Lumbricid macrofauna alter atrazine mineralization and sorption in a silt loam soil

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Received 16 July 2004; received in revised form 5 July 2005; accepted 28 September 2005
Available online 2 November 2005

Abstract

Atrazine is a widely used herbicide and is often a contaminant in terrestrial and freshwater ecosystems. It is uncertain, however, how the activity of soil macrofauna affects atrazine fate and transport. Therefore, we investigated whether earthworms enhance atrazine biodegradation by stimulating herbicide degrading soil microflora, or if they increase atrazine persistence by facilitating herbicide sorption. Short (43 d) and medium term (86 d) effects of the earthworms Lumbricus terrestris and Aporrectodea caliginosa on mineralization, distribution, and sorption of U-ring-14C atrazine and on soil C mineralization was quantified in packed-soil microcosms using silt loam soil. A priming effect (stimulation of soil C mineralization) caused by atrazine supply was shown that likely lowered the earthworm net effect on soil C mineralization in atrazine-treated soil microcosms. Although earthworms significantly increased soil microbial activity, they reduced atrazine mineralization to 14CO2–C from 15.2 to 11.7% at 86 d. Earthworms facilitated formation of non-extractable atrazine residues within C-rich soil microsites that they created by burrowing and ingesting soil and organic matter. Atrazine sorption was highest in their gut contents and higher in casts than in burrow linings. Also, gut contents exhibited the highest formation of bound atrazine residues (non-extractable atrazine). Earthworms also promoted a deeper and patchier distribution of atrazine in the soil. This contributed to greater leaching losses of atrazine in microcosms amended with earthworms (3%) than in earthworm-free microcosms (0.003%), although these differences were not significant due to high variability in transport from earthworm-amended microcosms. Our results indicated that earthworms, mainly by casting activity, facilitated atrazine sorption, which increased atrazine persistence. As a consequence, this effect overrode any increase in atrazine biodegradation due to stimulation of microbial activity by earthworms. It is concluded that the affect of earthworms of atrazine mineralization is time-dependent, mineralization being slightly enhanced in the short term and subsequently reduced in the medium term.

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Keywords: Herbicide; Earthworms; Atrazine degradation; Atrazine sorption; Soil C mineralization

1. Introduction

Atrazine (2-chloro-ethylamino-6-isopropylamino-s-triazine) is one of the most widely used herbicides in the world and it is frequently detected in terrestrial and freshwater ecosystems (IFEN, 2002). Consequently, there has been considerable research into the factors affecting the environmental fate of atrazine (Gevao et al., 2000). Since it cationic, atrazine has a high molecular affinity for soil organic matter-clay complexes (Khan, 1978) and its sorption to soils correlates positively with organic carbon content (Barriuso et al., 1992; Park et al., 2004). Sorption of atrazine to organic matter lowers its bioavailability (Demon et al., 1994; Houot et al., 1998), which increases its persistence despite its susceptibility to abiotic and biotic degradation (Radosevich et al., 1997).

In soils, atrazine degradation is mainly a result of microbial activity. A large variety of soil microorganisms degrade atrazine through co-metabolic processes that lead to the formation and accumulation of atrazine metabolites (Sheunert, 1992; Hickey et al., 1994) while others microorganisms derive nutrients and energy by completely mineralizing atrazine to CO2. These include various bacterial strains as Pseudomonas
(Yanze-Kontchou and Gschwind, 1994; Agrobacterium radiobacter J14a (Struthers et al., 1998), and Nocardioides sp. (Topp et al., 2000).

Soil macrofauna can also directly and indirectly affect atrazine fate and degradation. In particular, earthworms are keystone organisms that can significantly impact microbial communities and soil physicochemical properties. For example, earthworms bury and redistribute surface litter within the soil profile; ingest and intimately mix soil with organic matter during gut transit; alter soil structure through their burrowing and casting activities; and their metabolic wastes such as urine, mucus, and tissue enhance C- and N- availability (Lee, 1985; Edwards and Bohlen, 1996).

Accordingly, earthworm activity may affect atrazine dynamics in two ways. First, earthworms may change atrazine mobility and sorption. Earthworm burrows have been shown to enhance atrazine transport through the soil when storms heavy enough to initiate macropore flow occur shortly after atrazine application (Edwards et al., 1993). However, burial and incorporation of herbicide-coated residues into the soil by earthworms may also reduce the mobility of atrazine and delay its leaching (Farenhorst et al., 2000a). Additionally, since earthworms preferentially ingest fine soil particles (mostly clays) and organic matter that is intimately mixed with intestinal mucus (polysaccharides) during gut transit (Barois, 1992), their casts often display higher triazine sorption capacities than uningested soil (Bolan and Baskaran, 1996; Akhouri et al., 1997; Farenhorst and Bowman, 2000).

Secondly, on the other hand, earthworms may promote atrazine mineralization because they modify the size and diversity of soil bacterial and fungal communities (Clapperton et al., 2001; Tiunov and Dobrovolskaya, 2002; Orazova et al., 2003) and their metabolic activity (Binet et al., 1998; Tiunov and Scheu, 1999; 2000; Scheu et al., 2002). Thus, worms may stimulate pesticide-degrading bacteria, either because soil that has been burrowed by earthworms is more favorable for their growth through additional C and N resources, or because the bacteria are more widely dispersed and more likely to encounter the herbicide. The few studies that have reported on the role of earthworms on Atrazine mineralization have yielded inconsistent conclusions, with Meharg (1996); Gevao et al. (2001) reporting enhanced mineralization and Farenhorst et al. (2000b) noting reduced degradation.

Given these contradictory findings, our objective was to determine whether earthworms enhance atrazine mineralization by stimulating herbicide-degrading microflora or increase atrazine persistence by facilitating sorption and reducing transport. We also sampled representative soil microsites in order to determine how burrowing and casting by earthworms affect atrazine mineralization and sorption in a silt loam soil. A combination of two eco-physiological species of earthworms, the anecic species L. terrestris (Linne, 1758) and the endogeic species A. caliginosa (Savigny, 1826), was investigated in order to simulate realistic earthworm community conditions.

2. Materials and methods

2.1. Soil, litter, and earthworms

Silt loam soil material (sand 12, silt 75, clay 13%) was collected from the upper 30 cm of a maize (Zea mays L.) agroecosystem at the INRA experimental site in Vezin-le-Coquet, Brittany, France. The slightly acid (pH 6.4), low organic matter content (1.8%) soil was air-dried and sieved (2 mm) to remove stones and plant roots. It was then remoistened with distilled water to a gravimetric water content of 20% prior to building the soil columns. Maize leaves were collected from the soil surface at the same location and time. The leaves were dry-brushed to remove adhering soil and were cut into small pieces <0.5 cm². Earthworms were obtained at the same location by formalin extraction (Bouché and Aliaga, 1986). Immature L. terrestris and adult A. caliginosa were retained for usage as these species dominate the earthworm community in this agroecosystem (Binet and Le Bayon, 1999).

The earthworms were transferred to the laboratory where they were acclimated in the experimental soil for 10 d at 12 °C prior to placement in the soil columns.

2.2. Microcosm design

Twenty-four soil microcosms (PVC pipe, 9.4 cm internal diameter) as described by Binet and Tréhen (1992) were used. The microcosms were filled with 1.8 kg of remoistened soil and packed to a bulk density of 1.46 g cm⁻³, resulting in 15-cm-deep soil columns. Distilled water was then added to the columns to bring them to their water holding capacity of 28.1% w/w. Four treatments with six replications were applied to the microcosms: atrazine and earthworms (A⁺, Ew⁺), atrazine without earthworms, (A⁺, Ew⁻), earthworms without atrazine (A⁻, Ew⁺), and soil without atrazine and earthworms (A⁻, Ew⁻). Half the microcosms in each treatment were incubated for 43 d at 12 °C (mean spring and autumn air temperature) under a 12 h:12 h photoperiod with the remaining three microcosms incubated for 86 d.

Three earthworms, one immature L. terrestris (mean wt. 2.55 g) and two adult A. caliginosa (mean wt. 0.54 g), were placed in each Ew⁺ treatment microcosm. Total earthworm biomass averaged 3.63 g per microcosm (min 2.45 g; max 4.66 g). Two days after the earthworms moved into the soil, a 33 mg l⁻¹ solution of atrazine in water consisting of 17.9 mg l⁻¹ of U-ring-¹⁴C atrazine (Sigma-Aldrich, 95% radiochemical purity; sp. act 18.6 mCi mmol⁻¹) and 15.1 mg l⁻¹ of technical grade atrazine (Pestanal, Riedel-de-Haën) was sprayed uniformly on the surface of each A⁺ microcosm. The rate of application was equivalent to 1 kg a.i. ha⁻¹. Thus, each microcosm received a total of 0.7 mg of atrazine in 21 ml of water corresponding to ca 642 Bq g⁻¹ dry soil. Control (A⁻) microcosms received an equal volume of distilled water.

Maize leaf litter was added one day after atrazine application in order to minimize herbicide sorption by the litter. The litter was distributed uniformly on the top of each
column at a rate of 0.5 g every 2 weeks for a total of 1.5 g for the microcosms incubated for 43 d and 3.0 g for those maintained for 86 d. In the microcosms without earthworms (i.e. Ew− treatments), the litter was partially hand-buried using a spatula in order to simulate burial by earthworms and reduce fungal growth on the litter (Binet and Tréhen, 1992). Water was added to the microcosms in ~10 ml aliquots every 10–15 d to compensate for evaporation. A total of 50 ml was added to the microcosms incubated for 43 d and 80 ml for those incubated 86 d.

2.3. Sampling procedures

Atrazine and organic matter mineralization was continuously measured by placing CO₂ traps containing 30 ml of 0.5 M NaOH in the microcosms for 24 h every 2 d throughout the 43 and 86 d incubations. Similarly, surface casts were gathered every week, but the samples were pooled after 10, 25, 40, 53, 67, and 86 d in order to have sufficient material for analysis. At the conclusion of the incubations the microcosms were destructively sampled and the earthworms and any cocoons noted were removed and rinsed several times with distilled water. Radioactivity was then measured by mixing 1 ml of the rinsate with 5 ml of Hionic-Fluor scintillation cocktail (Packard) and performing liquid scintillation counting (LSC) using a Packard Tricarb 1900 (2×5 min counts) in order to determine earthworm and cocoon contamination by radio-labeled atrazine. The earthworms were then transferred onto humidified filter paper in Petri dishes for 24 h to purge their gut contents, which were retained for analysis.

Soil in microcosms with and without earthworms was sampled from 0–5 cm, 5–10 cm, and 10–15 cm depths. These 100 g samples did not include soil that was worked by the earthworms. In earthworm-amended microcosms the burrow linings were sampled separately by removing a 2-mm-thick layer with a fine spatula along the entire length of the burrows. Burrows along the walls of the microcosms were excluded from these samples as they were mainly half-burrows with discontinuous linings and contact between the wall of the PVC pipe and the soil may have contributed to atypical movement of the herbicide.

2.4. Analytical procedures

All soil samples were dried overnight at 60 °C, hand-crushed, and mixed in a mortar and pestle. Ten gram subsamples of the soil from each of the three depth intervals, 3–5 g of casts and burrow linings (depending on amount available), and the total amount of available gut contents were used for atrazine extraction and radioactivity measurements. In addition, organic carbon content of the soil, casts, and burrow linings was determined by Anne method (Page et al., 1982) at the INRA laboratory of Soil Science in Arras, France.

Radioactivity originating from the mineralization of the ring-labeled atrazine was determined by mixing 1 ml of NaOH from the CO₂ traps with 5 ml of Hionic-Fluor scintillation cocktail prior to LSC. Measured radioactivity as a percentage of applied radioactivity was used to calculate atrazine mineralization. The total amount of organic matter mineralized was determined by diluting 10 ml of NaOH from the traps with 10 ml of water and 10 ml of 1 M BaCl₂ (added to avoid additional sorption of atmospheric CO₂) and titrating to neutrality with 0.25 M HCl. The amount of CO₂–C evolved from the microcosms was calculated using the difference between the volume of HCl needed to neutralize the NaOH before and after placement in the traps. The amount of atrazine leached from the microcosms was calculated using the volume of gravitational drainage collected every 2 weeks and the measured radioactivity in a 1 ml sample mixed with 5 ml Hionic-Fluor cocktail and subjected to LSC (2×5 min).

The amount of ¹⁴C-atrazine retained in the soil was assessed by sequential extraction. Soil samples (0.3–0.5 g gut contents, 3–5 g casts and burrow linings or 10 g bulk soil) were first extracted by shaking with distilled water (10, 30 or 100 ml, respectively), for 1 h and then centrifuging for 20 min (20,000 or 11,000 revs/min, respectively). These extractions were repeated until radioactivity was no longer detected in the water. The water extracts were pooled and total radioactivity was determining by mixing 1 ml with 5 ml of Packard Ultimagold scintillation cocktail and performing LSC (3×10 min, Packard Tricarb 4430). Methanol–water extraction (1:1 by volume) was then performed on the samples to determine atrazine bound by hydrophobic links. Extraction and counting followed the same procedures used for the water extracts. Finally, non-extractable atrazine was determined by trapping ¹⁴C–CO₂ produced by combusting 50 mg of extracted soil using a Carlo Erba NA 1500 oxidizer followed by LSC. Combustion and counting efficiency were determined by processing a standard quantity of ¹⁴C radio-labeled glucose solution.

2.5. Statistical analysis

Non-parametric Mann–Whitney (U-tests) and Kruskal-Wallis tests were performed using MINITAB version 13.31 to assess: (i) atrazine impact on earthworm biomass, litter consumption, cast production, and epidermis contamination, (ii) earthworm effects on atrazine sorption, and (iii) atrazine leaching by comparing different microsites from microcosms with and without worms. ANOVA was performed on CO₂ data to determine atrazine and earthworm effects and possible interactions on soil respiration (MINITAB ver. 13.31). Linear regression was used to determine the relationships between the amount of organic carbon and the amount of non-extractable ¹⁴C-atrazine in the various microsites.

3. Results

3.1. General observations: earthworm mortality, biomass, and atrazine exposure

In microcosms that were inoculated with earthworms, but not treated with atrazine (A−, Ew−), no change in earthworm biomass or mortality was noted during the 86-d incubation.
In contrast, two endogeic A. caliginosa died during the 86-d period in microcosms that received atrazine. Total earthworm biomass also declined by 12% with A. caliginosa exhibiting a greater weight loss (−26%) than L. terrestris. Earthworms and cocoons collected in the upper 10 cm of soil were all contaminated by atrazine; atrazine sorption on worm epidermis was significantly higher at 86 d than at 43 d. The water used to rinse the epidermis of L. terrestris and A. caliginosa contained 256 and 153 Bq, respectively, (i.e. 0.03 and 0.018% of the rinse the epidermis of L. terrestris) whereas the cocoon rinsate contained < 20 Bq or 0.002% of the radio-labeled atrazine. Litter consumption (6.8 ± 0.7 mg d\(^{-1}\) g\(^{-1}\) fresh earthworm wt) and surface-cast production (0.18 ± 0.02 g d\(^{-1}\) g\(^{-1}\) fw) by earthworms were not significantly affected (\(P \leq 0.05\)) by atrazine exposure.

### 3.2. Carbon mineralization

The patterns of soil respiration were similar in all treatments throughout the 43- and 86-d incubations (Fig. 1), although there were significant differences among treatments in total CO\(_2\)–C output. The control (A\(^-\), Ew\(^-\)) microcosms had the lowest average microbial respiration rate (3.22 μg CO\(_2\)–C g\(^{-1}\) dry soil d\(^{-1}\)) for the 86-d incubation. Microbial respiration rate was slightly increased by the addition of atrazine (A\(^+\), Ew\(^-\)), although the total amount of C evolved was not significantly different (\(F = 0.01, P = 0.911\)) from the control. In contrast, the addition of earthworms significantly increased CO\(_2\) output (\(F = 22.78, P = 0.001\)) and resulted in the highest average respiration rate (5.43 μg CO\(_2\)–C g\(^{-1}\) dry soil d\(^{-1}\)). When atrazine was present in the earthworm-amended microcosms CO\(_2\) evolution was reduced to 4.89 μg CO\(_2\)–C g\(^{-1}\) dry soil d\(^{-1}\). Overall the presence of earthworms in microcosms without atrazine increased CO\(_2\) output 2.4 times more than the addition of earthworms to those amended with atrazine, indicating a significant atrazine \(\times\) earthworm interaction (\(F = 8.75, P = 0.018\)).

![Fig. 1. Cumulative respiration in microcosms with atrazine and earthworms (A\(^-\), Ew\(^-\)), atrazine without earthworms, (A\(^+\), Ew\(^-\)), earthworms without atrazine (A\(^-\), Ew\(^+\)), and soil without atrazine and earthworms (A\(^-\), Ew\(^-\)). The amount of carbon mineralized is expressed as mg CO\(_2\)–C per microcosm (i.e. 1500 g dry soil). Cumulative amounts at 43 and 86 d are indicated and bars depict standard errors.](image1)

### 3.3. Atrazine mineralization

Atrazine mineralization was similar in earthworm-free and earthworm-amended microcosms for the first 25 d of incubation (Fig. 2). Following an initial lag phase, the rate of mineralization peaked at 0.74% of labeled atrazine per day 11 d after application. After 25 d the atrazine mineralization stabilized at a lower rate in microcosms with earthworms compared to those that were earthworm-free. Cumulative mineralization at 86 d was 15.3% of applied radio-labeled atrazine in microcosms without earthworms, which was 31% higher than the 11.7% cumulative mineralization measured in earthworm-amended microcosms.

### 3.4. Atrazine distribution, sorption, and persistence

Earthworm activity resulted in the redistribution and compartmentalization of atrazine (Fig. 3). In general, irrespective of extractability fraction, \(^{14}\)C-atrazine concentrations were significantly higher in burrowed and ingested soil than in soil that was not worked by earthworms. The concentration of labeled atrazine in all fractions was highest in the gut content samples at 43 and 86 d with the next highest concentrations noted in casts (Fig. 3). Atrazine concentrations in all extractability fractions were highest in casts collected 14 d after atrazine application and generally declined with subsequent collections (Fig. 4). Atrazine concentrations in burrow linings were 11–52 times higher than in non-earthworm worked soil collected below 10 cm (Fig. 3).

Earthworm activity resulted in deeper translocation of atrazine even in those portions of the microcosms that were not worked by earthworms (Fig. 3). In microcosms without earthworms more radioactivity remained in the upper 5 cm of soil than in the lower layers, indicating minimal downward movement of atrazine after 86 d. In comparison, after 86 d there was significantly less radioactivity (1.8×) in the 0–5 cm layer and significantly more radioactivity in the 5–10 cm (2.5×) and 10–15 cm (25.6×) layers of the non-earthworm worked portions of the earthworm-amended microcosms than in the corresponding layers of the earthworm-free microcosms. Atrazine translocation to deeper layers in non-earthworm

![Fig. 2. Cumulative atrazine mineralization as a percentage of applied U-ring-\(^{14}\)C atrazine in microcosms with (A\(^-\), Ew\(^+\)) and without earthworms (A\(^-\), Ew\(^-\)). Bars depict standard errors.](image2)
worked portions of the microcosms also tended to progress with time. Comparisons of concentrations at 43 and 86 d using the Kruskal-Wallis test (95%, n=3) for microcosms without and with earthworms, respectively, indicated the following: 0–5 cm, \( P=0.275 \) and \( P=0.127 \); 5–10 cm, \( P=0.05 \) and \( P=0.827 \); 10–15 cm, \( P=0.05 \) and \( P=0.275 \). Conversely, total radioactivity declined from 43 to 86 d in almost all instances in casts, gut contents, and burrow lining material (Fig. 3). In particular, total \(^{14}\text{C}\)-atrazine in casts decreased progressively with time, with available (water extractable) atrazine decreasing more rapidly than bound (non-extractable) atrazine (Fig. 4). Non-extractable atrazine did increase from 43 to 86 d, however, in most microsites but not in casts and gut contents. For example, in microcosms amended with earthworms, it increased by 7% in unburrowed portions of the 5–10 and 10–15 cm layers and by 18% in burrow-linings. These observations suggest that formation of atrazine bound residues (non-extractable atrazine) increased with time but that its distribution became more heterogeneous in the presence of earthworms. The higher amounts of non-extractable atrazine in the 0–5 cm layer of earthworm-free microcosms compared to microcosms with earthworms (5% higher at 43 d, 12% higher at 86 d) supports this contention. Moreover, the amount of non-extractable \(^{14}\text{C}\) was positively correlated with C content in the unburrowed soil, casts, burrow-linings microsites at 43 d (\( r=0.87, P=0.05, n=9 \)), although the correlation was less strong at 86 d (\( r=0.63 \)).

3.5. Leachate volumes and atrazine transport

Earthworms increased atrazine losses in drainage through the 15-cm deep columns, although the effect was not significant due to high variability among replicates. Leachate volumes, however, were significantly higher from microcosms with earthworms than from those that were earthworm-free for all sampling dates except the first one 9 d after atrazine application (Table 1). In general, leachate volume decreased with time for both treatments and percolation ceased after 46 d in earthworm-free microcosms, but continued until the experiment was terminated at 86 d in microcosms with earthworms (Table 1). Total radioactivity recovered in leachate from microcosms with earthworms was 1000-fold greater than from those
Means within the same collection time followed by different letters are significantly different at P < 0.05 according to the U-Mann–Whitney test.

without earthworms (3 vs. 0.003% of labeled atrazine applied), but differences in transport were not statistically significant because 14C-atrazine concentrations were extremely variable in leachate from microcosms containing earthworms (Table 1).

4. Discussion

4.1. Atrazine mineralization

The rate of CO2 evolution from the microcosms increased with the addition of earthworms suggesting that they increased organic matter mineralization by stimulating soil microbial activity. Despite this increase in respiration, significantly less atrazine was mineralized after 86 d in microcosms amended with earthworms (11.7%) than in microcosms without earthworms (15.3%). These atrazine mineralization rates, measured at a constant temperature of 12 °C, were similar in magnitude to the 20% reported by Klint et al. (1993) after 90 d in a field soil, but were substantially greater than those reported by Farenhorst et al. (2000b) in soil columns amended with L. terrestris and maintained at 12 °C for 68 d (<3% mineralization) and Meharg (1996) for L. terrestris maintained in soil-filled flasks at 14–23 °C for 4 weeks (<1% mineralization).

Nevertheless, our finding that earthworms reduce atrazine mineralization was supported by the previous results of Farenhorst et al. (2000b) as they reported a slight difference in Atrazine mineralization between columns without and with the presence of L. terrestris (2.3 vs. 1.7%, respectively). On the other hand, Meharg (1996) found that L. terrestris doubled the rate of atrazine mineralization to CO2 and Gevao et al. (2001) noted that Aporrectodea longa significantly increased atrazine mineralization.

One possible explanation for these contradictory findings is that the effect of earthworms on atrazine degradation may be time-dependent. The short-term (≤4 weeks) studies by Meharg (1996); Gevao et al. (2001) indicated the earthworms enhanced atrazine mineralization whereas reduced mineralization with longer incubations were noted by Farenhorst et al. (2000b) and indicated by atrazine mineralization kinetics that shifted after 4 weeks in our present study (Fig. 2). Methodological differences between flask-based and soil column-based microcosms as well as factors such as earthworm biomass to soil ratio, earthworm species, and physicochemical properties of the test soil may have also contributed to the differences among studies. In fact, since atrazine is cationic, its sorption to humic matter and clay is pH dependent (Khan, 1978; Laird et al., 1992). Nevertheless, there were no apparent systematic differences in the soils used by Meharg (1996); Gevao et al. (2001) and the soils used by Farenhorst et al. (2000b) and in our study that would suggest differences in atrazine mineralization. Meharg (1996) postulated that mucilage secretion by earthworms promotes atrazine mineralization whereas Gevao et al. (2001) suggested that burrowing by earthworms enhanced the activity of bacteria capable of mineralizing atrazine. Definitive experiments are needed to test which of these two explanations can be substantiated.

4.2. Atrazine sorption and availability

Our results, based on microcosms that reasonably mimicked physicochemical relationships in the field, suggested that earthworms reduced atrazine availability by promoting binding to C-rich soil microsites, as indicated by positive correlations between non-extractable atrazine and microsite C contents. Earthworms have been shown to increase soil organic C content by incorporating plant materials and intestinal mucus into the soil (Lee, 1985; Lavelle and Spain, 2001). Since atrazine sorption is C-dependent (Khan, 1978; Demon et al., 1994; Houot et al., 1998) this should result in reduced atrazine bioavailability.

Our new findings were that (i) atrazine concentration and sorption was highest in earthworm gut contents and lowest in soil that was not worked by the earthworms and (ii) atrazine concentration in burrow linings was less than half that in excreted surface casts. The higher water content of gut contents and to a lesser extent of casts compared with burrow-linings and parts of soil not reworked by the worms may explained their higher concentration of available atrazine. Also, regarding the highest atrazine concentration in gut contents, we could not exclude an experimental bias due to a more favorable ratio water extractant: soil mass, that might result in a greater atrazine extraction. The decline of Atrazine concentrations in casts collected as far as time increased following atrazine application is likely a consequence of the enhanced atrazine losses in drainage through the 15-cm deep columns with earthworms (Table 1).
Atrazine sorption to earthworm burrow linings was first suggested by Edwards et al. (1992) and has been confirmed by Stehouwer et al. (1993, 1994), Farenhorst et al. (2000a), and our results. Farenhorst et al. (2000a) found that levels of extractable, radio-labeled atrazine in burrow linings were twice those of the surrounding soil. They also report that atrazine sorption (bound atrazine residue) was greater in soil inhabited by *L. terrestris* than in soil without earthworms; although they did not distinguish between burrow linings and casts in this instance. In the present study, we did not confirm a significant greater formation of bound atrazine residues in burrow-linings. Some of the differences between our results and those of Farenhorst et al. (2000a) may be related to the fact that we sprayed radio-labeled atrazine onto the soil surface before adding litter to the microcosms while they sprayed it onto the surface litter.

The higher sorption of atrazine to gut contents and casts than to burrow linings that we noted suggested that earthworm casting activity should have a more pronounced effect on atrazine persistence than burrowing. Burrow linings are composed primarily of fragmented litter pulled into the soil by earthworms, mucous and urea excreted by the earthworms, and some faecal material deposited during successive passages. Earthworm casts, however, are the result of intimate mixing of soil and litter in the worm gut along with the addition of large amounts of intestinal mucous and water (Barois and Lavelle, 1986). This probably results in more humic and colloidal organic matter in casts than in burrow-linings. The strong correlation of atrazine sorption to C content also suggests that litter feeding earthworm species that greatly increase the organic matter content of soil they ingest and burrow should have a major effect on atrazine fate.

Our results also showed that non-extractable, labeled atrazine residues increased from 43 to 86 d post-application, but not in casts and gut contents. Decreased atrazine availability with residence time was also observed in ‘no-till’ and ‘conventional-till’ soils by Radosevich et al. (1997). They postulated that in undisturbed, no-till soil reduced atrazine availability was related to enhanced sorption due to increased organic matter content and greater diffusion of soluble atrazine through micropores than in tilled soils. Thus, a careful study of changes in soil microporosity during gut transfer and the mixing of soil could provide insight in defining the mechanisms by which earthworm casting enhanced atrazine sorption. However, the high fraction of non-extractable atrazine in gut contents (Fig. 3) sustains that mucilage secretion during gut transit might be determinant as well. The two gut contents and cast samples were newly-formed samples at both 43 and 86 d compared with other microsites, that likely explained why they did exhibit any increase in non-extractable atrazine residues from 43 to 86 d post-application.

### 4.3. Atrazine distribution and transport

Earthworms were undoubtedly responsible for vertical redistribution of ¹⁴C-atrazine within the microcosms. Their burrows constituted preferential flow paths that contributed to the movement of atrazine through the microcosms, but high variability in burrowing activity among columns probably contributed to the lack of significant differences in herbicide losses in leachate among treatments. Differences in the way atrazine was applied to the microcosms (i.e. soil-applied vs. applied to litter) may explain why we found more pronounced atrazine mobility in presence of earthworms than did Farenhorst et al. (2000b).

#### 4.4. Impact of atrazine and earthworms on microbial respiration

Addition of atrazine to the microcosms in the absence of earthworms increased C mineralization, therefore it must have stimulated soil biological activity. Although some of this increased output of CO₂-C might have been the result of microbial metabolism of atrazine (Hickey et al., 1994; Entry et al., 1996; Fadullon et al., 1998; Struther et al., 1998), the amount of atrazine added to each microcosm (0.7 mg atrazine, 0.26 mg C) and the amount mineralized in 86 d (15.2%) was insufficient to account for the additional amount of C mineralized (94 mg C per microcosm). Soil microbial activity measured by CO₂ output and U-ring atrazine mineralization have been shown to be independent (Alvey and Crowley, 1995; Houot et al., 1998). Therefore, the additional mineralized C must have been derived from the soil organic matter and/or the litter added to the microcosms, probably as a result of changes in the composition and/or activity of the microbial communities following atrazine application. This accounts for the priming effect, i.e. the stimulation of soil organic matter mineralization after organic matter input to soil (Fontaine et al., 2003).

Nonetheless, our results indicated that addition of earthworms had a much greater effect (2.4 X) on C mineralization when the microcosms were not amended with atrazine. This was probably the result of the combination of (i) the priming effect caused by atrazine supply on soil C mineralization that partly masked the earthworm net effect and (ii) the possible atrazine toxicity on earthworms as it was found to be absorbed onto the earthworm epidermis and there was a net loss in earthworm biomass compared to microcosms that were not treated with atrazine.

### 5. Conclusions

Our experiment provided three important findings regarding the role of earthworms on the fate of herbicide in soil. First, earthworms facilitated atrazine sorption to the silt loam soil; especially within C-rich soil microsites that they produced, with earthworm gut contents and casts having a more pronounced effect on atrazine persistence (bound residues) than burrows. Second, atrazine sorption to these microsites reduced the bioavailability of atrazine to soil microorganisms, which resulted in significant reduced biodegradation. Third, the effect of earthworms on atrazine mineralization was time-dependent with mineralization being slightly enhanced in the short term (<4 weeks), but reduced in the medium term
(> 4 weeks). In addition, a priming effect (stimulation of soil C mineralization) caused by atrazine supply was shown that probably lowered the earthworm net effect on soil C mineralization in the soil treated with atrazine.

Acknowledgements

This research work was part of PEVS-CNRS program on the Fate of Pollutants in terrestrial ecosystems. It also received grants from the regional council of Brittany awarded to A. Kersanté & Dr F. Binet. The authors greatly thank Pr A.J. Morgan and Pr K. Butt for correcting the English language of the text, the anonymous referee for its helpful comments on the manuscript, and Michel Lefevre for technical assistance in setting-up the soil microcosms.

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