Denitrification in a Chinampa soil of Mexico City as affected by methylparathion

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Abstract
Chinampas are raised garden beds used for agriculture in the southeastern part of the Valley of Mexico since pre-Hispanic times. In modern times, large amounts of pesticides, such as methylparathion, have been used with an unknown effect on soil processes, such as denitrification and the ratio of N_2O-to-N_2. Soil from a Chinampa was amended with nitrate (NO_3^-) with or without chloramphenicol known to inhibit *de novo* synthesis of enzymes, with or without acetylene (C_2H_2) known to inhibit the reduction of nitrous oxide (N_2O) to dinitrogen (N_2) and spiked with or without methylparathion. Methylparathion increased the concentration of NO_2^- and removal of NO_3^- from soil, but increased the emission of N_2O and N_2. The concentration of NO_2^-, the removal of NO_3^- production, and the N_2O and N_2 emission rates were generally larger when the aerobic conditioning of the soil was less than 14 days. It was found that methylparathion increased the denitrification process and showed no inhibitory effects on emissions of N_2O or N_2.

Key words
Chloramphenicol; dynamics of mineral N; nitrous oxide to dinitrogen ratio; organophosphorous pesticide

Introduction

In Xochimilco (Mexico City), agriculture is done in a unique way called '*Chinampa'* since pre-Hispanic times. *Chinampa* is a pre-Columbian form of agriculture whereby sediment from canals is collected regularly and applied to human-made islands forming raised garden beds called chinampas. They contributed greatly to the development of indigenous cultures that occupied the southeastern part of the Valley of Mexico, i.e. Tenochtitlan. The soils are deep and discontinuous and due to the human influence some authors classify them as Anthrosols (INECOL 2002).

Chinampas are intensively cultivated whereby different pesticides, such as clorpiriphos, methylparathion, malathion, lindane and atrazine, are used in large quantities (CICOPLAFEST 2008). Little information is available how methylparathion might affect soil processes. However, Zhang *et al.* (2006) found that in a soil contaminated with methylparathion the diversity and structure of microbial communities changed.

Negative effects on non-targeted soil microorganisms have been reported when pesticides are applied and microbial activity and diversity is reduced in soil (Johnsen *et al.* 2001; Locke and Zablotowicz 2004; IPCC 2007b). Nevertheless, other studies found that fumigation increased organic matter degradation rates (Locke and Zablotowicz 2004). Recently, it has been reported that fumigation with pesticides increased nitrous oxide (N_2O) emissions (Spokas and Wang 2003; Spokas *et al.* 2005; Spokas *et al.* 2006). This suggests that pesticides might affect the denitrification process. However, little information exists about how these pesticides might affect the gaseous products of the denitrification process, i.e. the N_2O-to-N_2 ratio.

Denitrification is a respiratory microbial process by which oxides of nitrogen serve as electron acceptors for respiratory electron transport in anaerobic conditions. As a result nitrate (NO_3^-) is reduced to nitrite (NO_2^-) and then gaseous products mainly N_2O and dinitrogen (N_2) (Knowles 1982; Simek *et al.* 2000). Denitrification has been studied intensively as it is an important contributor to the emission of N_2O. N_2O is a strong greenhouse gas with a global warming potential that is 300 times more powerful than carbon dioxide (for time horizon of 100 years) (IPCC 2001).

De novo synthesis of the reductases involved in denitrification process is inhibited by chloramphenicol while acetylene (C_2H_2) inhibits the activity of nitrous oxide reductase (Smith and Tiedje 1979). As such, the N_2O-to-N_2 ratio of the denitrification process can be determined when C_2H_2 is used while chloramphenicol at low

concentrations allows to study the denitrification capacity of the soil upon sampling (Knowles 1982; Pell *et al.* 1996). The objective of this study was to determine the effect of the pesticide methylparathion on dynamics of the denitrification process in a Chinampa soil using acetylene and chloramphenicol as selective inhibitors.

Methods
Soil samples were collected in the Chinampa of Xochimilco in San Gregorio Atlapulco (Xochimilco, Mexico City, Mexico).

As part of a study into the effects of methylparathion on soil processes one Chinampa soil was sampled. The sampling site of 4500 m^2 was divided in three equal plots. Soil was sampled by augering the 0-15 cm layer of the three plots with a stony soil auger diameter 7 cm (Eijkelkamp, Nl). The soil of each plot was pooled and sieved (5 mm). Sub-samples of soil were amended with or without methylparathion and incubated aerobically at 22±2°C for 28 days. At the onset of the experiment and every seven days, soil was amended with or without acetylene (C_2H_2) or chloramphenicol and incubated anaerobically for 48 h while dynamics of NO_3^-, NO_2^- and N_2O were monitored, after 0, 6, 24 and 48 h, three sub-samples of each treatment ($n = 4$), plot ($n = 3$) and amended with or without pesticide ($n = 2$) were selected at random for assays of NO_3^-, NO_2^- and N_2O. The headspace of each flask was sampled and analysed for N_2O. The concentrations of N_2O were corrected for gas dissolved in the water (Moraghan and Buresh 1977). After measurement of N_2O, samples were analysed for NO_2^- and NO_3^- as described earlier. Data for NO_2^- were corrected for the formation of NO_2^- through the degradation of chloramphenicol (Dendooven *et al.* 1994).

Emission of N_2O was regressed on elapsed time using a linear regression model which was forced to pass through the origin but allowed different slopes (production rates) for each treatment. This approach is supported by theoretical considerations that no N_2O was produced at time zero and the atmosphere in the flask contained no N_2O as the headspace was flushed with N. Production of NO_2^- and NO_3^- was regressed on elapsed time using a linear model that was not forced to pass through the origin and allowed different slopes (production rates) for each treatment.

Results
The concentration of NO_3^- decreased over time (Table 1). In the SMP soil, day of sampling or treatment had no significant effect on the decrease in concentration of NO_3^- (Table 1). In the CMP soil, the decrease in the concentration of NO_3^- was slowest in soil conditioned aerobically for 7 days and the fastest for soil incubated for 28 days, while treatment had no significant effect on it. Methylparathion had no significant effect on the concentration of NO_3^- in soil (Table 2).

No clear pattern emerged in the concentrations of NO_2^- during the different anaerobic incubation. In the SMP soil, the concentration of NO_2^- (mean of all treatments) was significantly larger at day 0 and 7 than at day 14 and 28. In CMP soil, the mean concentration of NO_2^- was significantly larger at day 7 than at day 14 and 28, but significantly lower than at the onset of the experiment ($p < 0.05$) (Table 1). In the SMP soil, the mean concentration of NO_2^- was largest when amended with C_2H_2 plus chloramphenicol and lowest in the unamended SMP soil. In CMP soil, the concentration of NO_2^- increased significantly when amended with chloramphenicol compared to soil not amended with chloramphenicol. The concentration of NO_2^- was significantly larger in the CMP soil than in the SMP soil ($p < 0.05$) (Table 2).

Table 1. Effect of time of aerobic conditioning at 25 °C, i.e. 0, 7, 14 and 28 days, and treatment, i.e. control, acetylene, chloramphenicol, acetylene+chloramphenicol, on mean concentrations of NO_2^- (mg N/kg soil) and NO_3^- and N_2O production rates (mg N/kg soil/h) in soil amended or not with methylparathion incubated anaerobically at 25 °C for 48 h.

	NO_2^- concentration			Concentration of NO_3^-		Emission of N_2O	
	SP [a]	CP [b]		SP	CP	SP	CP
Time of conditioning	— (mg N/kg soil) —			———— (mg N/kg soil/h) ————			
Day 0	.3 A [c]	8.8 A		-0.39 A	-0.61 BC	0.014 A	0.032 B
Day 7	.1 A	.4 B		-0.27 A	-0.11 A	0.011 B	0.043 A
Day 14	.4 B	.7 C		-0.26 A	-0.26 AB	0.009 B	0.022 C
Day 28	.9 B	.2 C		-0.42 A	-0.83 C	0.010 B	0.016 C
LSD [d]	.8	.0	SEE [e]	0.19	0.19	0.001	0.003
Treatment							
Control	.1 C	.5 B		-0.23 A	-0.33 A	0.007 B	0.020 B
Acetylene (C_2H_2)	.2 B	.4 B		-0.25 A	-0.50 A	0.014 A	0.038 A
Chloramphenicol	.8 BC	1.9 A		-0.39 A	-0.53 A	0.008 B	0.018 B
Chloramphenicol+(C_2H_2)	.6 A	2.3 A		-0.42 A	-0.59 A	0.014 A	0.034 A
LSD (P<0.05)	.8	.0	SEE	0.19	0.21	0.001	0.003

[a] SP: without methylparathion,
[b] CP: with methylparathion,
[c] Values with the same capital letter are not significantly different within the column at $p < 0.05$,
[d] LSD: Least significant difference ($p < 0.05$),
[e] SEE: Standard error of the estimates ($p < 0.05$).

The emission of N_2O increased over time and resembled a zero order kinetic. However in the CMP soil amended with C_2H_2, the emission of N_2O increased after 24 h. In the SMP soil, the N_2O emission rate was significantly larger at the onset of the experiment than when conditioned aerobically for 7, 14 or 28 days (Table 1). In CMP soil, the highest N_2O emission rate was found when conditioned aerobically for 7 days and lowest when incubated aerobically for 14 or 28 days. The emission of N_2O was significantly larger in the CMP soil than in the SMP soil ($p < 0.05$) (Table 2).

The production of N_2 increased over time and resembled a zero order kinetic. However, in CMP soil conditioned for 7 days, the emission of N_2 increased after 24 h. The emission of N_2 was significantly larger in the CMP soil than in the SMP soil ($p < 0.05$) (Table 2).

Table 2. Effect of metylparathion on mean concentrations of NO_2^- (mg N/kg soil) and NO_3^-, N_2O and N_2 production rates (mg N/kg soil/h) of soil conditioned for 0, 7, 14 or 28 days and left untreated or treated with acetylene, chloramphenicol, acetylene+chloramphenicol, and incubated anaerobically at 25 °C for 48 h.

	NO_2^- concentration		NO_3^- production rate	N_2O emission	N_2 emission	N_2 emission + chloramphenicol
	— (mg N/kg soil) —		———— (mg N/kg soil/day) ————			
- Methylparathion	9.9 B [a]		-0.19 A	0.011 B	0.009 B	0.005 B
+ Methylparathion	13.2 A		-0.54 A	0.028 A	0.015 A	0.012 A
LSD [b] (P<0.05)	1.6	SEE [c]	0.22	0.001	0.002	0.001

[a] Values with the same capital letter are not significantly different within the column at $p < 0.05$,
[b] LSD: Least significant difference ($p < 0.05$),
[c] SEE: Standard error of the estimates ($p < 0.05$).

Conclusions

Pesticides inhibit or stimulate the denitrification process. Methylparathion when added to a Chinampa soil stimulated the denitrification process. It increased the concentration of NO_2^- and removal of NO_3^- from soil, and increased the emission of N_2O and N_2. Additionally, methylparathion did not affect the N_2O-to-N_2 ratio.

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