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Earthworms highly increase ciprofloxacin mineralization in soils

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Abstract

This report shows that earthworms increase up to eight times the mineralization of the antibiotic ciprofloxacin in soils. Antibiotics are extensively used and disseminated in environmental compartments. Antibiotics may enter food chains and thus induce resistance in environmental and human commensal bacteria. The antibiotic ciprofloxacin is suspected to induce significant adverse effects on soil microbial processes, with possible consequences on soil functions. Nevertheless, little is known concerning the fate of ciprofloxacin in soils. Here, we studied the mineralization and distribution of the [2-¹⁴C]-ciprofloxacin in soil-plant-water systems where ciprofloxacin was applied by amendment of spiked pig slurry. Results show that a very weak microbial mineralization of the antibiotic, lower than 0.01 %, occurred after 84 days of incubation. By contrast, the addition of earthworms increased from 5 to 8 times ciprofloxacin mineralization during the following 84 days incubation. In addition, earthworm activity induced the transfer of 40 % of radioactive compounds from the upper to the lower layer of soil, modifying the distribution of the antibiotic within the soil profile. We conclude that earthworms can be used efficiently to mineralize ciprofloxacin and modify its distribution in soils. As a consequence, earthworms change the exposure of soil organisms to ciprofloxacin, and, in turn, the eco-toxicological impact of the antibiotic.

Keywords Antibiotics, Fluoroquinolone, Persistence, Bioturbation, Ecotoxicological assessment, Pig slurry

Introduction

Fluoroquinolones are a major class of antibiotics world- wide used for human and veterinary medicine. Excreted from cattle and humans in urine and faeces, they enter the environment directly through grazing livestock or indirectly through the use of manure, slurry and sewage sludge as fertilizers of agricultural soils (Thiele-Bruhn 2003; Ding and He 2010). Runoff and wastewater are then responsible for the contamination of aquatic ecosystems. There is a growing concern about the potential impact of antibiotic residues on the environment, as well as on human health. Among fluoroquinolones, ciprofloxacin is widely detected in the environment following its own use, or as the main metabolite of enrofloxacin.

The effects and fate of ciprofloxacin on the soil ecosystem are poorly known, thus underlining the ecotoxicological relevance of this antibiotic. The quinolone has been shown to be genotoxic, and Mersch-Sundermann et al. (1994) discussed early possible mutagenic effects in eukaryotic cells. Effects of ciprofloxacin on several microbial biomarkers have been reviewed by Ding and He (2010). Recently, Girardi et al. (2011) reported an inhibition of soil respiration, as well as a shift in microbial abundance and diversity of microbial communities after application of ciprofloxacin, although at high amounts of contamination. By contrast, considering realistic levels of antibiotic contamination of pig slurry used to amend French soils, we failed to demonstrate any significant effects of the antibiotic on the microbial community, as well as on enzymatic biomarkers of the white-rot fungus *Trametes versicolor* maintained in spiked liquid culture (Igel-Egalon et al. 2012). For many years, ciprofloxacin has been measured in aquatic and terrestrial environments, and its concentration in soils ranges from 6 to 750 $\mu\text{g kg}^{-1}$ according to the type of soil and way of contamination (Golet et al. 2002; Kemper 2008; Martinez-Carballo et al. 2007). The antibiotic appears poorly biodegradable and adsorbs onto soil particles with respect to their physic-chemical properties and mineralogy (Girardi et al. 2011; Vasudevan et al. 2009; Wu et al. 2010). As a consequence, it is hypothesized that ciprofloxacin can easily accumulate into the soil. Recently, Wen et al. (2011) reported the accumulation and elimination of ciprofloxacin in the redworm *Eisenia fetida* grown in quartz sands spiked with an aqueous solution of antibiotics. Ciprofloxacin was also measured in several subcellular fractions obtained from the earthworms. Here, we assessed the fate of ^{14}C -labelled ciprofloxacin entering an agricultural soil according to several scenarios of contamination. The first originality of our approach was to include in microcosms soil-dwelling macroorganisms well known for their ability in bioturbation. They are

intended to model a realistic scenario of soil-plant-water system (SPWS). Our second originality was the use of relevant species obtained from French soil (and not from rotting vegetation or compost as *Eisenia*) maintained in their real growing environment, the soil. We hypothesized that the efficiency of microbial communities for biotransformation of recalcitrant chemicals can be enhanced by the engineering role of earthworms. To our knowledge, no other study reported the action of earthworms on the fate of ciprofloxacin has been published.

Materials and methods

Chemicals and reagents

Chemicals and solvents were of analytical grade. All chemicals were purchased from Sigma-Aldrich (France). [2-¹⁴C]-ciprofloxacin (555 MBq mmol⁻¹) has been obtained from Moravek Biochemicals and Radiochemicals (Isobio, Fleurus, Belgium). Solvents were purchased from Carlo-Erba (Val de Reuil, France).

Soil characteristics

The silt loam (Luvisol, FAO classification) was collected in the 10–20 cm layer of an experimental site (La Cage, INRA, Versailles, France). It comprised 21.6 % sand, 61.1 % silt and 17.3 % clay. Its content in organic carbon was 0.98 %, and its pH_{water} was 7.1. Its water holding capacity (WHC) was 15.64 ± 0.21 % at pF = 2.5 and 6.79 ± 0.21 % at pF = 4.2. The soil was roughly homogenized, sieved at 2 mm, and used without storage for the preparation of the soil–plant–water systems (SPWSs).

Pig slurry sampling and characterization

The pig slurry, referred to here as green slurry, was from the quarantine livestock building of the IFIP (French Pig Institute) at Romillé (Brittany, France). The animals were cross-bred from Large White and Landrace and weighed 40-100 kg (fattening pigs). They were fed an unchanging cereal formula consisting of wheat (44 %), barley (30 %), soybeans (18 %), molasses (2 %) and amino acids and minerals (6 %). They did not receive antibiotics.

The pig slurry resulting from about three months of manure accumulation from 20 pigs was

homogenized in a pit (about 25 m³) using a submersible pump for about 20 min. Then, 20 L of slurry was collected and stored at 4 °C for 1 day until use.

The slurry pH (7.5), dry matter content ($26.2 \pm 0.1 \text{ g kg}^{-1}$), chemical oxygen demand ($36 \pm 2 \text{ g O}_2 \text{ L}^{-1}$), total Kjeldahl nitrogen ($3.05 \pm 0.03 \text{ g kg}^{-1}$) and total ammonia-N ($2.3 \pm 0.1 \text{ g kg}^{-1}$) were determined according to standard methods (APHA 1992). All parameters were analysed in triplicate. These data are in agreement with previous reports on pig slurry characterization in French husbandry (Burton and Turner 2003).

Design of the soil–plant–water system design

Here, we used the soil-plant-water system (SPWS) developed by our team (Ghanem et al. 2006), and set-up according to Igel-Egalon et al. (2012). Four experimental treatments have been prepared: non-amended control soil, soil amended with slurry spiked with low and high amounts of antibiotic and finally, soil spread with acetic solution of the antibiotic.

Three days before the beginning of the experiments, 1 kg dry soil fractions were prepared and moistened to 50 % of WHC. They were added to each SPWS as lower layers. For the control soil, 1 kg was similarly prepared and added to the SPWS as upper layer. Concerning the slurry-spiked treatments, 25 mL pig slurry was spread on 1 kg of soil which was added to the SPWS. That amount of slurry corresponded to the equivalent dose of 30 T dw ha⁻¹. The low-spiked and the high-spiked treatments received pig slurry previously spiked with labelled ciprofloxacin (42 and 420 kBq, respectively) to ensure a final concentration of 25 and 250 $\mu\text{g kg}^{-1}$ dry soil in the upper layer, respectively. Finally, in the fourth SPWS, the upper layer of soil was spread with an acetic solution of labelled ciprofloxacin (420 kBq) to ensure a final concentration of 250 $\mu\text{g kg}^{-1}$ dry soil. Afterwards, total soil was moistened to 80 % of WHC and the SPWSs were closed. The four SPWSs were incubated for 168 days under 16 h light at 20 °C and 8 h darkness at 18 °C. Soil moisture was checked every week and adjusted.

After 56 days of incubation, three seeds of wheat were sowed in each SPWS. After 84 days of incubation, four mature specimens of oligochaeta (two *Aporrectodea caliginosa* Savigny 1826 and two *Aporrectodea longa* Savigny 1826), previously collected from the same plot as the soil used to fill the SPWSs, were introduced in each SPWS. They were still alive and active at the end of the experiment.

Analytical procedures

We measured the mineralization, the partitioning and the leaching of antibiotic in the soil and the associated biological response in terms of soil microbial activity and transfer in wheat.

Regarding antibiotic mineralization, a stream (0.5 L min^{-1}) of wet air was continuously flushed through the SPWSs to allow $^{14}\text{CO}_2$ trapping in NaOH solutions. NaOH solutions were changed every 7 days and $^{14}\text{CO}_2$ was determined by liquid scintillation counting. In addition, two soil cores (1.5 cm diameter and 20 cm depth) were extracted to determine extractable and bound ^{14}C in the layers of the soil and soil/slurry mixtures at the beginning of the experiment, and after 84 days of incubation. At the end of the experiment (168 days of incubation), soils corresponding to the location to the previous upper and lower layers were separated, and samples were collected in each of them to measure the influence of earthworm bioturbation. Ultrasonic extraction was performed to recover radioactive compounds from 10 g fresh soil samples during 20 min, in the presence of 40 mL acidified acetonitrile (ACN) (Martinez-Carballo et al. 2007). The suspension in ACN was kept overnight and shaken during 1 h prior to centrifugation. After removal of the extract, another sequence comprising addition of ACN, shaking and centrifugation was performed. The two extracts were finally pooled and filtered. To assess the leaching of ciprofloxacin, water was sprayed after 70 days of incubation onto the soils to mimic 20-mm rainfalls and to allow recovery of leachates. Wheat seedlings were harvested and dried after a 28-day period of growth (84 days of incubation). The radioactivity was measured in all liquid fractions (extracts, leachates) by liquid scintillation counting. Non-extractable radioactivity (soil, soil/slurry samples and seedlings) was determined by combustion, followed by liquid scintillation counting.

After 28, 56 and 84 days of incubation, dehydrogenase activity (DHA; E.C. 1.1.1.1), indicating the level of global metabolic process in the soil, was measured according to an adapted protocol from Schaefer (1963). That activity was intended to evidence a possible effect of the antibiotic on the soil microbial activity. One millilitre distilled water and 250 μL aqueous solution of 1,3,5-triphenyltetrazolium chloride (TTC) at 40 g L^{-1} were mixed with 1 g fresh soil in 30-mL centrifuge tubes. Controls were prepared without TTC. Incubation was performed at $37 \text{ }^\circ\text{C}$ for 16 h. After that period, 5 mL ethanol was added to the mixture which was shaken for 5 min in the dark. The medium was incubated at $37 \text{ }^\circ\text{C}$ for 2 h, and shaken after 1 h. Finally, TTC was added to the control and all the mixtures were centrifuged 5 min at 6,000g. The absorbance of the supernatant (red colour in case of formazan formed) was measured at 485 nm. Results were expressed in mU g^{-1} dry soil.

Availability of ciprofloxacin in the soil solution

The amounts of ciprofloxacin in the soil solution were determined using the protocol of Gaillardon and Dur (1995) adapted by Kollmann et al. (2003). Aliquots of slurry and soil (250 μ L + 750 mg) or soil alone (1 g) were spiked with labelled (4.2 kBq) ciprofloxacin corresponding to 2.5 lg antibiotic, the highest amount was used for SPWS incubation. After adding the chemical, the solvent (acetone) was left to evaporate for 30 min. The chemicals were then absorbed onto the slurry and soil for 24 h at 4 °C before these spiked aliquots were mixed with 9 g non-spiked soil. Soil/slurry and soil samples (10 g equivalent dry matter) were placed in 5-cm diameter Petri dishes to give a 3-4 mm thick layer, and moistened dropwise to achieve 80 % of WHC. Although earthworms have not been added, the dishes were incubated in similar conditions than SPWSs, and radioactivity determined according to the above protocols. It was hypothesized that no transformation of the antibiotic occurred during that period.

Concentrations of ciprofloxacin in the soil solution were determined at the beginning of the incubation (just after soil spiking) and after 84 and 168 days of incubation. Two superposed 42.5 mm diameter glass micro fibber filters GF/A (Whatman) were laid on the soil surface and a slight pressure was applied for 10 s to favour wetting of the filters. The upper filter was then recovered. The volume of soil solution and the dissolved radioactivity retained in the filter were determined by weighing and liquid scintillation counting.

Results and discussion

Mineralization of ciprofloxacin in soils

The mineralization of ciprofloxacin was monitored in the four soil–plant–water systems (SPWSs) (Fig. 1). No mineralization occurred in the SPWS containing the reference soil. By contrast, traces of mineralization (less than 0.01 % of initial amount of radioactivity) were detected during the first week of incubation in the three SPWSs containing ¹⁴C-ciprofloxacin. That situation corresponded likely to the mineralization of impurities contained in the commercial solution of labelled ciprofloxacin (purity of 98.5 %). Then, mineralization increased very slightly to a maximal value of 0.019 % of initial radioactivity at day 84 for the lower spiking amount, and 0.007–0.010 % for the highest amount of contamination. Interestingly, earthworm introduction in the soil at day 84 led to a

sevenfold increase in antibiotic mineralization, reaching 0.058–0.080 % of initial radioactivity after the next 84-day period, depending on the SPWS considered. Several explanations can be provided concerning that last result. Earthworm bioturbation can affect the fate of contaminants by changing their mobility and sorption to the soil in modifying the quality and dynamics of soil organic matter (Ngo et al. 2012). It can also provide hot spots of microbial activity (Monard et al. 2008). Finally, earthworms can be efficient degraders directly by their own enzymatic equipment (Achazi et al. 1998), or indirectly because of the microflora present in their intestine (Shin et al. 2005). However, ciprofloxacin mineralization remained low. Repeated application of the antibiotic (i.e. by slurry amendment) is exceeding its degradation rates, ensuring the accumulation of the antibiotic on certain soil layers (Kemper 2008).

Dehydrogenase activity values measured in the soil samples along the incubation ranged from 0.03 to 0.12 mU g⁻¹ dry soil. They were not enhanced after wheat growth, indicating the lack of effect of the rhizosphere. They were in the range of values previously reported in the similar soil (Igel-Egalon et al. 2012) spiked with lower amounts of antibiotics (at the ng kg⁻¹ level). Here, no clear relationship has been evidenced between dehydrogenase activity, the type of soil treatment or the presence of ciprofloxacin. Our results evidenced the persistence of a microbial activity of the soil in the presence of antibiotic, and the poor mineralization did not seem linked with any toxic effect on soil microorganisms. Similar low levels of mineralization rates have been recently reported in soil using the OECD assay 307 (Girardi et al. 2011). It was reported to be sensitive to the contamination rate. Taken together, these results reveal a poor ability of soil endogenous microflora to mineralize ciprofloxacin. To our knowledge, microbial or fungal strains able to metabolize ciprofloxacin have not been identified from environmental media to date.

Distribution and transfer of labelled carbon among the compartments of the system

The mass-balance analysis of labelled carbon was performed in the two SPWSs treated with the highest amount of ciprofloxacin at the beginning of the experiment, and then after 84 and 168 days of incubation (Table 1). Just after soil spiking, labelled carbon in the two SPWSs was mainly associated with the solid phase of the soil (77–78 % of initial labelled carbon applied), and only 21 % of ciprofloxacin could be extracted. That result confirmed the strong and rapid sorption of the antibiotic onto the soil particles, without effect of the type of spiking. These initial values are in accordance with recent soil studies using radiochemicals (Girardi et al. 2011).

After 84 days of incubation including the simulated rainfall event, a more detailed analysis showed

that the main fraction of radioactive carbon was stabilized in the upper soils, probably as non-extractable residues. In parallel, the amount of extracted labelled carbon decreased to less than 0.6 % of initial radioactivity in the upper layers of soil. Nevertheless, a part of the radioactivity was measured in the lower layers of soil, showing a significant mobility of the antibiotic after the rainfall. Radioactive material was not detected in the extracts of that layer of soil. The strong sorption of antibiotics is described as governed by soil organic matter, soil minerals and soil pH (Thiele-Bruhn 2003). In our experiment, mobility of ciprofloxacin within the two layers of soil may be closely linked with mobility of dissolved organic matter (here endogenous and exogenous) or minerals. Clays of the studied soil contained 60 % smectite (montmorillonite) with a high CEC (100-150 cmol_c kg⁻¹) and the pH of the soil was 7.1. These properties were reported by Wu et al. (2010) and Vasudevan et al. (2009) to ensure a strong adsorption of the zwitterionic form of ciprofloxacin on the minerals.

After 168 days of incubation, earthworm activity resulted in a strong mixing of the upper and lower layers in the SPWSs, as evidenced by the observation of developed burrow systems through the glass wall of the experimental devices and a final packing of the soils. Analysis of the radioactivity confirmed that observation. 51.7 and 38.4 % of the radioactivity were detected in the soil fractions corresponding to the previous upper and lower layers of the soil amended with the spiked slurry. These values were 48.0 and 43.2 % in the case of the soil spread with the antibiotic. Radioactive carbon was never detected in the organic extracts obtained after 168 days of incubation of soils. The lack of published data concerning the action of earthworms on the fate of antibiotics prevents the comparison of our innovative results with other studies.

We studied the mobility of labelled carbon to soil leachates after rainfall simulation at 70 days of incubation, and to wheat seedlings during the 56-84-day period (Table 1). Radioactivity was detected in low amounts when expressed in % of the applied dose of labelled carbon, in leachates from the spiked soils. When expressed as concentrations of equivalent ciprofloxacin, the values were 0.46 and 0.16 µg L⁻¹ corresponding to 1.39 and 0.48 nM in leachates. Ciprofloxacin was also absorbed by wheat seedlings during their early growing period. All the wheat seeds were germinated 7 days after sowing, and the seedlings provided after 28 days of growth identical dry bio-mass whatever the soil treatment (around 70 mg dry biomass). Expressed as % of the applied dose or calculated as concentrations (µg kg⁻¹ dry biomass), values of absorbed radioactivity were very low, suggesting a weak transfer to that higher plant.

Finally, total amounts of labelled carbon measured ranged from 88 to 100 % of initial amounts applied. They were around 90 % after 161 days. Experimental shifts (¹⁴CO₂ trapping, soil

combustion...) may occur, but we cannot exclude any bioaccumulation of labelled carbon in earthworms. Finally, because the amounts of radioactive compounds in the liquid fractions were very low and prevented any qualitative analysis using HPLC coupled to radioactivity detection, we attributed labelled carbon to non-transformed ciprofloxacin.

Availability of ciprofloxacin in the soil solution

That experiment was performed to evidence variations of ciprofloxacin availability in the soil solution with respect to time, in the SPWSs spiked with the highest amount of ^{14}C -chemical (Fig. 2). Because of the previous results demonstrating the recalcitrance of the antibiotic, we hypothesize that labelled carbon corresponded to untransformed ciprofloxacin. Just after soil spiking, ciprofloxacin concentration measured in the soil solution was $0.57 \pm 0.07 \mu\text{g L}^{-1}$ when it was provided by the slurry, and $0.33 \pm 0.07 \mu\text{g L}^{-1}$ when it was directly spread. That difference between the soil solutions from two soils with different ways of contamination was not observed in the mass-balance analysis of SPWSs based on solvent extraction. In that previous experiment, using the % of labelled carbon extracted at the beginning of the experiment, we were able to calculate a concentration of ciprofloxacin reaching 6 ng L^{-1} in the organic extracts. Taken together, these results allow us to conclude that only a weak fraction of the solvent extractable antibiotic was available in the soil solution. Then, amounts of ciprofloxacin were 0.41 ± 0.03 and $0.36 \pm 0.02 \mu\text{g L}^{-1}$ after 168 days of incubation. Statistical analysis using two-way ANOVAs showed significant crossed effects ($P = 0.05$) of the type of contamination and length of the incubation on ciprofloxacin availability in the soil solution.

Our results demonstrated the low availability of ciprofloxacin for soil organisms. When expressed in molarity, average antibiotic concentrations in the soil solutions ranged from 0.81 to 1.90 nM. These values can be used to design further experiments for the assessment of antibiotic toxicity on (micro)-organisms in liquid cultures.

Conclusion

From the present study based on the use of radiolabelled ciprofloxacin, it is concluded that the antibiotic introduced in a French Luvisol through spiked pig slurry or direct spreading undergoes a very weak microbial mineralization under aerobic conditions, and may stabilize in the soil as non-extractable residues. Nevertheless, its mineralization and distribution in soils were affected by the

action of earth- worms through their engineering activity. Taken together, our results suggest that the action of these macroorganisms may be studied in more details, because they change the exposure of soil organisms to ciprofloxacin, and, in turn, the possible eco-toxicological impact of the antibiotic.

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References

- Achazi RK, Flenner C, Livingstone DR, Peters LD, Schaub K, Scheiwe E (1998) Cytochrome P450 and dependent activities in unexposed and PAH-exposed terrestrial annelids. *Comp Biochem Physiol C* 121:339–350
- APHA (1992) Standard method for the examination of water and wastewater, 18th edn. American Public Health Association, Washington p 1110
- Burton CH, Turner C (2003) Manure management, treatment strategies for sustainable agriculture, 2nd edn. Silsoe Research Institute, Wrest Park p 451
- Ding C, He J (2010) Effect of antibiotics in the environment on microbial populations. *Appl Microbiol Biotechnol* 87:925–941
- Gaillardon P, Dur JC (1995) Influence of soil moisture on short-term adsorption of diuron and isoproturon by soil. *Pestic Sci* 45:297–303
- Ghanem A, Dubroca J, Chaplain V, Mougin C (2006) Fate of herbicides and nonylphenol in soil–plant–water systems amended with contaminated sewage sludge. *Environ Chem Lett* 4:63–70
- Girardi C, Greve J, Lamshöft M, Fetzner I, Miltner A, Schäffer A, Kästener M (2011) Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. *J Hazard Mater* 198:22–30

- Golet EM, Strehler A, Alder AC, Giger W (2002) Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. *Anal Chem* 74:5455–5462
- Igel-Egalon A, Cheviron N, Hedde M, Hernandez-Raquet G, Mougine C (2012) Impact of antibiotics from pig slurry on soil microbial communities, including the basidiomycete *Trametes versicolor*. *Environ Toxicol* 27:129–136
- Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol Indic* 8:1–13
- Kollmann A, Brault A, Touton I, Dubroca J, Chaplain V, Mougine C (2003) Impact of nonylphenol surfactants on fungi following the application of sewage sludge on agricultural soils. *J Environ Qual* 32:1269–1276
- Martinez-Carballo E, Gonzalez-Barreiro C, Scharf S, Gans O (2007) Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environ Pollut* 148:570–579
- Mersch-Sundermann V, Hauff K-H, Braun P, Lu W, Hof H (1994) DNA damage caused by antibiotic drugs: quinolones. *Int J Oncol* 5:855–859
- Monard C, Martin-Laurent F, Vecchiato C, Francez AJ, Van- denkoornhuysse P, Binet F (2008) Combined effect of bioaugmentation and bioturbation on atrazine degradation in soil. *Soil Biol Biochem* 40:2253–2259
- Ngo P-T, Rumpel C, Doan T, Jouquet P (2012) The effect of earthworms on carbon storage and soil organic matter composition in tropical soil amended with compost and vermicompost. *Soil Biol Biochem* 50:214–220
- Schaefer R (1963) L'activité déshydrogénasique comme mesure de l'activité biologique globale des sols. *Annales de l'Institut Pasteur* 105:326–331
- Shin K-H, Lim H, Ahn J-H, Khil J, Cha C-J, Hur H-G (2005) Anaerobic biotransformation of dinitrotoluene isomers by *Lactococcus lactis* subsp. *lactis* strain 27 isolated from earthworm intestine. *Chemosphere* 61:30–39
- Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils—a review. *J Plant Nutr Soil Sci* 166:145–167
- Vasudevan D, Bruland GL, Torrance BS, Upchurch VG, MacKay AA (2009) pH-dependent ciprofloxacin sorption to soils: interaction mechanisms and soil factors influencing sorption. *Geoderma* 151:68–76
- Wen B, Huang R, Wang P, Zhou Y, Shan X-Q, Zhang S (2011) Effect of complexation on the

accumulation and elimination kinetics of cadmium and ciprofloxacin in the earthworm *Eisenia fetida*. Environ Sci Technol 45:4339–4345

Wu Q, Li Z, Yin HH, Tie L (2010) Adsorption and intercalation of ciprofloxacin on montmorillonite. Appl Clay Sci 50:204–211

Table 1 Mass-balance analysis at the beginning of the experiment, and after 84 and 168 days of incubation, in the compartments of our soil-plant-water systems filled with soil amended with slurry spiked with the high amounts of antibiotic, or soil spread with the high amount of antibiotic

Compartment	% of initial labelled carbon applied					
	At the beginning		After 84 days		After 168 days	
	spiked	spread	spiked	spread	spiked	spread
Mineralized	0	0	0.007	0.010	0.073	0.058
Upper soil layer						
-non-extracted	78.021	77.378	79.060	85.312	51.709	47.988
- extracted	21.527	21.488	0.560	0.570	n.d.	n.d.
Lower soil layer						
-non extracted	n.m.	n.m.	8.602	13.556	38.399	43.178
- extracted	n.m.	n.m.	n.d.	n.d.	n.d.	n.d.
Leachates (70 days)	n.m.	n.m.	0.014	0.005	n.m.	n.m.
Wheat seedlings (56-84 days)	n.m.	n.m.	<0.001	0.002	n.m.	n.m.
Total labelled carbon measured	99.548	98.866	88.236	99.445	90.608	91.166

^a if not indicated otherwise

n.d. not detected

n.m. not measured

Fig. 1 Mineralization of [2-¹⁴C]-ciprofloxacin in control soil (filled circle), soil amended with slurry spiked with the low (open circle) and high (filled inverted triangle) amounts of antibiotic, and soil spread with the high amount of antibiotic (open triangle) during the 168 days of incubation. Wheat seedlings were allowed to grow between 56 and 84 days of incubation. Earthworms have been introduced in the soils after 84 days of incubation. They increased the mineralization rate of the antibiotic

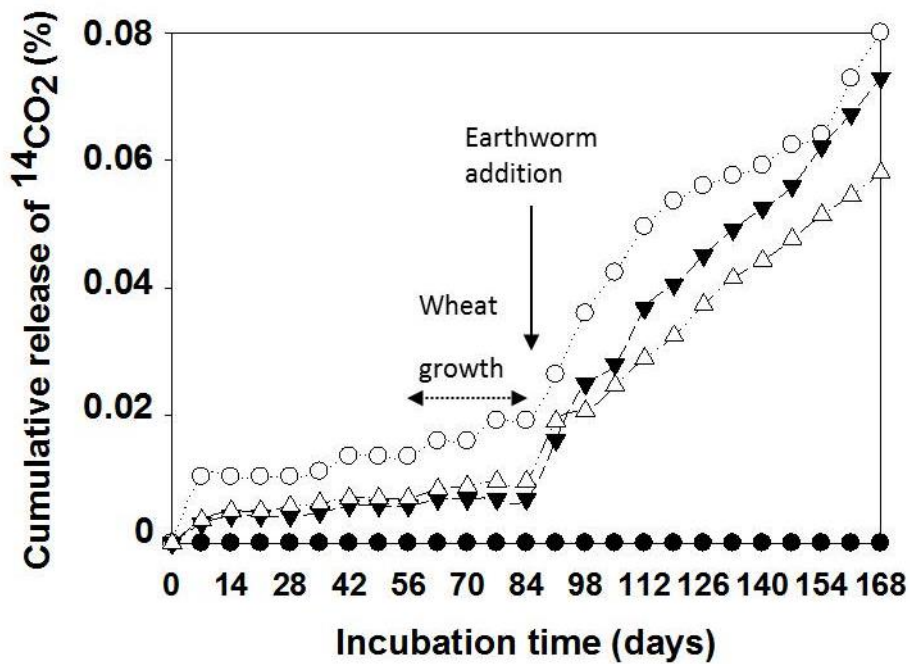


Fig. 2 Availability of [2-¹⁴C]-ciprofloxacin in the soil solution at the beginning of the experiment, and after 84 and 168 days of incubation. Statistical analysis using two-way ANOVAs showed significant crossed effects (P = 0.05) of the type of contamination [soil amended with slurry spiked with the high (filled circle) amount of antibiotic and soil spread with the high amount of antibiotic (open circle)] and the length of the incubation on ciprofloxacin bioavailability in the soil solution. The results are expressed as means of triplicate, with standard deviation lower than 10 % of the mean

