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Effect of long-term liquid pig manure application on atrazine mineralization in a soil cultivated with maize

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Abstract Soil samples were collected in plots from a field experiment in maize monoculture receiving 0, 60 and 120 m^3 ha^{-1} liquid pig manure (LPM) for 19 years. Soils were sampled from the 0- to 20-cm layer in August and October 1997 and in June, July and September 1998. Subsurface samples were also evaluated in September 1998. Laboratory soil radiorespirometry was used to evaluate atrazine mineralization using [U-*ring*-^{14}C]-atrazine mixed with commercially available product. The effect of atrazine dose (50, 100 and 500 mg atrazine kg^{-1} soil) was evaluated on soils sampled in August 1997. For the other sampling dates, the soils were spiked with 50 mg atrazine kg^{-1} soil. No LPM dose effect on atrazine mineralization was obtained in the different experiments. Increasing atrazine dose to 500 mg kg^{-1} decreased significantly the mineralization rate (R_i) and the maximum of atrazine mineralized (MAX), while the time needed to mineralize 50% of MAX (DT-50%) was not significantly affected. Sampling time had a significant effect on atrazine mineralization. Atrazine mineralization in the soils sampled in June 1998 showed lower R_i and MAX than in the soils sampled at the other dates. Atrazine mineralization in subsurface soils (20–60 cm) was very variable and quite high in some samples. This may be due to atrazine pre-exposure in subsoils resulting from atrazine deep movement by preferential flow.

Keywords Atrazine mineralization · Corn · Herbicide · Hog manure

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Introduction

Pesticide degradation in soils has been studied in great detail, especially for compounds like atrazine that are not primarily used as microbial growth substrates. However, the behavior of s-triazine herbicides in soils amended with organic materials has been investigated less and is not fully understood (Rouchaud et al. 1994; Alvey and Crowley 1995; Topp et al. 1996; Barriuso et al. 1997).

The application of organic amendments resulted in either a stimulation of atrazine degradation rates by enrichment of selected atrazine-degrading microbes, or a modification of both the rates and pathways of atrazine degradation (Alvey and Crowley 1995; Entry and Emmingham 1995; Topp et al. 1996; Houot et al. 1998). Soil amendment with corn meal stimulated the dissipation of freshly sprayed atrazine, but did not significantly affect the dissipation of aged residues (Dzantor et al. 1993). Some reports suggested that the application of organic amendments, such as cow manure and liquid pig manure, increased the herbicides adsorption onto the soil organic matter (Rouchaud et al. 1994; Businelli 1997). However, Shapir and Mandelbaum (1997) observed with soils from Israel that the higher organic matter content in the upper soil level did not result in a sorption-related decrease in degradation rates. An inhibition of atrazine mineralization has been demonstrated in soils receiving different organic amendments when a high level of NO_3^--N was added as inorganic N (Alvey and Crowley 1995). Atrazine degradation by cell suspensions of a bacterial isolate M91–3, tentatively identified as a *Ralstonia* sp., was also inhibited by the presence of NH_4^+ or NO_3^- (Gebendinger and Radosevich 1999). These findings suggest that atrazine may serve as nitrogen source for those established indigenous populations in soil.

In the present work, we investigated the effect of the long-term application of different rates of liquid pig manure (LPM) on atrazine mineralization in a soil with a long history of continuous maize planting and atrazine application.

Materials and methods

Site description and soil sampling

The soil samples were collected from field plots at the St-Lambert experimental research station of the Institut de Recherche et de Développement en Agroenvironnement (IRDA) near Quebec city, Canada (46°05′N, 71°02′W, altitude 110 m). The soil is a silt loam (32% sand, 52% silt and 16% clay) that belongs to the Le Bras soil series (Typic Humaquept, fine mixed, frigid). The plots are in maize (*Zea mays* L.) monoculture and have received LPM annually at different rates (0, 30, 60, 90 and 120 m^3 ha^{-1}) since 1979. Each treatment is repeated three times in the field in a completely randomized block design. The soil has a history of atrazine application at a yearly rate of about 1.2 kg a.i. ha^{-1}. A permanent meadow on the same soil type adjacent to the plots receiving LPM was also sampled to compare the results obtained with the ones from a soil with no history of atrazine or LPM application. A subsample of the meadow soil was also sterilized in the autoclave for 60 min on three consecutive day and the results obtained were compared to those of the non-sterilized soil.

Soils were sampled from the field at two different times in 1997 and at three different times in 1998. Table 1 summarizes the treatment schedules and sampling dates for each year. Atrazine was applied in corn post-emergence as the commercial formulation AATREX liquid 480 SU (Novartis Crop Protection) at a rate of 1.2 kg a.i. ha^{-1}. LPM was also applied in corn post-emergence, when the plants were 15–25 cm tall, by injection into the soil to a depth of 5–7 cm. In 1997, the soils were sampled in the plots receiving all the different LPM doses. In 1998, there was a change in the field protocol and only one dose of LPM (60 m^3 ha^{-1}, which is the agronomic recommended dose) was applied to the plots that received different doses of LPM the previous years, while the control plots (0 m^3 LPM ha^{-1}) remained. In June 1998, prior to the application of LPM and atrazine in the field, the plots treated with 0, 60 and 120 m^3 LPM ha^{-1} the previous years were sampled. The results obtained from these samples can be compared to the ones obtained from the samples collected in 1997 to evaluate the long-term effect of LPM on atrazine mineralization. In July and September 1998, only the plots receiving 0 and 60 m^3 ha^{-1} in 1998 and the previous years were sampled and used for incubation experiments. These different sampling times allow the evaluation of LPM dose effect on atrazine mineralization, as well as the effect of time after LPM application on atrazine mineralization in soils receiving LPM at given doses for many years. The soil samples were collected between corn rows with a hand auger of 2.5-cm diameter, and put in sterile bags. The soils were sampled to a depth of 20 cm, except in September 1998 where the soils were sampled up to 60 cm deep, in depth increments of 20 cm (0–20 cm, 20–40 cm and 40–60 cm). Each sample was a composite of 15 subsamples taken following an X pattern in each plot. The samples were transported on ice to the laboratory, wet sieved to 2 mm to remove coarse particles and stored at 4°C for no more than 3 weeks. Prior to the start of the incubations, the soils were removed from the cold and stored at room temperature for 18 hours.

Table 1 Field treatments schedule and soil sampling dates

	1997	1998
Maize seeding date	May 29	May 28
Liquid pig manure (LPM) application date	June 19	June 30
Doses of LPM applied	0, 30, 60, 90, 120 m^3 ha^{-1}	0, 60 m^3 ha^{-1}
Field atrazine application date	June 11	July 9
Maize harvest date	October 14	October 13
Soil sampling date	August 4	June 2
	October 3	July 15
		September 17

Table 2 Selected characteristics of the soils sampled in August 1997. Means of three replicate plots (± SD). Means *followed by the same letter in the same line* are not significantly different (P =0.05)

Characteristics	Liquid pig manure added (m^3 ha^{-1})				
	0	30	60	90	120
C_{org} (g kg^{-1})	21.1 a (3.3)	21.3 a (3.1)	23. 5 a (0.7)	26.7 b (0.5)	22.6 a (3.2)
N total (g kg^{-1})	2.4 b (0.3)	1.9 a (0.1)	2.1 ab (0.1)	2.5 b (0.1)	2.1 ab (0.3)
$N\text{-}NO_3$ (mg kg^{-1})	7.9 a (2.8)	27.8 a (24.9)	17.7 a (3.6)	49.7 b (0.7)	47.6 b (24.1)
pH water (1:1 v/v)	6.3 b (0.1)	6.0 a (0.1)	6.1 a (0.1)	6.0 a (0.1)	6.1 a (0.1)
CEC ($cmol_c$ kg^{-1})	11.1 a (1.3)	11.4 a (0.6)	12.3 a (0.3)	13.2 b (0.1)	12.0 a (1.8)

Soil analysis

At the beginning of the experiment (August 1997), the soils were characterized for selected properties (Table 2). Soil pH was evaluated in water using a 1:1 soil:water ratio and a combined glass electrode. Soil organic C was quantified using the Walkley and Black procedure (McKeague 1978). Total N was evaluated using the micro-Kjeldahl method (Bremner 1965a). Exchangeable NO_3-N was evaluated with 2 N KCl extraction followed by steam-distillation (Bremner 1965b).

Gravimetric soil water content, microbial biomass and microbial activity were evaluated on soils sampled at different dates. The fumigation-extraction method of Vance et al. (1987) was used to evaluate soil microbial biomass, using a conversion factor (k_{ec}) of 0.45 (Wu et al. 1990). Microbial activity was estimated by titration of the CO_2 trapped in a solution of 1 N NaOH using 0.5 N HCl after 6 days incubation as described by Gan et al. (1998).

Atrazine mineralization

Atrazine mineralization was evaluated by radiorespirometry. The soils were incubated in the laboratory with [U-*ring*-^{14}C]-atrazine mixed with commercially available atrazine (AATREX liquid 480 SU, Novartis Crop Protection). The radiolabelled atrazine (specific activity of 305.6 Mbq $mmol^{-1}$ and radiochemical purity greater than 98%) was a gift from Novartis Crop Protection, Greenboro, N.C. The radiolabelled atrazine was first diluted in methanol 90% (v/v), then mixed with unlabelled product to the desired concentrations.

A quantity of moist soil at 70% field capacity corresponding to 20 g dry soil was placed in 125-ml sterile flasks. A $^{14}CO_2$ trap made of 4 ml 1 N NaOH was placed in each flask. The soil was then spiked uniformly with the solution containing the marked atrazine to a given final concentration and the flask was immediately sealed. For the samples collected in August 1997, the soils were spiked with three different atrazine concentrations (50, 100 and 500 mg kg^{-1}) which correspond to specific ^{14}C activity of 11.56, 23.12 and 115.6 kBq per flask, respectively. For all the other sampling dates, the soils were spiked with 50 mg atrazine kg^{-1} soil. The flasks were done in duplicate. The soils were incubated at constant temperature (22±0.5°C) and kept at 70% field capacity for 42 days. Every 3 days during the incubation period, the flasks were opened and the NaOH solution replaced. The $^{14}CO_2$ trapped by the NaOH solution was quantified by placing 1-ml aliquot of trapping solution into 7 ml Beckman Ready-Safe aqueous scintillation cocktail (Beckman Instruments, Fullerton, Calif.) and counting on a LKB-Wallac 1217 liquid scintillator.

Modeling and statistics

The model selected to describe the mineralization data was the general saturation model developed by Morgan et al. (1975). This is a four-parameter model that has been successfully used by Parkin et al. (1991) and Parkin and Shelton (1992) to describe carbofuran hydrolysis in soils. The main equation is:

$$P = \frac{bg + (MAX)t^c}{g + t^c} \quad (1)$$

where *P* is the cumulative $^{14}CO_2$ formation, MAX is the asymptote (plateau), *b* is the ordinate intercept, *c* is the apparent kinetic order of the reaction as *t* approaches 0, and *g* is a characteristic constant for the system (Parkin et al. 1991). When no pesticide is present initially (as is the case in the present study, since no [U-*ring*-^{14}C]-atrazine was present in the soil initially), *b* is equal to 0 and Eq. 1 becomes a three parameter model:

$$P = \frac{(MAX)t^c}{g + t^c} \quad (2)$$

Parameter estimates for Eq. 2 were obtained from the $^{14}CO_2$ production curves by nonlinear regression (StatMost for Windows; DataMost, Salt Lake City, Utah) using the Lavenburg-Marquardt algorithm (Press et al. 1992) for least-square minimization.

The parameters obtained using this model are used to calculate the time it takes for 50% of the mineralized compound to be lost as $^{14}CO_2$ (DT-50%, days) and the maximum rate of pesticide mineralization (R_i, % day^{-1}). DT-50% is obtained using the following equation:

$$DT - 50\% = g^{1/c} \quad (3)$$

while R_i is given by:

$$R_i = \frac{((MAX) * c * g * (g*(c-1)/(c+1))^{(c-1)/c}}{(g + g*(c-1)/(c+1))^2} \quad (4)$$

Statistical analyses were performed using SAS (SAS Institute 1998). Analyses of variance were performed using the mixed procedure (PROC MIXED) of SAS. For comparison of samples collected at different dates on the same plot, repeated measure ANOVA was used, and the covariance structure for sampling times was selected using the Akaike criteria. Significant differences between treatment means were detected using the LSMEANS statement with the DIFF option in SAS. Normality and variance homogeneity were tested using the Shapiro-Wilk test. These statistical analysis were performed separately on DT-50%, R_i and MAX.

Results and discussion

Mineralization curves

Typical atrazine mineralization curves obtained in the present study are presented in Figs. 1, 2 and 3. The results reported are sometimes quite variable, which is not uncommon for this type of experiment (Alvey and Crowley 1996; Ostrofsky et al. 1997; Parkin and Shelton 1992). Therefore, different symbols were used in the figures to show the results obtained from the different field replicates. Thus the curves with the same symbols are for laboratory-replicated flasks on the same soil sample. This was done in order to determine the source of the variability observed. We can see that, in some cases, considerable variations from replicated flasks are observed, while others give identical results. In other cases, the variability seems to come from the different field replicates.

Generally, the kinetics of $^{14}CO_2$ production curves from [U-*ring*-^{14}C]-atrazine amended soils, were sigmoidal (Figs. 1, 2), which is typical of microbial growth with pesticide used as a C and/or N source (Focht and Shelton 1987; Jacobensen and Pedersen 1992; Shapir et al. 2000). Similar sigmoidal atrazine mineralization curves have been observed in other studies with soils having a history of atrazine application (Gan et al. 1996; Shapir et al. 2000). Detectable amounts of $^{14}CO_2$ (0.1–1.2% of initial [U-*ring*-^{14}C]-atrazine) were measured after only 3 days of incubation in soils with a history of atrazine application (Figs. 1, 2). Similar short mineralization lag time periods were observed by others in atrazine pre-exposed soils (Shapir et al. 2000; Yassir et al. 1999). The lag time period is usually indicative of growth or adaptation of the microbial population to the pesticide (Parkin et al. 1991) and may represent the time needed for the atrazine degraders to proliferate and accumulate the critical cell mass needed to mineralize a sufficient amount of atrazine to be detected experimentally (Shapir et al. 2000). The short mineralization lag time periods observed in the present study, are thus indicative that the soil microbial population is well adapted to the atrazine compound. The meadow soil, which had not received any atrazine for the past 17 years exhibited nearly linear $^{14}CO_2$ production with time, with less than 1.5% of the initial atrazine mineralized after 42 days (Fig. 3). When substrate concentrations are low, or conditions are not conducive to microbial growth, apparent linear kinetics may be observed (Parkin and Shelton 1992). For the meadow soil, the substrate (atrazine) is not limiting and the very low mineralization observed is most probably due to the inability of the microorganisms to mineralize this substrate. Other studies have reported very low atrazine mineralization (<2% of the amount applied) in soils with no history of atrazine application (Kruger et al. 1993; Miller et al. 1997; Shapir et al. 2000; Willems et al. 1996). The absence of atrazine mineralized in the sterile soil indicates that microbial degradation is the main reaction responsible for atrazine mineralization, as was found in other studies (Alvey and Crowley 1995; Issa and Wood 1999).

Effect of LPM and atrazine doses on atrazine mineralization

The mineralization curves for the soils sampled in August 1997 and spiked with different amount of atrazine are presented in Fig. 1 for two doses of LPM added (0 and 60 m^3 ha^{-1}). The results obtained for the other LPM doses are similar. The simple visual comparison of the effect of the different treatments on atrazine mineralization is difficult from these curves because of the variability observed. For quantitative comparison, the decomposition parameters estimated from the $^{14}CO_2$ production curves

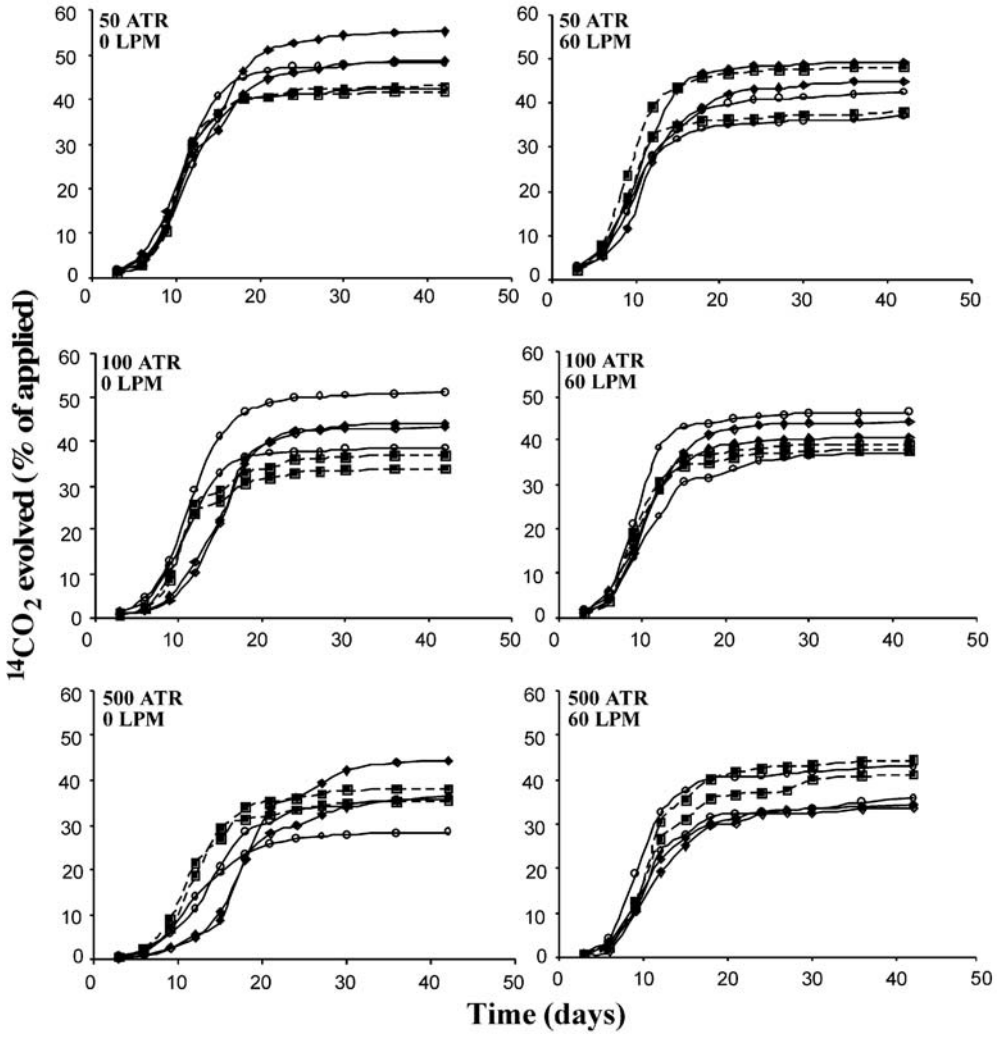

Fig. 1 Cumulative $^{14}CO_2$ production from [U-^{14}C-ring]-atrazine amended soils for samples collected in August 1997 from plots receiving, annually, 0 and 60 m^3 ha^{-1} liquid pig manure (*LPM*) and spiked with different doses of atrazine (*ATR*, in mg kg^{-1} dry soil). The three field replicates are plotted with *different symbols*. The *curves with the same symbol* are for the duplicated laboratory flasks

using Eqs. 2, 3 and 4 are used (Table 3). The model describes the $^{14}CO_2$ production curves very well, with r^2 ranging from 0.97 to 0.99 (data not shown). The summary rate parameters obtained (DT-50%, R_i and MAX) were normally distributed. Statistical analysis revealed no significant differences in the decomposition parameters for the different LPM doses for a given atrazine concentration (Table 3). This may be due to the sampling time, which is about 6 weeks after LPM application (Table 1). The effect of LPM dose and sampling time on atrazine mineralization will be discussed in more details in the following section.

Differences in the decomposition parameters were observed with the different atrazine doses added to the soil, and statistical analysis revealed significant atrazine dose effects for all decomposition parameters. Increasing atrazine concentrations resulted in a significant decrease of the maximum mineralization rate (R_i) within a given LPM dose (Table 3). Averaged over the different LPM doses, R_i is 4.9%, 4.3% and 3.2% day^{-1} for 50, 100 and 500 mg atrazine kg^{-1}, respectively. The time needed to mineralize half of MAX (DT-50%) has a slight tendency to be longer with increasing atrazine concentration (Table 3). DT-50% for the treatment with no LPM added was significantly longer when 500 mg atrazine kg^{-1} was added to the soil compared to the other two-atrazine concentrations. However, the difference represents only 2–3 days. We would expect a longer DT-50% with higher atrazine concentration added because of the decrease in R_i obtained. Since the DT-50% is not decreased substantially with increasing atrazine concentration, the decrease in R_i is probably not important enough to make a difference in the final result.

The proportions of maximum atrazine mineralized (MAX) are decreased with increasing atrazine concentration (Table 3). Values averaged over the different LPM doses are 43.9%, 40.6% and 37.7% of the amount applied, mineralized for 50, 100 and 500 mg atrazine added per kg of soil, respectively. The total amount of atrazine mineralized increases with the increasing amount of atrazine added to the soil. This increase in the mass mineralized with increasing amount of atrazine added shows that the soil microbial population has a great potential for atrazine mineralization. The total amount

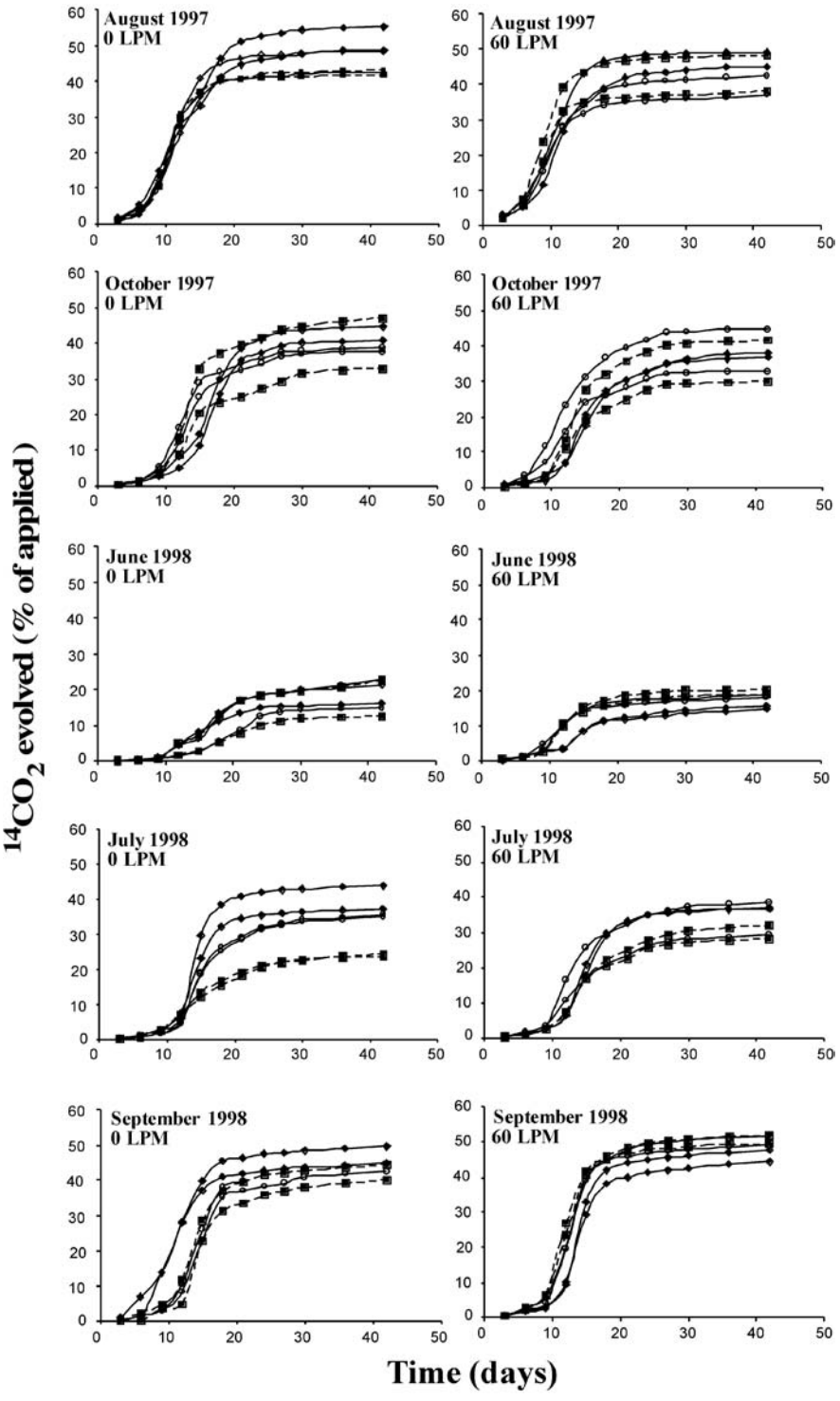

Fig. 2 Cumulative $^{14}CO_2$ production from [U-^{14}C-ring]-atrazine amended soils from plots receiving 0 and 60 m^3 ha^{-1} liquid pig manure (*LPM*) and sampled at different times. The three field replicates are plotted with *different symbols*. The *curves with the same symbol* are for the duplicated laboratory flasks

mineralized is influenced by the degradation processes involved, with the formation of degradation by-products, and by the availability of the parent compound and the degradation by-products in soil, which depend on the system used on sorption and formation of bound residue. Gan et al. (1996) observed an increase in soil microbial activity at higher atrazine concentration, which suggests that some microbial species may have used atrazine as a N and C source. These authors suggest that the presence of biodegradable pesticide may lead to the proliferation of active microbial communities and a concurrent increase in the decomposition of the applied pesticide. Since the application of different atrazine concentrations in the soil resulted in a proportional increase in the mass mineralized, MAX is probably mostly influenced by substrate availability in this soil. Gan et al. (1996) also showed that

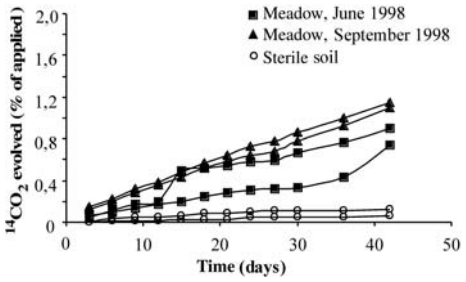

Fig. 3 Kinetics of $^{14}CO_2$ evolution from [U-^{14}C-ring]-atrazine amended soil samples collected from a meadow with no history of atrazine application and compared to a sterile soil. The *curves with the same symbol* are for the duplicated laboratory flasks

Table 3 Comparison of mean value of atrazine *DT-50%*, maximum rate (R_i) and saturation (*MAX*) as a function of liquid pig manure application dose for soils sampled in August 1997 spiked with different atrazine concentrations

Liquid pig manure (m^3 ha^{-1})	DT-50%[a] (days)	R_i[a] (% day^{-1})	MAX[a] (%)
50 mg atrazine kg^{-1} ξ			
0	11.06 (0.68) a	5.30 (0.91) d	46.57 (5.66) d
30	9.87 (1.61) a	5.01 (1.15) d	44.95 (3.57) cd
60	9.76 (0.93) a	5.02 (0.98) d	42.78 (5.35) bcd
90	12.83 (2.82) ab	4.26 (1.21) bcd	42.39 (3.84) bcd
120	10.06 (1.15) a	4.93 (0.84) d	42.99 (2.99) bcd
100 mg atrazine kg^{-1}			
0	11.99 (1.99) a	4.32 (0.79) bcd	41.54 (6.32) abcd
30	10.05 (2.07) a	4.35 (1.55) bcd	39.88 (7.35) abcd
60	9.55 (0.59) a	4.72 (1.15) cd	41.09 (3.64) abcd
90	12.17 (1.73) ab	3.42 (0.88) ab	39.09 (4.21) abc
120	10.64 (1.77) a	4.50 (1.05) bcd	41.18 (3.88) abcd
500 mg atrazine kg^{-1}			
0	14.08 (2.84) b	2.92 (0.61) a	37.29 (5.20) ab
30	10.44 (2.53) a	3.56 (0.94) ab	38.54 (5.54) abc
60	10.39 (0.72) a	3.81 (0.90) abc	38.10 (4.66) abc
90	12.32 (1.23) ab	2.83 (0.48) a	34.72 (5.04) a
120	12.43 (1.49) ab	3.08 (0.77) a	39.69 (6.27) abcd

[a] *Numbers in the same column followed by the same letter* are not significantly different at P =0.05; standard deviation in *parentheses*

hydroxylated atrazine degradation products were more persistent (in proportion) with increasing atrazine concentration in two soils. Since atrazine is labeled on the triazine ring, the accumulation of hydroxylated products would result in a decrease in the final proportion of mineralized atrazine, which could also explain the decrease in MAX with increasing atrazine concentrations in the present study.

Effect of LPM dose and sampling time on atrazine mineralization

Some kinetic curves for atrazine mineralization in soils sampled at different times in 1997 and 1998 and spiked with 50 mg kg^{-1} of [U-*ring*-^{14}C]-atrazine are presented in Fig. 2. Only the results obtained from the samples collected in plots receiving annually 0 and 60 m^3 ha^{-1} LPM are presented. The results obtained from the other plots are similar. All the curves are sigmoïdal in shape as observed previously. Again, no effects of the LPM doses on atrazine mineralization are visible from the kinetic curves for the soils sampled at different times, while sampling time seems to affect the maximum of atrazine mineralized. The mineralization lag time period also seems to be slightly affected by the sampling time, being longer in June and shorter for the other sampling times (Fig. 2). However, for most treatments and sampling times, the atrazine mineralization was essentially completed after 25 days of incubation.

Table 4 Comparison of mean values of atrazine *DT-50%*, maximum rate (R_i) and saturation (*MAX*) as a function of liquid pig manure application dose and sampling time in 1997 and 1998 for soils spiked with 50 mg atrazine kg^{-1}

Liquid pig manure (m^3 ha^{-1})	DT-50%[a] (days)	R_i [a] (% day^{-1})	MAX[a] (%)
August 1997			
0	11.06 (0.68) bc	5.30 (0.91) f	46.57 (5.66) c
60	9.76 (0.93) a	5.02 (0.98) ef	42.78 (5.35) c
120	10.06 (1.15) ab	4.93 (0.84) df	42.99 (2.99) c
October 1997			
0	14.99 (1.40) d	4.53 (1.31) def	44.86 (12.58) c
60	13.29 (1.73) d	3.39 (0.78) cd	37.20 (5.39) bc
120	14.73 (1.35) d	3.36 (1.08) c	35.9 (4.74) bc
June 1998			
0	17.71 (2.39) e	1.29 (0.39) a	18.57 (3.87) a
60	12.61 (1.50) cd	1.54 (0.62) ab	17.13 (2.05) a
120	14.57 (1.29) d	2.31 (0.81) abc	20.30 (3.04) a
July 1998			
0	14.69 (0.56) d	3.77 (2.06) cde	32.76 (7.26) b
60	14.12 (0.61) d	3.25 (1.04) bc	33.27 (4.07) b
September 1998			
0	13.09 (1.75) d	5.15 (0.34) ef	43.10 (3.60) c
60	12.75 (0.82) cd	6.86 (0.42) f	47.87 (3.06) c

[a] *Numbers in the same column followed by the same letter* are not significantly different at P =0.05; standard deviation in *parentheses*

The decomposition parameters obtained from the data in Fig. 2 using Eqs. 2, 3 and 4 are presented in Table 4 and are used for quantitative comparison. Again, the model used describes the $^{14}CO_2$ production curves very well, with r^2 ranging between 0.82 and 0.99 (data not shown). Summary decomposition parameters (DT-50%, R_i and MAX) are also normally distributed. Again, no significant effect of LPM dose on the different decomposition parameters was observed. This is in agreement with the results reported by Rouchaud et al. (1994), who observed no effect of LPM and cow manure applied to field plots on atrazine and metolachlore field dissipation.

However, the sampling date has a significant effect on the decomposition parameters describing atrazine mineralization. Statistical analysis revealed that R_i was signif-

Table 5 Estimation of the microbial biomass, microbial activity, and measure of the gravimetric water content (θ_g) in the soil samples collected at different times. *Numbers followed by the same letter* are not significantly different at P =0.05; standard deviation in *parentheses*. Data not available for the October 1997 sampling date. *NA* Not available

Liquid pig manure (m^3 ha^{-1})	Biomass-C[a] (μg g^{-1})	C-CO_2 [b] (μg g^{-1})	θ_g (kg H_2O kg^{-1} soil)
August 1997			
0	285.0 (44.3) a	126.9 (15.3) cde	0.24 (0.03) a
30	395.7 (12.1) bc	146.9 (43.3) e	0.28 (0.02) a
60	415.1 (28.4) c	131.9 (17.5) de	0.28 (0.02) a
90	404.0 (31.1) bc	152.5 (7.1) e	0.29 (0.02) a
120	562.6 (25.2) d	153.1 (8.8) e	0.27 (0.03) a
June 1998			
0	286.6 (18.8) a	69.5 (17.2) a	0.27 (0.03) a
60	349.7 (16.8) bc	94.0 (23.9) abc	0.29 (0.01) a
120	393.6 (36.1) bc	103.9 (18.4) bcd	0.29 (0.03) a
Meadow[c]	679.4	121.5	0.38
July 1998			
0	277.5 (61.1) a	81.7 (17.9) ab	0.25 (0.01) a
60	337.4 (52.3) ab	104.7 (6.1) bcd	0.28 (0.01) a
Meadow	537.7	143.6	0.43
September 1998			
0	346.2 (39.8) abc	NA	0.26 (0.04) a
60	428.4 (39.6) c	NA	0.28 (0.01) a
Meadow	864.3	NA	0.34

[a] Fumigation-extraction method (Vance et al. 1987)
[b] Titration of CO_2 trapped after 6 days of incubation (Gan et al. 1998)
[c] No replicates for the meadow soil

icantly less at the beginning of the 1998 season (soils sampled in June 1998) as compared to the values obtained from soils sampled at the end of the 1997 and 1998 seasons (Table 4). Lower R_i and higher DT-50% are indicative of lower mineralization activity. Evaluation of microbial biomass and activity in the soils sampled at the different dates shows a lower activity in June and July of 1998 as compared to August 1997 (Table 5).

While some studies showed no seasonal variation in pesticide mineralization (Ostrofsky et al. 1997), others have demonstrated an effect (Parkin and Shelton 1992; Barriuso and Houot 1996). Barriuso and Houot (1996) observed that atrazine mineralization varied in soils sampled at different times during the season. They speculated that a seasonal variation in the responsible microbial activity could explain the results. Parkin and Shelton (1992) also found a seasonal effect, with carbofuran DT-50% increasing and R_i decreasing with sampling time during the growing season (from June to October). They related this variation to the gravimetric soil water content, which was higher in June and lower in October and affected the microbial carbofuran degradation activity. In the present study, soil water content was controlled in the soil incubation flasks to avoid the effect of this factor on atrazine mineralization. However, we wondered if the water content in the field at the moment of sampling could have affected the results obtained. The gravimetric water content (θ_g) of the soil samples at the time of sampling is presented in Table 5. Since θ_g is constant for the different sampling dates, it doesn't contribute to explain the variability observed in decomposition parameters. The presence of a crop may also be a factor responsible for variations in pesticide mineralization rate with sampling time during the growing season, although Alvey and Crowley (1996) showed no effect of maize on atrazine mineralization rate. However, they observed that soils planted with maize increased the survival of an atrazine degrading consortium and enhanced the transformation of atrazine to hydroxyatrazine.

The maximum proportion of atrazine mineralized (MAX) at the end of the incubation period is also strongly influenced by the sampling time (Table 4). Averaged over the different LPM doses, MAX values are 43.9% (August 1997), 38.2% (October 1997), 18.7% (June 1998), 33.0% (July 1998) and 45.9% (September 1998). As mentioned in the previous section, this parameter is influenced by mineralization activity and substrate availability. Strong variations in the maximum proportion of atrazine mineralized for a given incubation period is reported in the literature. Miller et al. (1997) found that 36% of the ^{14}C-ring-labeled atrazine was converted to $^{14}CO_2$ after 22 weeks in the 0- to 5-cm soil layer of a loamy soil. Yassir et al. (1999) observed that [U-*ring*-^{14}C]-atrazine mineralization in 15 soils with a long history of atrazine application reached 65–85% of the initial radioactivity. Houot et al. (2000) obtained mineralized atrazine values ranging from less than 25% to 80% in different French and Canadian soils with a history of atrazine application. In their study, mineralization was positively correlated with soil pH, with mineralization values less than 25% in soil with pH less than 6.0, followed by a rapid increase of total mineralization with an increase in soil pH value. In the present study, the soils have initial pH values (Table 2) between 6.0 and 6.3 and the maximum mineralization observed falls within the range reported by Houot et al. (2000) for other pre-exposed soils with similar pH.

Depth variation in atrazine mineralization

Results of atrazine mineralization studies with depth are presented in Fig. 4. Atrazine mineralization at depths of 20–40 cm and 40–60 cm is more variable than in the 0- to 20-cm soil layer (Fig. 2), as shown by the results from the three field replicates, with little variability in duplicated flasks. Such variability in pesticide degradation with subsurface samples have been observed by others for samples taken at similar and at greater depths (Klint et al. 1993; Issa and Wood 1999). According to Issa and Wood (1999), this variability would indicate that substantial local variation in the size or activity of the microbial population exists in subsurface samples. In general, the lag time is longer in deep samples as compared to surface samples, which is indicative of the adaptation of the

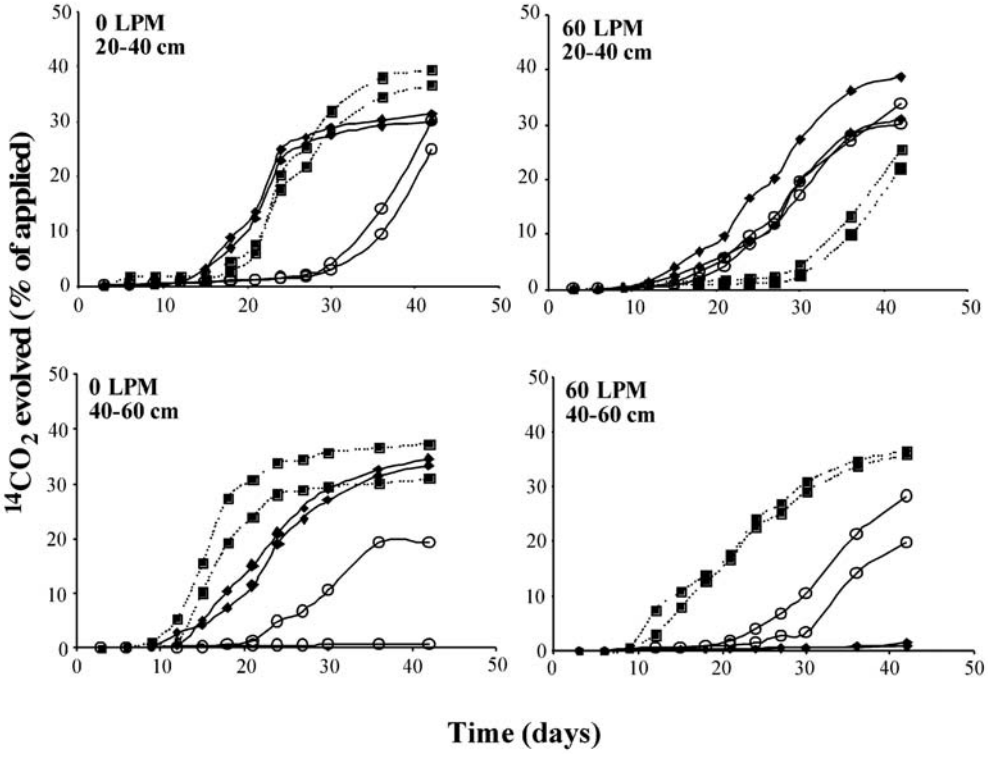

Fig. 4 Kinetics of $^{14}CO_2$ evolution from [U-^{14}C-ring]-atrazine amended soil samples collected September 1998 in the subsurface at two different depths from field plots receiving two doses of liquid pig manure (*LPM*). The three field replicates are plotted with *different symbols*. The *curves with the same symbol* are for the duplicated laboratory flasks

microflora to the presence of the pesticide in the surface samples. However, mineralization is surprisingly high deep in the soil, as compared to the results obtained in other studies where almost no atrazine mineralization was observed (Kruger et al. 1993; Miller et al. 1997; Shapir and Mandelbaum 1997). This may be explained by a pre-exposure of sub-surface microflora to atrazine migrating by preferential flow movement, which was demonstrated to occur in this soil (Fortin et al. 2002). The most important factor that governs atrazine mineralization in soils is the presence of an atrazine-mineralizing population (Shapir and Mandelbaum 1997). The type and activity of microorganisms capable of atrazine mineralization and their physical environment may have considerable influence on atrazine degradation and are likely to be responsible for much of the variation in the rate of degradation observed at different depths (Issa and Wood 1999).

In this study, soil sampled from plots receiving LPM for the past 19 years showed no LPM dose effect on atrazine mineralization. This may be due in part to the adaptation of the soil microflora to the atrazine compound due to annual atrazine application to this soil, which masked any LPM effect. However, the sampling period during the growing season affected atrazine mineralization, with significant differences in the maximum amount of atrazine mineralized (MAX) and in the mineralization rate (R_i), with lower values obtained in the soils sampled in June 1998. These results show that care should be given to the sampling date with respect to soil treatments and crop size when reporting atrazine degradation data in the literature. Variability in atrazine mineralization was observed with soil samples collected in the sub-surface. In some of the sub-surface samples, the atrazine mineralization was quite high as compared to results reported in the literature.

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