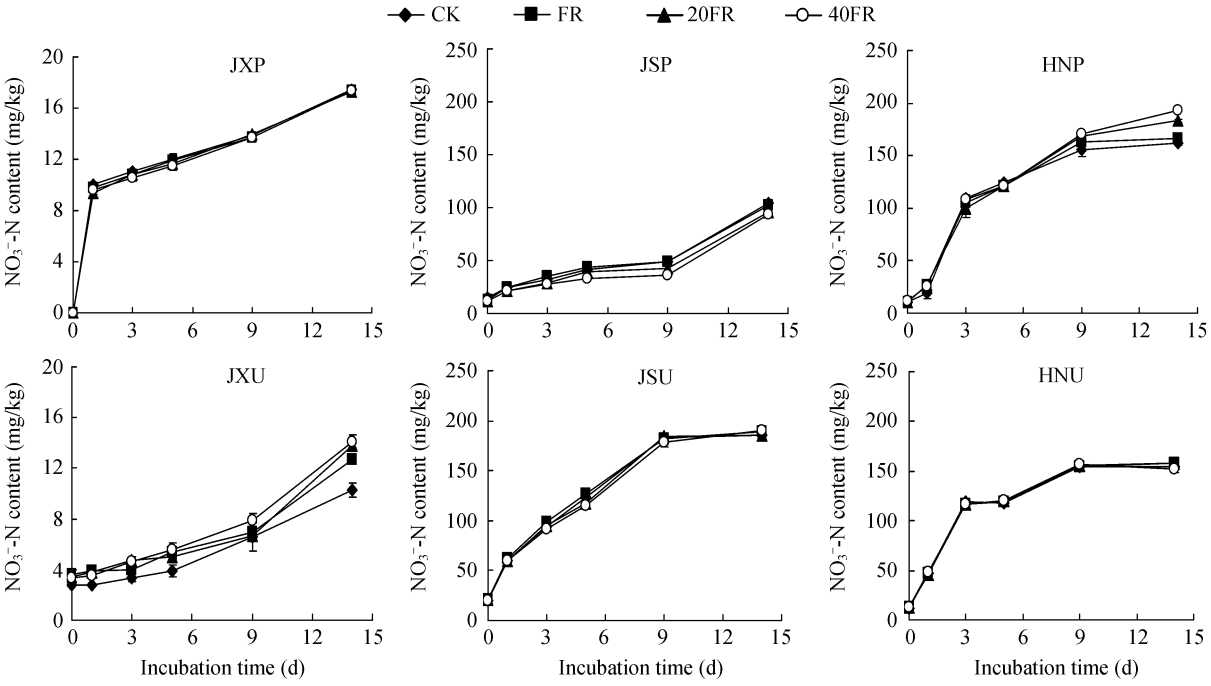

Fig. 5 Changes in nitrate concentrations in 150 mg NH_4^+-N/kg amended soil samples with carbendazim added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d incubation period at 25°C and 60% WHC.

entire incubation period (Fig. 8). For JXP soil, denitrification in the 20FR and 40FR systems was significantly inhibited between day 3 and day 9, but then recovered to control levels at day 14. The nitrate content of JSP samples that lacked chlorothalonil was fully consumed with 5 d incubation, whilst application of chlorothalonil at the 20FR and 40FR levels caused a marked inhibition of denitrification and required an additional 4 d to reach this point. Chlorothalonil at the 20FR and 40FR levels inhibited denitrification in HNP and HNU soils, and the degree of inhibition increased with incubation time, whereas no appreciable effects of chlorothalonil addition occurred in JXU and JSU samples independent of the application rate. In contrast with chlorothalonil, all application rates of carbendazim exerted little or no effects on denitrification in the soils (Fig. 9).

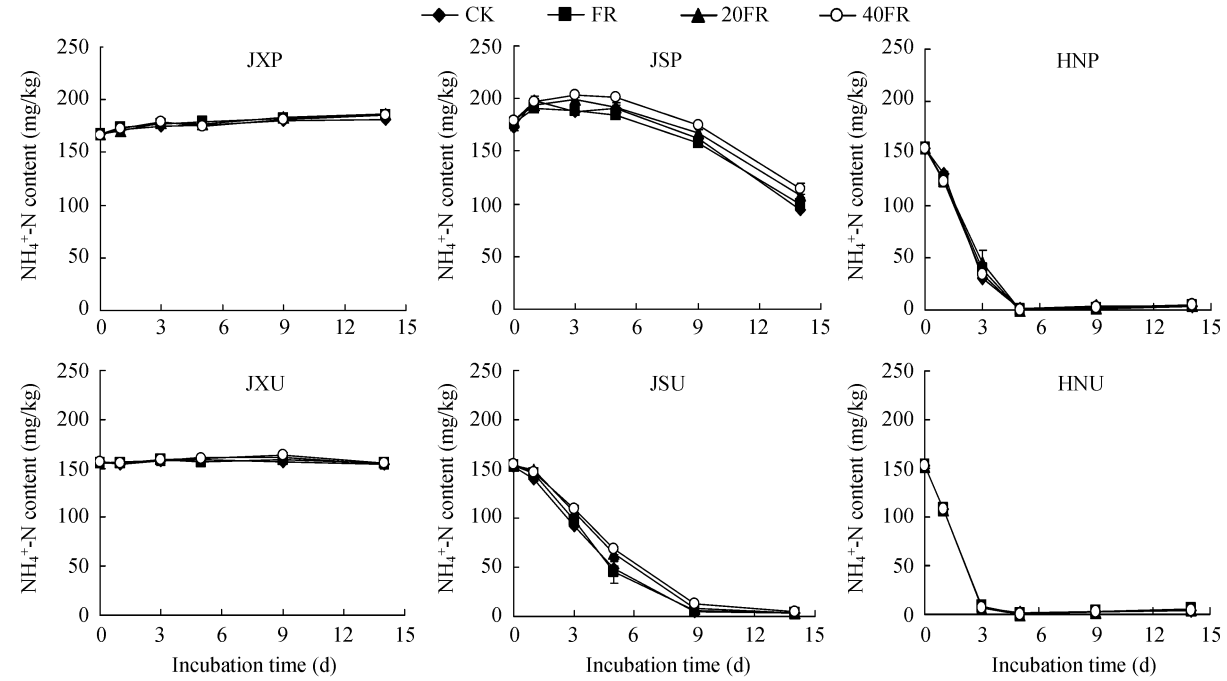

Fig. 6 Changes in ammonium concentrations in 150 mg NH_4^+-N/kg amended soil samples with carbendazim added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d incubation period at 25°C and 60% WHC.

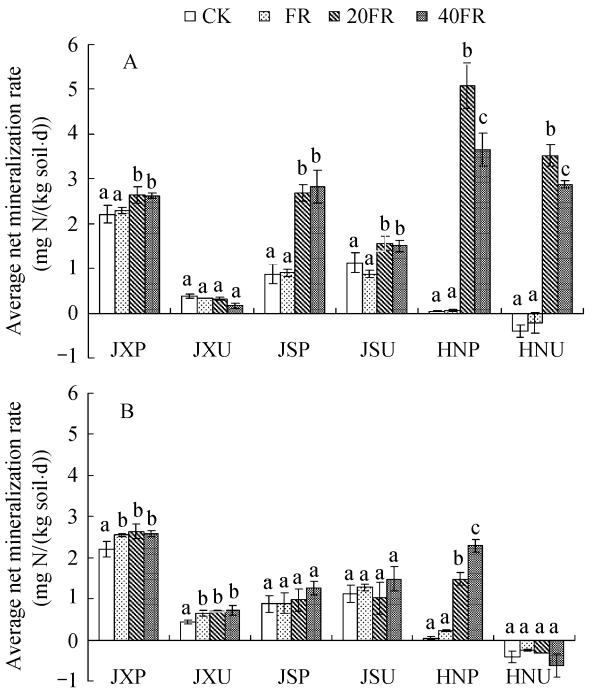

Fig. 7 Average net mineralization rate in 150 mg NH_4^+-N/kg amended soil samples with chlorothalonil (A) and carbendazim (B) added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d incubation period at 25°C and 60% WHC. Bars indicate the standard deviation of the mean of three replicates. Different letters indicate a significant difference ($p < 0.05$, Duncan).

2.5 Effect of chlorothalonil addition on degree of inhibition of nitrification and denitrification

To allow comparison of the inhibitory effects of chlorothalonil addition on nitrification and denitrification between different soils, the average net nitrification and denitrification rates and the inhibitory degree over the first 3 d of the incubation period were calculated (Table 2). The results indicate that there was no significant difference in the impact of chlorothalonil on nitrification compared to denitrification at the FR level, with the exception of soil JXU. Conversely, at the 20FR and 40FR levels, the inhibitory effects on nitrification were much greater than that on denitrification.

3 Discussion

Our results indicated that the effects of fungicides on nitrification and denitrification in soils varied not only with the fungicides used and their dosage, but also with the soils properties. This confirms the findings of other previous studies that soil type impacts upon the behaviour of pesticides in the soil (Beulke and Malkomes, 2001; Chen and Edwards, 2001; Kim *et al*., 2002; Monkiedje and Spiteller, 2002). Carbendazim was much less effective in inhibiting nitrification and denitrification than chlorothalonil and, evidently, the chemical nature of fungicides determines their effects on N transformation in soil (Monkiedje and Spiteller, 2002). The inhibitory effects of chlorothalonil on nitrification were much greater than its effects on denitrification (Table 2), which agrees with the results of Martínez-Toledo *et al*. (1998) and Kinney *et al*. (2005) who reported that denitrifiers are more tolerant to fungicides than nitrifiers.

3.1 Effect of fungicides on nitrification

In general, nitrification is significantly weaker in soils with low pH (Hankinson and Schmidt, 1988; Katyal *et al*, 1988; Hayatsu and Kosuge, 1993). The number of nitrifiers is less and their activity is weaker in acid soils

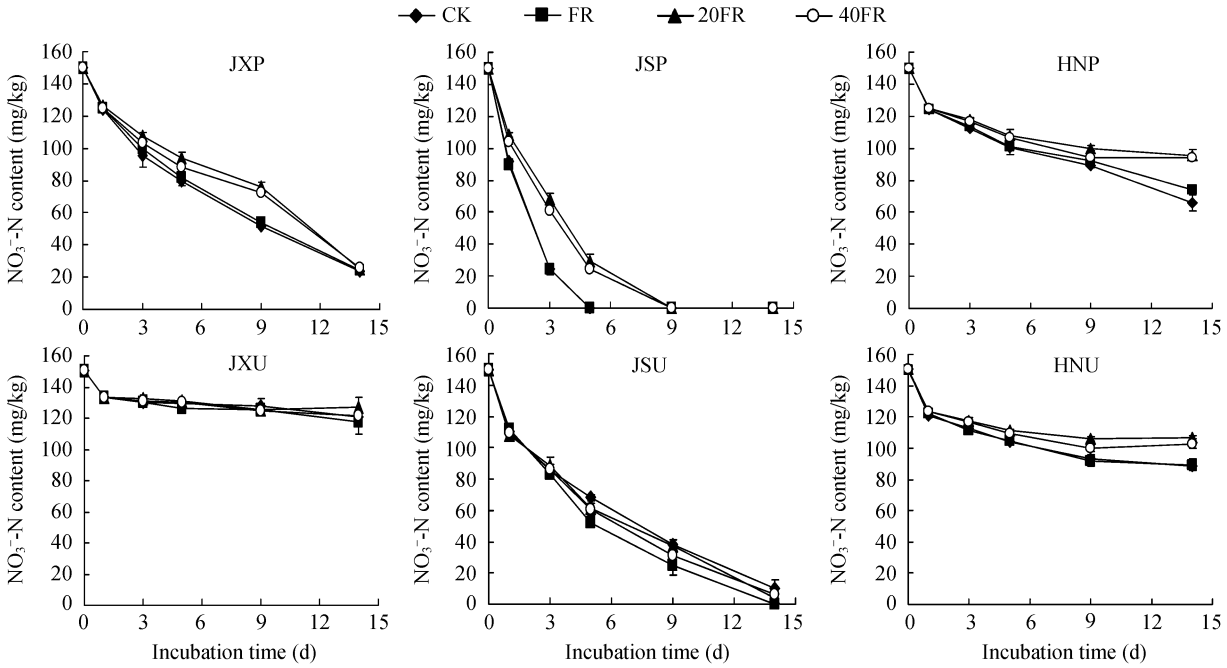

Fig. 8 Changes in nitrate concentrations in 150 mg NO_3^--N/kg amended soil samples with chlorothalonil added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d flooded incubation period at 25°C.

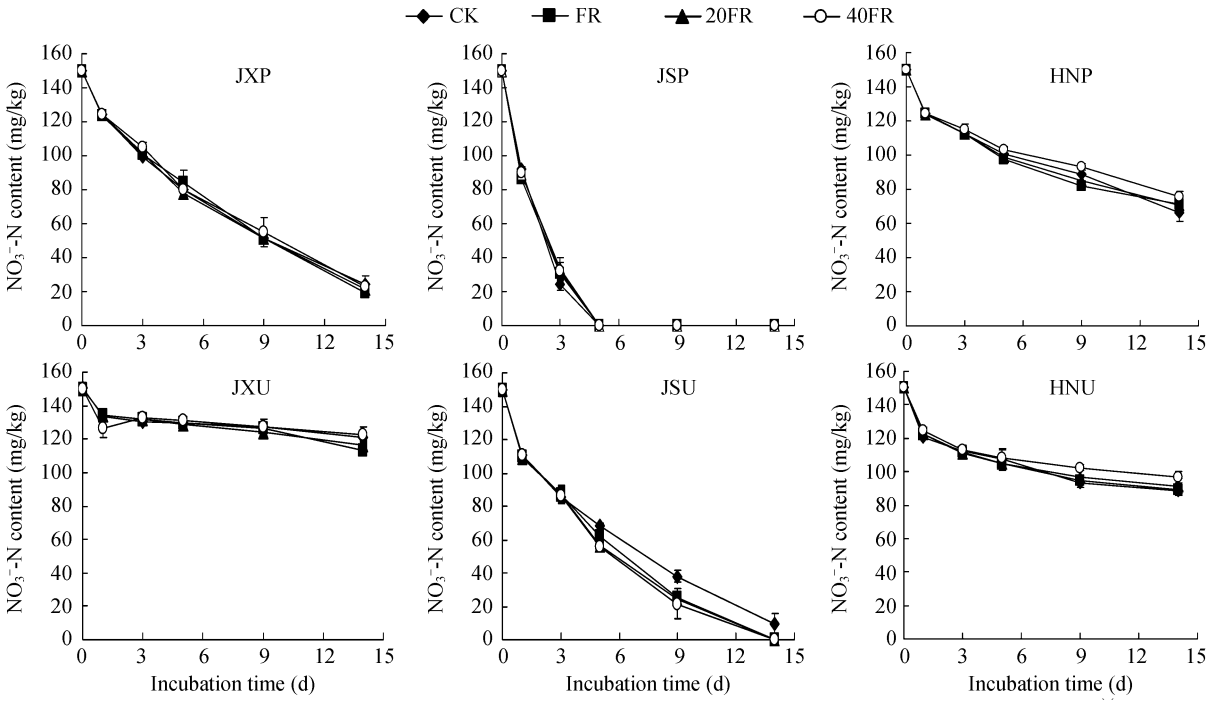

Fig. 9 Changes in nitrate concentrations in 150 mg NO_3^--N/kg amended soil samples with carbendazim added at rates of 0 (CK), 5.5 (FR), 110 (20FR) and 220 (40FR) mg/kg over a 14-d flooded incubation period at 25°C.

than in neutral or alkaline soils (De Boer and Kowalchuk, 2001; Bååth and Anderson, 2003) and active nitrification under acid conditions may be attributed to heterotrophic nitrification (Focht and Verstraete, 1977; Papen and von Berg, 1998), predominantly performed by fungi (Kester *et al.*, 1997). This scenario also appeared to be the case in the soils studied. The nitrification rates in the soils without fungicide addition was positively and significantly correlated with soil pH ($p < 0.001$). Our results indicated that acidic soils were also more susceptible to inhibition by chlorothalonil addition than neutral and alkaline soils. For the soils JXP and JXU with a pH < 5.0, evidence of nitrification activity almost vanished at the 20FR and 40FR levels of chlorothalonil (Figs. 3 and 4) supports this argument. Chlorothalonil is designed to kill fungi and has highly toxic effect on fungal populations in soil (Sigler and Turco, 2002). Consequently, the fact that nitrification in acid soils was inhibited to a larger degree than that

Table 2 Comparison of the degree of inhibition of nitrification and denitrification by chlorothalonil at day 3

Soil	Treatment	N_3 (mg N/(kg·d))	D_3 (mg N/(kg·d))	N_I (%)	D_I (%)	$\|N_I\| - \|D_I\|$
JXP	CK	3.68 a	17.0 a	0.00 a	0.00 a	
	FR	3.35 b	16.6 a	9.09 b	2.08 a	7.01
	20FR	2.50 c	14.2 b	32.1 c	16.2 b	15.9*
	40FR	2.59 c	15.6 b	29.6 c	9.50 c	20.1*
JXU	CK	0.18 a	6.79 a	0.00 a	0.00 a	
	FR	0.07 b	6.86 a	47.8 b	-1.13 a	46.5**
	20FR	–0.15 c	6.11 a	200 c	3.71 a	196**
	40FR	–0.16 c	6.56 a	200 c	3.47 a	197**
JSP	CK	5.38 a	41.9 a	0.00 a	0.00 a	
	FR	5.38 a	41.9 a	3.87 a	-0.20a	3.66
	20FR	1.91 b	27.3 b	65.8 b	34.7 b	31.1*
	40FR	1.42 b	29.6 b	74.3 b	29.2 b	45.1**
JSU	CK	24.4 a	21.4 a	0.00 a	0.00 a	
	FR	23.5 a	22.3 a	3.53 a	–3.75 a	–0.22
	20FR	14.1 b	20.6 a	42.1 b	4.08 a	38.0*
	40FR	11.7 c	21.2 a	51.9 c	0.93 a	51.0**
HNP	CK	32.8 a	12.6 a	0.00 a	0.00 a	
	FR	30.0 a	12.2 a	6.49 a	3.25 a	3.24
	20FR	2.77 b	10.6 b	91.6 b	15.7 b	75.9**
	40FR	1.75 c	11.1 b	94.7 b	12.1 b	82.6**
HNU	CK	35.7 a	12.6 a	0.00 a	0.00 a	
	FR	36.2 a	12.8 a	–1.25 a	–1.87 a	–0.62
	20FR	15.2 b	11.0 b	57.3 b	12.9 b	44.4**
	40FR	11.1 c	11.2 b	68.9 c	10.8 b	58.1**

Letters indicate a significant difference at $p < 0.05$ between treatments applied to the same soil (Duncan's multiple range test). A significant difference in the degree of inhibition of nitrification compared to denitrification (t-test, * $p < 0.05$, ** $p < 0.01$). N_3: net nitrification rate on day 3; D_3: net denitrification rate at day 3. N_I: the degree of inhibition of nitrification by fungicide addition; D_I: the degree of inhibition of denitrification by fungicide addition.

in neutral and alkaline soils highlights the important role played by fungi in the nitrification of acid soils. On the other hand, although some acid tolerant autotrophs could be responsible for nitrification in acid soils (De Boer and Kowalchuk, 2001), the soil bacteria *Nitrosomonas* sp. and *Nitrobacter* sp. responsible for oxidation of ammonium to nitrate via nitrite have been reported as being among the most susceptible to changes in soil environment (Nakos, 1980; Martínez-Toledo *et al*., 1998).

Furthermore, soil pH affected the recovery of nitrification activity. Previous studies have reported that the growth of nitrifying bacteria can be inhibited by fungicide application (Martínez-Toledo *et al*., 1998), but Cycoń *et al*. (2006) found that the number of nitrifying bacteria decreased in soil treated with the highest concentration of the fungicide tebuconazole only on the first day after treatment, whilst a higher number was present at day 28. Similar patterns also have been reported by Wainwright and Pugh (1974). These results suggest that the reduction in nitrification by fungicides is temporary and that nitrification activity may recover soon after application to relatively high levels. In our present study, only the soils HNP and HNU showed this kind of response at the 20FR and 40FR levels of chlorothalonil application, probably because the two soils are alkaline and nitrification activity was strong. In the other neutral and acid soils, nitrification activities did not completely recover by the end of the 14 d incubation period, particularly in the case of JXU and JXP soils in which nitrification was almost completely inhibited during the whole incubation period (Figs. 3 and 4).

3.2 Effect of fungicides on mineralization

Application of chlorothalonil at the 20FR and 40FR rates promoted a significant amount of mineralization except in soil JXU (Fig. 7A). Chen and Edwards (2001) and Chen *et al*. (2001) also indicated that chlorothalonil could enhance net mineralization rate, which is consistent with our results. Moreover, the chlorothalonil itself might not be the best source of energy for soil microbes. The degradation of chlorothalonil increased with pH, especially at pH > 8 (Szalkowski and Stallard, 1977; Katayama *et al*., 1995), and the favourable environmental conditions for chlorothalonil degradation were 60% WHC and an incubation temperature of 25–30°C (Sato and Tanaka, 1987). Evidently, the soils HNP and HNU were the most suitable for the degradation of chlorothalonil. The products of degradation may be suitable for use by microbes, or serve as nutrient and energy sources. This would stimulate further microbial activity and a significant increase in net N mineralization, promoting a further considerable increase in nitrification as more substrate became available. Low soil pH was unsuitable for the degradation of chlorothalonil (Regitano *et al*., 2001). Therefore, a low pH and organic content may be the reason why chlorothalonil did not stimulate mineralization in the soil JXU. Carbendazim also increased the net mineralization rate but only in three soils (Fig. 7B). Similar promoting effects of other fungicides on mineralization also have been reported in previous studies (Monkiedje *et al*., 2002; Monkiedje and Spiteller, 2002; Cyco *et al*., 2006).

4 Conclusions

In this study, we investigated the effects of applying the fungicides chlorothalonil and carbendazim on nitrification and denitrification in six soils differing in pH. The results showed that chlorothalonil at higher rate was toxic to nitrification activity, particularly in very acid soils, whereas had little effect on denitrification. Carbendazim had essentially no effect on nitrification and denitrification. It is common practice to apply fungicides several times throughout the growing season, which might lead to high residual concentrations in the soil. Therefore, chlorothalonil might be able to help in conserving soil N by inhibiting nitrification and by promoting mineralization. However, further studies are needed to evaluate the full and long-term effects of fungicides on soil fertility and the environment.

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