Journal of Hazardous Materials 198 (2011) 22–30

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities

Cristobal Girardi[a,*], Josephine Greve[b], Marc Lamshöft[c], Ingo Fetzer[d], Anja Miltner[a], Andreas Schäffer[e], Matthias Kästner[a]

[a] UFZ – Helmholtz Centre for Environmental Research, Department of Environmental Biotechnology, Permoserstraße 15, 04318 Leipzig, Germany
[b] Minnesota State University, Mankato, MN 56001 8400, USA
[c] Institute of Environmental Research (INFU), TU Dortmund University, Otto-Hahn-Str. 6, NRW 44221 Dortmund, Germany
[d] UFZ – Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Permoserstraße 15, 04318 Leipzig, Germany
[e] Department of Environmental Biology and Chemodynamics, Institute for Environmental Research (Biology V), RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

ARTICLE INFO

Article history:
Received 29 April 2011
Received in revised form
27 September 2011
Accepted 2 October 2011
Available online 10 October 2011

Keywords:
Ciprofloxacin
Radiotracer
Biodegradation
Bioavailability
Toxicity

ABSTRACT

While antibiotics are frequently found in the environment, their biodegradability and ecotoxicological effects are not well understood. Ciprofloxacin inhibits active and growing microorganisms and therefore can represent an important risk for the environment, especially for soil microbial ecology and microbial ecosystem services. We investigated the biodegradation of ^{14}C-ciprofloxacin in water and soil following OECD tests (301B, 307) to compare its fate in both systems. Ciprofloxacin is recalcitrant to biodegradation and transformation in the aqueous system. However, some mineralisation was observed in soil. The lower bioavailability of ciprofloxacin seems to reduce the compound's toxicity against microorganisms and allows its biodegradation. Moreover, ciprofloxacin strongly inhibits the microbial activities in both systems. Higher inhibition was observed in water than in soil and although its antimicrobial potency is reduced by sorption and aging in soil, ciprofloxacin remains biologically active over time. Therefore sorption does not completely eliminate the effects of this compound.

1. Introduction

Antibiotics are designed to be refractory to biodegradation and to act effectively even at low doses. Recently, the concern about potential ecological impacts of synthetic antibiotics increased because they may inhibit key environmental processes mediated by microorganisms like nutrient regeneration, carbon and nitrogen cycles and pollutant degradation [1].

The most widely prescribed fluoroquinolone antibiotic is ciprofloxacin (CIP) [2], which is active against a broad spectrum of Gram-negative and Gram-positive bacteria [3]. It is frequently detected in the environment and proven to be genotoxic [4]. Also, it is the main metabolite of enrofloxacin, a commonly used veterinary fluoroquinolone [2]. Of the administered dose of CIP in humans, 45–62% is excreted unmetabolised via urine and 15–25% via faeces [5]. Thus, CIP can reach the environment by sewage, discharges from sewage treatment plants, leaching from landfills, its release from pharmaceutical industries, livestock activities and application of sewage sludge, manure or treated waste water to agricultural land [6–9]. Therefore, antibiotics enter the soil, but their fate and effects on this ecosystem are unknown [2].

CIP concentrations in the environment range from ng L^{-1} to mg L^{-1}. Larsson et al. [10] reported concentrations up to 31 mg L^{-1} in the effluents of a wastewater treatment plant (WWTP) for pharmaceutical industries in India. During wastewater treatment, 80–90% of CIP is removed via sorption to sludge, which stabilizes the substance [2]. Therefore, digested sludge contains CIP (around 3 mg kg^{-1}; [5]). In soil, reported concentrations range from 0.37 mg kg^{-1} to 0.40 mg kg^{-1} [11,12], underlining the ecotoxicological relevance of CIP in soil. CIP is not readily biodegradable [4]. It also strongly sorbs to soil [2,13], mostly by cation exchange [14]. Therefore, the soil can act as a reservoir of this and other antibiotics [15].

The effects of CIP on microbial communities in wastewater, streamwater and marine and salt marsh sediment were studied thoroughly [4,16–18]. It reduced algal diversity at environmentally relevant concentrations [19]. However, nothing is known about its effects on soil microbial communities [2], and standardised studies on its degradation in soil, e.g. OECD 307 tests, are missing. This information is needed to estimate the fate of these compounds and to perform accurate risk assessments.

* Corresponding author. Tel.: +49 341 235 1753; fax: +49 341 235 1471.
E-mail address: cristobal.girardi-lavin@ufz.de (C. Girardi).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.10.004

Exposure of bacteria in the environment may contribute to spreading antibiotic resistance to pathogens [7]. Fluoroquinolone resistant *Campylobacter jejuni* was found in poultry husbandry [20]. Furthermore, antibiotics in sewage can inhibit the microbiota of WWTP [21,22] and thus reduce the waste water treatment efficiency. Composting is used to degrade organic contaminants such as pesticides, PAHs and PCBs in sewage sludge before its application to soils [23]. However, the effectiveness of this process and the fate of the antibiotics themselves remain unclear.

We hypothesise that CIP is not degraded in water or soil and that it can pose a risk for the environment. Therefore, the aims of this study were (1) to directly compare the biodegradation of radiolabeled CIP in water and in soil following the OECD tests 301B and 307 [24,25] in order to produce a database for extrapolating biodegradation data obtained in water-based tests to soil; (2) to obtain a mechanistic overview of CIP's biodegradation and (3) to elucidate the potential effects of this antibiotic on soil microbes and their activities.

2. Materials and methods

2.1. Chemicals and soil material

All chemicals were analytical or reagent grade obtained from VWR (Darmstadt, Germany) or Sigma–Aldrich (Munich, Germany) if not specified otherwise. Ciprofloxacin hydrochloride (99% purity) was purchased from Biotrend Chemicals (Zurich, Switzerland), [2-^{14}C] ciprofloxacin (radiochemical purity 99.4%; specific activity 20 mCi mmol^{-1}) from Hartmann Analytic GmbH (Braunschweig, Germany), sodium acetate-U-^{14}C ($\geq$98 atom% ^{14}C, 50 mCi mmol^{-1}) from Biotrend GmbH (Cologne, Germany).

The soil experiments were performed with soil samples (21% clay, 68% silt, 11% sand, TOC 2.1%, total N 0.17%, pH 6.6 and water holding capacity 37.5%) from the A horizon of a Haplic Chernozem from the agricultural long-term experiment "Statischer Düngungsversuch" (Bad Lauchstädt, Germany). The plot has been fertilised with farmyard manure (30 t/ha) every second year since 1902 [26].

2.2. Incubations in mineral medium

Four different incubations were performed to test the biodegradability in aqueous systems according to the OECD guideline 301B [24]: (1) standard mineral medium (MM) with [2-^{14}C] ciprofloxacin; (2) sterilised MM with [2-^{14}C] ciprofloxacin (sterile control to account for abiotic degradation processes); (3) MM with ^{14}C-acetate (positive control); and (4) MM with ^{14}C-acetate and unlabelled CIP (inhibition test). Each bottle was inoculated with diluted fresh activated sludge (10 mg L^{-1} of suspended solids [SS], sterilised by autoclaving for the sterile control) from a municipal wastewater treatment plant (Klärwerk Rosental, Leipzig, Germany). The final concentration of ciprofloxacin or acetate was 20 mg L^{-1}, the radioactivity added was 10 kBq per system. 300 mL of the spiked MM were incubated in 500 mL Schott bottles in the dark at 20 °C for 29 days. Samples were periodically flushed with air to provide O_2. The gas leaving the bottles was passed through 1 M NaOH (2 × 20 mL) to trap $^{14}CO_2$. The bottles were destructively sampled after 12 and 29 days. Samples were filtered through 0.22 μm cellulose filters to determine the radioactivity in the medium and SS.

2.3. Soil incubation experiments

Biodegradation experiments in soil were based on the OECD guideline 307 [25]. The soil was sieved to 2 mm and amended with stabilised sludge at 1.8 g kg^{-1} soil (dry weight) from a local wastewater treatment plant (Klärwerk Rosental, Leipzig, Germany). Two different incubations were performed: (1) soil with [2-^{14}C] ciprofloxacin, (2) sterilised soil (autoclaved 3 times on 3 consecutive days) with [2-^{14}C] ciprofloxacin (sterile control). The radiolabeled mixture was initially added to 10% of the soil. Then the spiked soil was thoroughly mixed with the remaining 90% with a pastry blending machine. The final concentration of ciprofloxacin was 20 mg kg^{-1} of soil (60% of WHC); the radioactivity added was 10 kBq per system. Twenty grams of soil were incubated in 500 mL Schott bottles in the dark, at 60% WHC and at 20 °C for 90 days. Samples were flushed with air every 3 days and the CO_2 produced was trapped in two NaOH traps as described above. The bottles were destructively sampled after 17, 32, 60 and 93 days.

2.4. Extractable and non-extractable residues in soil

2.4.1. Soil extractions by ASE

Five grams of soil were mixed in a 33 mL stainless steel extraction cell with Hydromatrix™ (Varian Inc., Santa Clara, USA) and extracted with a mixture of 63% ethyl acetate, 25% methanol and 3% ammonium hydroxide using an ASE 200 accelerated solvent extraction system (Dionex, Sunnyvale, USA) at the following operating conditions: extraction temperature, 100 °C; extraction pressure, 120 bar; preheating period, 5 min; static extraction period, 30 min; number of extraction cycles, 5; solvent flush, 50% of the cell volume; and nitrogen purge, 120 s. A subsample of the extract was removed for ^{14}C analysis and the remaining sample was diluted with MilliQ water until <5% solvent content for purification and chemical analysis.

2.4.2. Non-extractable residues

In order to determine the initial total radioactivity in soil and the ^{14}C in non-extractable residues after extraction by ASE, soil samples were combusted in a biooxidizer (Biological oxidizer OX 500, Zinsser Analytic, Frankfurt, Germany; [27]). The CO_2 produced during combustion was trapped in Oxysolve 400 (Zinsser Analytic) and analysed by liquid scintillation counting (LSC) with a Wallac 1414 scintillation counter (Perkin Elmer Wallac GmbH, Freiburg, Germany).

2.5. Radioactivity measurements

The radioactivity in liquid samples (NaOH traps, MM, SS, and soil extracts) was determined by LSC after addition of Ultima Gold™ scintillation cocktail (Perkin Elmer).

2.6. Chemical analyses

Filtered MM samples were analysed by thin layer chromatography (TLC) on silica plates developed with a dichloromethane–methanol–2-propanol–25% NH_3 (3:3:5:2) mixture [28]. The radioactivity on the TLC plates was determined with a Linear TLC Analyser LB284 (Berthold GmbH & Co KG, Bad Wildbad, Germany; [27]).

The diluted soil extracts were acidified to pH 3 and purified by solid phase extraction (SPE; [13]). Samples were passed through a 500 mg anion-exchange cartridge (Waters, Taunton, USA), stacked on top of a 500 mg hydrophilic–lipophilic balance cartridge (Waters) and eluted with methanol/NH_3 6%. The samples were concentrated under nitrogen and resuspended for analysis.

The extracts were analysed by reversed phase liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a Thermo Fisher Surveyor HPLC-system (Thermo Fisher Scientific, Waltham, USA) equipped with a Luna PFP(2) column (150 × 2 mm, 3 μm particle size; Phenomenex, Aschaffenburg, Germany). Ten microliters samples were separated with the following gradient

Table 1
SRM data, retention time, LOD and LOQ of CIP.

Compound	Retention time (min)	Precursor ion (m/z)	Product ions (m/z)	Collision energy (eV)	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)
CIP	19.07	332.1 332.1	314.1 288.0	−19 −17	5	15

program: 90% A (solvent A: 1 mM ammonium acetate and 0.1% HCOOH in water) for 2 min, linear gradient to 50% A over 23 min, and to 100% within the next 1 min. Subsequently, the column was rinsed with 100% B (solvent B: 0.1% HCOOH in methanol) for 5 min, and then the system was returned to 90% A within 1 min where it was held for 5 min before the next run was started. The mobile phase flow rate was 0.3 mL min^{-1}; the column temperature was 26 °C. The mass spectra were acquired using a TSQ Quantum Ultra AM mass spectrometer (Thermo Fisher) with a HESI-II ion source operating in positive mode. Nitrogen was both the drying and the nebulizer gas, and argon (1.5 bar) was the collision gas. The capillary temperature for the TSQ Quantum was 250 °C, and the vaporizer temperature 350 °C. The MS/MS parameters (tube lens, collision energy) were optimized in continuous flow mode for maximum sensitivity for product ions, and the two most sensitive SRM (selected reaction monitoring) transitions were determined for each molecule (for instrument parameters and SRM data for CIP, see Table 1).

The main metabolites were identified by ESI-HR-MS with an LTQ-Orbitrap Spectrometer (Thermo Fisher) according to [28].

2.7. Toxicity study in pure culture

The EC_{50} for *Pseudomonas putida* mt-2 (a soil isolate) in pure culture was determined as described by [29].

2.8. Inhibition studies in soil

Soil incubations with ciprofloxacin hydrochloride at different concentrations (0 mg L^{-1}, 0.2 mg L^{-1}, 2 mg L^{-1} and 20 mg L^{-1}) were performed in a Sapromat® E BOD Measuring Unit (H + P Labortechnik, Oberschleissheim, Germany) to study the effects of CIP on soil respiration and soil microbial community structure (by terminal restriction fragment length polymorphism [T-RFLP] analysis using universal primers for the 16S rRNA). The samples were incubated for 113 days, but soil respiration was only recorded through day 77.

2.9. DNA extraction, T-RFLP analyses and detection of resistance genes

Total DNA was extracted from 0.5 g of soil using the UltraClean® Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA). 16S rDNA was amplified by PCR with the primers 27f [30] and 1378R [31]. The reaction conditions for the 25 μL reaction (Hot Start Taq PCR master mix, Qiagen) were: 15 min at 95 °C, 32 cycles of 30 s at 94 °C, 30 s at 52 °C, 1.2 min at 72 °C, and a final extension for 10 min at 72 °C. PCR products were purified using the QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany), and quantified by a NanoDrop ND-100 device (NanoDrop Technologies. USA). The PCR products were digested with MspI (Fermentas, St. Leon-Rot, Germany) and the resulting T-RFs were analysed [32].

Resistance genes *qnr*A, *qnr*B and *qnr*S were amplified with the same PCR reagents and appropriate primers [33]. The reaction conditions for the 25 μL reaction were: 10 min at 95 °C, 35 cycles of 1 min s at 94 °C, 1 min s at 52 °C, 45 s at 72 °C, and a final extension at 72 °C for 10 min. PCR products were purified as described above.

2.10. Data analysis, mass balance and statistical analysis

All results are presented as means of triplicate experiments with standard deviation. Mineralisation and radioactivity in medium or soil, SS and extractable and bound residues were quantified on each sampling date. The recovery was calculated to set up a complete mass balance.

To visualise the changes caused by CIP on the soil microbial communities, non-metric multidimensional scaling analysis (MDS) were performed using the Bray–Curtis distance and Jaccard index measure on the T-RFLP data [34]. A two-way PERMANOVA was used to test between groups and ANOSIM within treatment differences of the T-RFLP data results.

Due to the unequal number of replicates for biotic and abiotic incubations, the Kruskal–Wallis test was used to identify differences in mineralisation, non-extractable residues (NER), ER, soil respiration and metabolite formation. For soil respiration data, 95% confidence intervals were calculated based on results from triplicates, assuming a balanced normal distribution. Differences were regarded statistically significant for all tests if $p < 0.05$. Statistical tests were conducted with the software packages PAST [35] and R [36].

3. Results

3.1. Biodegradation study in aqueous and soil systems

3.1.1. Mass balance

No mineralisation was observed in the aqueous system over 29 days of incubation (Fig. 1) and only the parent compound was detected by TLC analysis at that time (Fig. S1). Consistently, the radioactivity in the MM remained high. Radioactivity in SS was similar for biotic and abiotic incubations (Table S1). CIP is therefore recalcitrant to degradation under the OECD 301 test conditions.

In soil, however, some mineralisation (0.9% of the added CIP after 93 days; $p < 0.05$) was observed (Fig. 1). The contribution of biotic and abiotic processes was approximately equal. Until day 6 the degradation rate was relatively high (around 0.03% day^{-1}), thereafter, it was low but constant (0.008% day^{-1}).

The extractability by ASE decreased over time. On day 0, 39% and 46% of the initial ^{14}C amount was extracted in the biotic and abiotic incubations, respectively (Fig. 2). The extractability decreased to 12% and 15% after 93 days.

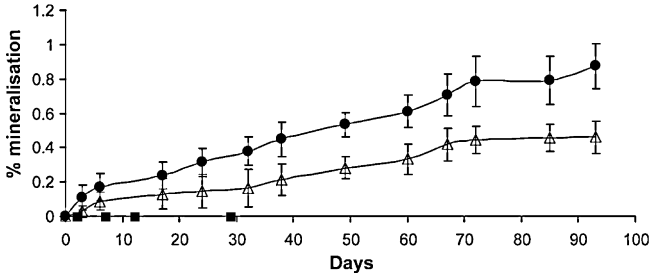


Fig. 1. Mineralisation of [2-^{14}C]-ciprofloxacin in mineral medium and soil. (●) Soil, (△) sterile soil and (■) mineral medium. Percentages refer to the total radioactivity applied. Standard deviations are not visible if smaller than the symbols.

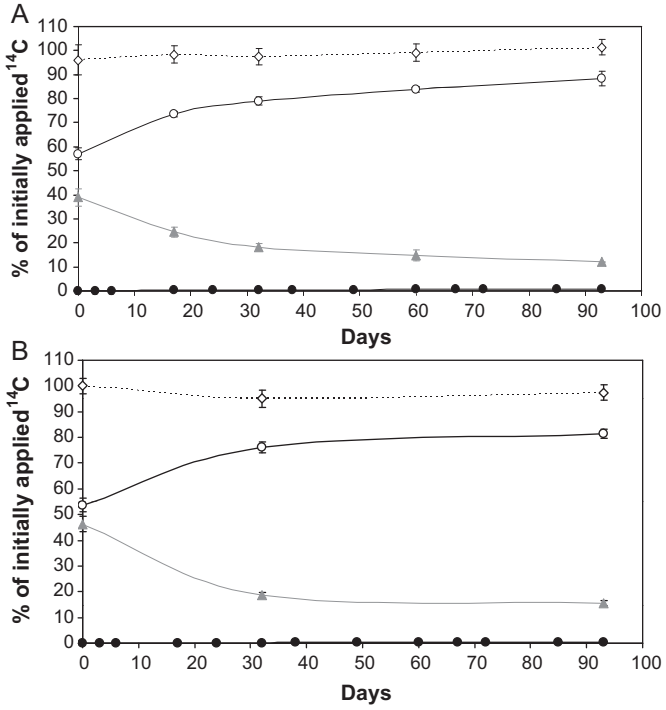


Fig. 2. Degradation of [2-^{14}C]-ciprofloxacin in soil under biotic (A) and abiotic conditions (B). (●) Mineralisation, (▲) extractable amount, (○) non-extractable residues and (◊) recovery. Standard deviations are not visible if smaller than the symbols.

Non-extractable residues on day 0 accounted for 57% and 54% of the applied radioactivity for biotic and abiotic systems, respectively, and over time increased to 88% (biotic) and 81% (abiotic) of the initial ^{14}C amount on day 93. NER formation slowed down after 30 days, but increased steadily until day 93 (Fig. 2). CIP thus strongly sorbs to soil and aging increases NER formation. Overall, the extractability of ciprofloxacin-derived radioactivity and NER were not statistically different between the biotic and abiotic systems ($p > 0.05$). Total recoveries ranged from 93 to 101% (Table S2).

3.1.2. Ciprofloxacin and its metabolites

Extractable CIP in the soil declined over time in both biotic and abiotic incubations (Table 2), consistent with ^{14}C extractability. This decline was more pronounced in biotic incubations (10.5% of initially extracted CIP on day 93) than in abiotic incubations (25.2%; $p < 0.05$). Two known metabolites of ciprofloxacin [37] (F9, F6; Tables 2 and 3) and one unknown, M311, of m/z 311, were found in low amounts at all the sampling times (including time 0). Unfortunately, the information obtained from the analysis was not sufficient to propose a chemical structure for M311. The amounts of F6 were similar in both experiments ($p > 0.05$) and the amount of F9 slightly more abundant under biotic conditions (Table 2; $p < 0.05$).

3.2. Induced effects on the sludge and soil microbial community

3.2.1. Inhibition tests in MM and soil

To test the effect of CIP on the general microbial activity in aqueous systems, the inhibition of acetate mineralisation by CIP was analysed. Without CIP, acetate mineralisation started immediately, and, after 29 days, 70% of the acetate was mineralised. In the presence of CIP, acetate was slowly degraded after a 5-day lag phase (Fig. S2). At the end of the experiment, mineralisation was inhibited by 75% compared to the control without CIP (Table 4).

Soil respiration was used as an indicator of microbial activity. The inhibition of soil respiration by CIP was lower than in the aqueous system, but also decreased with time. After 2 days, cumulative soil respiration was inhibited by approximately 70% at all three CIP concentrations, as opposed to only roughly 35% at the end of the experiment (Table 4).

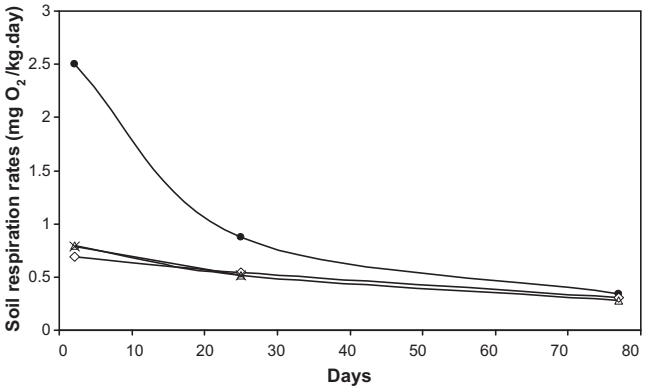


Fig. 3. Soil respiration rates in the inhibition test. (●) 0 mg kg^{-1} CIP, (◊) 0.2 mg kg^{-1} CIP, (Δ) 2 mg kg^{-1} CIP, (x) 20 mg kg^{-1} CIP.

Microbial activity was thus strongly inhibited both in aqueous systems and soil. Even though concentrations in the soil solution were much higher than in MM (Table 4), it seems that CIP was more toxic in aqueous media than in soil. One explanation might be the higher diversity of microorganisms in soil. More important, however, seems to be the reduced bioavailability of CIP in soil, which potentially reduces the toxicity of this compound. Moreover, in the concentration range studied, CIP toxicity did not depend on its concentration (Fig. S3); this suggests that the maximum effect was already obtained with the lowest concentration studied. The decrease of inhibition in soil over time can be explained by the aging of the compound and by the adaptation of microorganisms.

The reduction of soil respiration rates by CIP was most evident during the first month, whereas later respiration rates of CIP treatments were similar to the controls (Fig. 3). Ciprofloxacin mainly reduced the microbial activity at the beginning of the experiment, because it is a biostatic compound that targets growing microorganisms.

T-RFLP analyses were applied to study the effects of CIP on the soil bacterial community. MDS analyses of soil bacterial communities (Fig. 4) revealed a shift in both microbial abundance and microbial diversity after the application of CIP. The relevant factors driving this change were CIP and time. Statistical analysis revealed that these two factors were uncorrelated ($p = 0.431$), demonstrating that both factors were acting independently. The samples clustered into four groups. Two of them comprised the controls and the other two the CIP treatments at the early (days 3, 14 and 29) and late (days 65 and 113) stages of incubation. The microbial community stabilised after 65 days of incubation. PERMANOVA analysis confirmed a significant difference ($p < 0.001$) between control and CIP treatments. However, the difference between the three CIP treatments was not significant ($p = 0.67$), as confirmed by ANOSIM analysis. Although the effect of CIP on microbial communities was evident, no clear concentration effect was detectable, which is consistent with the effect on soil respiration.

Moreover, when Jaccard index was used instead of Bray–Curtis for the analysis of the changes in the community, significant differences were also found between the control and the treatments (data not shown), which indicates a shift in species composition.

3.2.2. EC_{50} for bacteria (Pseudomonas putida)

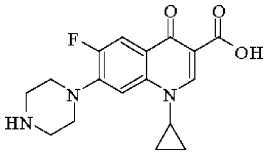
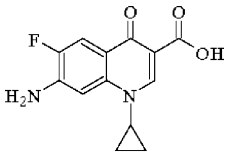
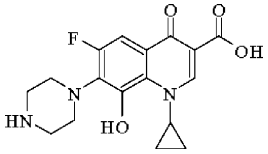
The inhibition study of CIP in pure culture of *P. putida* mt-2 revealed an EC_{50} of 0.25 mg L^{-1} (Fig. 5). At 1 mg L^{-1}, growth was

Table 2
Ciprofloxacin and metabolites relative abundance (F6 and F9) in purified soil extracts.

	Time (days)	% of initial $^{14}C_2$-ciprofloxacin[a]		
		CIP	F6	F9
Biotic	0	100 (±13.1)	4.70 (±0.918)	3.28 (±0.501).
	17	38.66 (±0.982)	6.49 (±1.26)	13.84 (±6.10)
	32	46.5 (±13.1)	1.85 (±0.144)	3.01 (±0.260)
	60	20.76 (±1.30)	1.48 (±0.245)	3.23 (±0.867)
	93	10.46 (±2.87)	0.674 (±0.177)	4.43 (±2.73)
Abiotic	0	100 (±5.82)	8.12 (±2.63)	4.14 (±0.488)
	32	44.3 (±3.38)	1.51 (±0.464)	0.916 (±0.087)
	93	25.17 (±7.65)	0.854 (±0.043)	0.793 (±0.043)

Values in brackets (±) represent the standard deviation of the average of triplicates.
[a] 100% corresponds to initially measured amount of ciprofloxacin in the time 0 extract.

Table 3
Accurate masses of $[M+H]^+$ and chemical structures of ciprofloxacin (332 m/z) and metabolites F9, 7-Amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (263 *m/z*); F6, 1-cyclopropyl-7-(1-piperazinyl)-6-fluoro-1,4-dihydro-8-hydroxy-4-oxo-3-quinolinecarboxylic acid (348 *m/z*) and M311 (311 *m/z*) in soil (structures from Wetzstein et al. [37]).

Compound	$[M+H]^+$ [*m/z*] (experimental)	$[M+H]^+$ [*m/z*] (theoretical)	Calculated formula	Chemical structure
CIP	332.14042	332.14050	$C_{17}H_{19}N_3O_3F$	
F9	263.08260	263.08265	$C_{13}H_{12}N_2O_3F$	
F6	348.13540	348.13541	$C_{17}H_{19}N_3O_4F$	
M311	311.12787	Not available	$C_{14}H_{13}O_4N_3$	Not determined

completely inhibited. In contrast to these results, soil microorganisms were still active at this concentration and acetate was mineralised at a much higher concentration in the OECD inhibition test (Fig. S2 and Table 4). One explanation for this difference is the higher microbial diversity in activated sludge and soil compared to the pure culture. Additionally, the fraction of CIP adsorbed to sludge (10% of initial ^{14}C; Table S1) and soil (up to 88%, Table S2) could also contribute to the reduced toxicity against microorganisms.

3.2.3. *Ciprofloxacin resistance genes in soil*

To assess the adaptation of soil microbiota to CIP, samples from different incubation times were tested for CIP resistance genes (*qnr*A, *qnr*B, *qnr*S). The genes, *qnr*A and *qnr*B, were not detected in any of the samples (Fig. 6). Gene *qnr*S was not detected in either the control (non-amended) or in incubations with CIP after 3 days. In contrast, it was detected in samples with 20 mg kg^{-1} CIP from days 14, 29 and 65; in samples with 0.2 mg kg^{-1} CIP from day 29;

Table 4
Microbial activity inhibition in soil and water at different concentrations and times.

Time (days)	% of inhibition (compared to control)			
	Soil			MM
	0.2 mg kg^{-1} CIP	2 mg kg^{-1} CIP	20 mg kg^{-1} CIP[a]	20 mg L^{-1} CIP
2	70.9 (±12.0)	69.1 (±3.78)	71.5 (±15.6)	99.1 (±4.03)
7	56.4 (±3.02)	56.7 (±3.77)	56.8 (±3.23)	87.8 (±0.81)
12	45.9 (±1.19)	47.0 (±2.14)	48.3 (±1.33)	86.8 (±1.7)
20	46.9 (±4.14)	49.0 (±4.78)	50.4 (±7.99)	79.4[c]
End[b]	33.1 (±1.49)	36.8 (±4.06)	n.a.	74.9 (±11.2)

n.a.: not assessed.
Values in brackets (±) represent the standard deviation of the average of triplicates.
[a] The concentration in the soil solution corresponded to 45.8 mg L^{-1} and 12.5 mg L^{-1} on days 0 and 77, respectively.
[b] End of the experiment was on day 29 for water and on day 77 for soil.
[c] Estimated by linear interpolation.

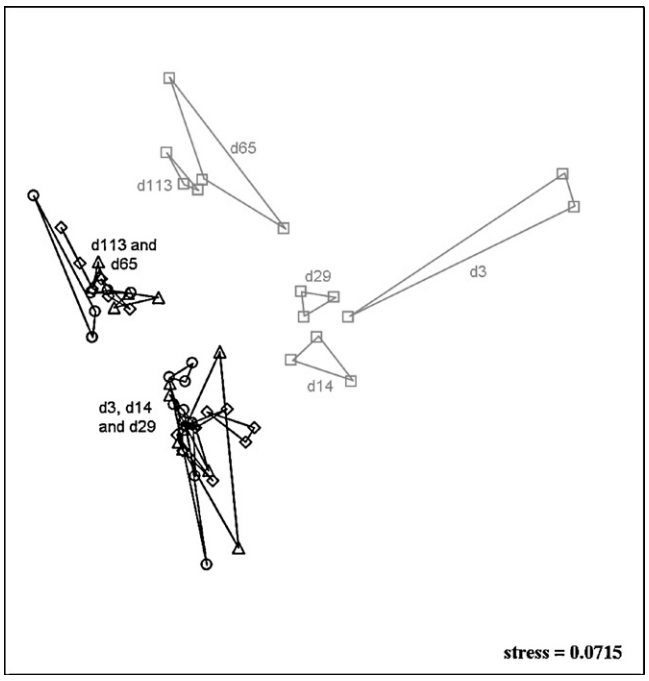


Fig. 4. T-RFLP analysis of bacterial 16S rRNA from bacteria in soil. Non-Metric Multidimensional Scaling plot using Bray-Curtis similarity measure of the bacterial communities after 3, 14, 29, 65 and 113 days of incubation with different concentrations of ciprofloxacin. (□) 0 mg kg^{-1} CIP, (◊) 0.2 mg kg^{-1} CIP, (Δ) 2 mg kg^{-1} CIP, (○) 20 mg kg^{-1} CIP. The closer two communities are in the plot, the more similar they are. Groups of triplicates are connected by polygons.

and in samples with 2 mg kg^{-1} after 65 and 113 days of incubation. The low intensity of the *qnr*S bands indicates a low copy number of this gene in soil.

4. Discussion

Our data provides reliable information from OECD degradation tests to estimate the fate of CIP in aqueous media [24] and in a typical agricultural soil [25]. Based on a combined approach of CIP degradation data and its induced effects on microbial communities, we evaluated the risk of this antibiotic for the environment. We obtained a general overview on the degradation process, quantified mineralisation and carbon distribution during degradation, and analysed metabolites and the effects of CIP on microbial communities.

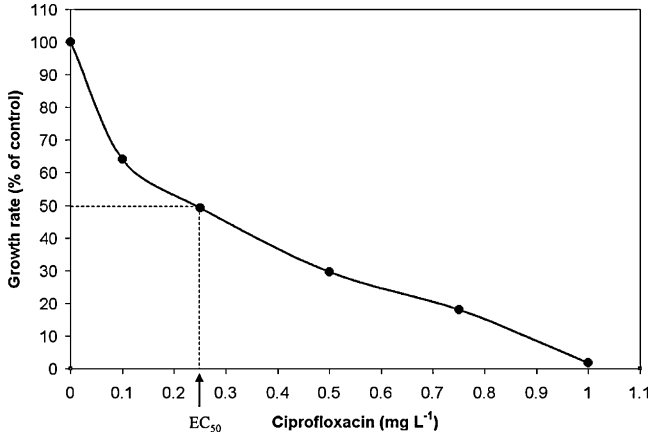


Fig. 5. Effect of CIP on the growth of *Pseudomonas putida* mt-2 in pure culture.

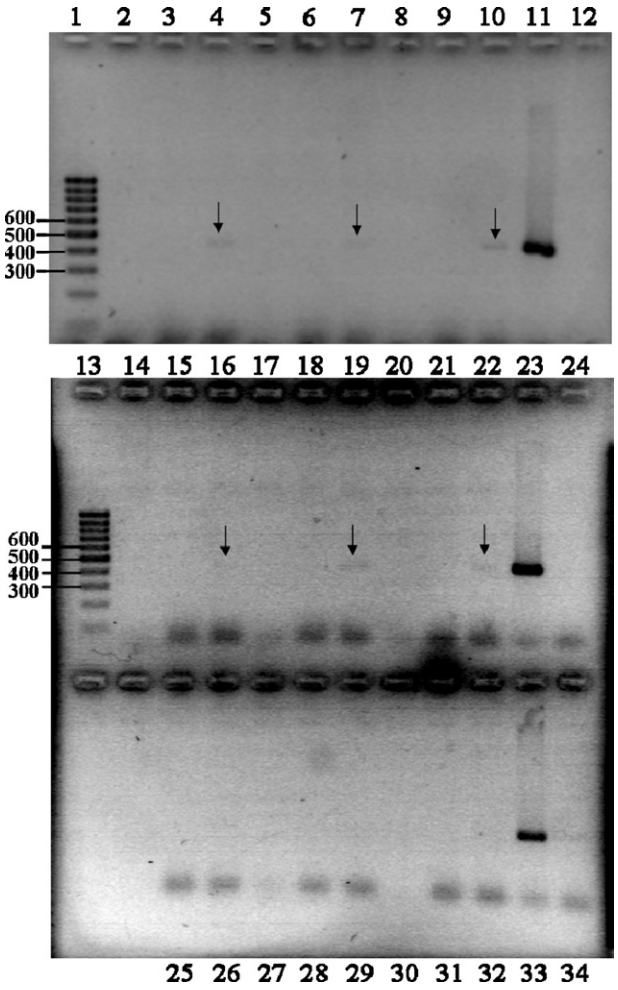


Fig. 6. PCR of CIP resistance genes *qnr*A (580 bp), *qnr*B (264 bp) and *qnr*S (428 bp) from soil incubations with ciprofloxacin. Agarose gel electrophoresis (2%). Lanes: 1 and 13, Molecular size marker; 2, 3 and 4, *qnr*B, *qnr*A, *qnr*S respectively from CIP 20 mg kg^{-1} day 14; 5, 6 and 7, *qnr*B, *qnr*A, *qnr*S respectively from CIP 0.2 mg kg^{-1} day 29; 8, 9, 10; *qnr*B, *qnr*A, *qnr*S respectively from CIP 20 mg kg^{-1} day 29; 14, 15 and 16, *qnr*B, *qnr*A, *qnr*S respectively CIP 2 mg kg^{-1} day 65; 17, 18 and 19, *qnr*B, *qnr*A, *qnr*S respectively from CIP 20 mg kg^{-1} day 65; 20, 21, 22, *qnr*B, *qnr*A, *qnr*S respectively from CIP 2 mg kg^{-1} day 113; 25, 26, 27 *qnr*B, *qnr*A, *qnr*S respectively from CIP 20 mg kg^{-1} day 3; 28, 29 *qnr*A, *qnr*S from non spiked soil day 3; 30, 31, 32 *qnr*B, *qnr*A, *qnr*S from non spiked soil day 113; 11, 23 and 33 *qnr*S in resistant strain (positive control); 12, 24 and 34 no DNA (negative control).

4.1. Ready biodegradability and fate in soil

No degradation was observed in water because CIP is highly toxic and inhibits the microbial activity. It is resistant to abiotic degradation reactions such as hydrolysis [8]. Therefore, CIP is recalcitrant to degradation under ready biodegradability test conditions.

In the biotic soil systems, however, 0.9% of the added CIP was mineralised after 93 days. Thus, contrary to the well-accepted rule that degradation of antibiotics is hampered by fixation to the soil matrix [8], more ciprofloxacin was degraded in soil than in water under the used conditions. Sorption to soil particles may have reduced the bioavailability and thus the effective toxicity of CIP [38]. An association of the bioactive functionalities to soil exchange sites is particularly efficient in this respect [39]. The biotic mineralisation may be performed by fungi [37], archaea, yeasts [40] or CIP-resistant bacteria.

Similar low mineralisation levels (0.49–0.58%) were reported for 4 mg kg^{-1} sarafloxacin (another fluorquinolone) after 80 days of incubation in various soils [41]. The authors attributed the low

mineralisation to the strong sorption and the resulting low bioavailability, but did not test inhibition of microbial activity. Our results indicate that mineralisation was low mainly because the compound is toxic.

Although CIP can be degraded, its strong sorption to soil, particularly to humic acids (see Supplementary Material S4), makes it highly persistent, while abiotic and microbial degradation are less important. In our experiment, NER formation was similar in biotic and abiotic incubations, suggesting that it occurred mainly abiotically. This is consistent with the low mineralisation under biotic conditions. The decline of extractable CIP is thus mainly governed by sorption and formation of NER. The metabolites we found at day 0 have to be formed by fast abiotic reactions with soil components, which is consistent with the relatively high initial abiotic mineralisation rate (Fig. 1). The unknown metabolite M311 was also detected during the biodegradation of norfloxacin by white-rot fungi (Prieto et al., personal communication), and thus can be regarded as a degradation product of CIP. The detected metabolites F6 and F9 were reported to be produced by the brown rot fungus *Gloeophyllum striatum* [37]. In our incubations, degradation pathways mediated by hydroxyl radical attack [37] were most common. Metabolite F9 can either be formed by the loss of the piperazine moiety or be formed by photodegradation [42], which can proceed rapidly in surface water [43]. Additionally, sarafloxacin was degraded abiotically immediately after contact to soil by surface-catalyzed hydrolysis or by oxidation resulting in a polar transformation product [41].

CIP proved to be extensively degraded by soil fungi [37,44], but these results were obtained under special artificial conditions (i.e. one fungal species degrading CIP in pure culture with optimal growth conditions) and may not be relevant for the real environment. Golet et al. [5] proposed an initial phase of biodegradation followed by long term persistence in soil. They explained this by the aging of CIP residues in soil or by reaching the biodegradation concentration threshold, but they did not consider the toxicity of CIP and how it inhibits soil microbial activity.

4.2. Toxicity and bioavailability

In general, toxicity was higher in water than in soil. Sorption of toxicants is one of the main mechanisms controlling toxicity via reduction of bioavailability [38]. Toxicity in soil declines with time because of aging and transformation to less toxic molecules. Sorption and desorption of compounds in soil systems play key roles for their environmental fate, even though sorption of CIP to soil does not completely inactivate this compound.

Ciprofloxacin persists even after stabilisation of activated sludge under methanogenic conditions [5]. However, it may lose its antibiotic potential under such conditions [16]. We detected some transformation products in soil. According to Wetzstein et al. [37,45], CIP loses its antibacterial activity by defluorination, decarboxylation, hydroxylation or oxidation of the amine moiety. These processes, however, are unlikely to occur in our soil. In general, the decline in the antimicrobial potential was slow and incomplete as previously reported for sludge [46]. Possible reasons are incomplete transformation of the molecule and the stability of the fluorine substituent at the aromatic C-6 position [47], which is crucial for the antibiotic potency [2].

Enrofloxacin labelled in the piperazine moiety or the carboxyl group, which are suggested as good indicators for the antibiotic activity and degradability of the compound, was extensively degraded [37,45]. Our conservative approach, using the label in one of the most stable carbon positions of the molecule, contradicted this extensive degradation and provided consistent results of both low degradation and inactivation of the antibiotic.

Bioavailability influences the effects of antibiotics on the soil microbial community. The presence of multivalent cations was reported to inhibit the antimicrobial potential of fluoroquinolones [48]. This may partly explain the lower toxicity in soil, even though the concentration of CIP in the soil solution was higher than in the mineral medium. Furthermore, some soil microorganisms are naturally tolerant towards antibiotics [49], such as some pseudomonades [50], while others can acquire resistance. In addition, the large microbial diversity in soil may be responsible for the weaker effects of antibiotics in soil than in water [51]. The antibiotic does not target Archaea and fungi and many soil bacteria are dormant and thus not sensitive to bacteriostatic antibiotics [52]. All these reasons may account for the difference in toxicity towards "active" activated sludge bacteria and soil bacteria.

Ciprofloxacin inhibited the indigenous microbial activity in soil (Fig. 3), in particular the growing or active bacteria. This is consistent with its bacteriostatic mechanism of action, which inhibits the DNA gyrases involved in DNA replication, recombination and transcription [53]. The results highlight the important potential impacts of CIP on microbial ecosystem services, such as nutrient recycling. Although readily sorbed to the soil matrix, CIP was already fully effective at the lowest concentration employed, which is similar to what was described for sulfadiazine [54]. Since microorganisms are living attached to soil particles, the CIP sorbed on soil surfaces may be still bioavailable and thus toxic for microorganisms. Another explanation can be the fact that the maximum effect on target bacteria was already obtained with the lowest concentration studied. Presumably, the microorganisms inhabiting our soil have a lower EC_{50} than *P. putida* mt-2. Therefore, it would be interesting to know more about the effect of lower concentrations, even those below the detection limit of chemical analysis (5 $\mu g\,kg^{-1}$, Table 1).

4.3. Implications for environmental risk assessment of ciprofloxacin

Predicted environmental concentration for CIP in soil indicated the need for assessing its environmental fate and effects [5]. The present study was conducted using environmentally relevant CIP concentrations [10–12]. It provided strong evidence for a high persistence of CIP in both our aqueous system and in soil, and demonstrated the negative effects of fluoroquinolones on soil and water ecosystems. These results contradict with the previous assessments of low persistence and low ecological risk of CIP [37,45].

The EC_{50} of CIP varies over a wide range. EC_{50} of 0.006 $mg\,L^{-1}$ and 0.61 $mg\,L^{-1}$ were reported for sewage sludge bacteria [46,55]. The EC_{50} for *Microcystis aeruginosa* (cyanobacteria) was 0.005 $mg\,L^{-1}$ but 2.97 $mg\,L^{-1}$ for *Selenastrum capricornutum* (algae; [55]). Moreover, at relevant environmental concentrations [10,11] CIP has phytotoxic effects on the aquatic plant *Lemna gibba* EC_{25} 271 $\mu g\,L^{-1}$; [56]. These results are in the same range as the EC_{50} 0.25 $mg\,L^{-1}$. We determined for *P. putida* mt-2 and reported CIP concentrations in soil [12]. Therefore, according to the European legislation [57] CIP can be classified as "very toxic to aquatic organisms" (EC_{50} below 1 $mg\,L^{-1}$) and "toxic to soil organisms". These results and the strong inhibition of the soil microbial activity, as well as the induced shift in the microbial community abundance and composition reported here, underline the strong antibacterial power of CIP and its hazardous consequences on the environment. Moreover, CIP derived NER apparently are still toxic to soil bacteria. Our results agree with a life cycle assessment of pollutants in waste water, which showed that CIP contributes significantly to ecotoxicity in terrestrial and freshwater systems [58].

Moreover, the *qnr*S CIP resistance gene appeared after 14 days of exposure, independent of the CIP concentrations studied. Resistance development is promoted by continuous exposure of bacteria

to concentrations below therapeutic levels [2]. This is exactly what occurs in soil, where due to the reduced bioavailability, bacteria are exposed to lower effective concentrations of CIP. Therefore soils can be an important source of resistant bacteria that can transfer the corresponding genes to other bacteria living in ground or drinking water. Under appropriate conditions, these genes can eventually be transferred to pathogenic microorganisms [59].

Consequently, our knowledge on the fate and effects of pharmaceuticals in the environment and their degradation products must be improved for proper risk assessment, e.g. for the normally occurring mixtures of pharmaceuticals which have stronger effects than single compounds [60,61]. Improved strategies to remediate sludge and manure contaminated with antibiotics or to restrict their application to agricultural fields are needed to avoid the input of these compounds into the soil ecosystem.

5. Conclusion

This work contributes to the proper environmental risk assessment of fluoroquinolone antibiotics, for which insufficient and contradictory data on their environmental fate and effects are available. We clearly demonstrated that CIP is persistent and affects the microbial communities and activities in soil. Therefore, much more attention has to be given to antibiotic contamination in soil, which often has been neglected. Furthermore, we conclude that for toxic compounds in soil, reduced bioavailability results in reduced effective toxicity and higher biodegradation compared to aqueous systems. However, even if soil has a buffering capacity against toxic compounds, it does not inhibit their antimicrobial activity completely. Compound dissipation with low or without mineralisation indicates abiotic NER formation, which may contain toxic parent compound and/or its primary metabolites.

Fluoroquinolones have significant effects on environmental processes and ecosystems services. The effects of these compounds on specific soil processes such as nutrient or carbon cycles, and the adaptation of the microbial communities to continuous application of these compounds with manure or sewage sludge still need to be studied.

Acknowledgements

The study was supported by the European Commission funding the RAISEBIO project (Contract: MEST-CT-2005-020984) under Human Resources and Mobility Activity (6th Framework programme) in particular the fellowship of C. Girardi. The stay of J. Greve at the UFZ was funded by the DAAD-RISE program. The authors thank Paula Martinez, Kerstin Ethner and Hermann Heipieper for their contribution in the experimental setup and analyses.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.10.004.

References

[1] J. Ollivier, K. Kleineidam, R. Reichel, S. Thiele-Bruhn, A. Kotzerke, R. Kindler, B.-M. Wilke, M. Schloter, Effect of sulfadiazine-contaminated pig manure on abundance of genes and transcripts involved in nitrogen transformation in the root-rhizosphere complexes of maize and clover, Appl. Environ. Microbiol. 76 (2010) 7903–7909.
[2] Y. Picó, V. Andreu, Fluoroquinolones in soil—risks and challenges, Anal. Bioanal. Chem. 387 (2007) 1287–1299.
[3] R. Davis, A. Markham, J.A. Balfour, Ciprofloxacin – an updated review of its pharmacology, therapeutic efficacy and tolerability, Drugs 51 (1996) 1019–1074.
[4] K. Kummerer, A. Al-Ahmad, V. Mersch-Sundermann, Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test, Chemosphere 40 (2000) 701–710.
[5] E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder, W. Giger, Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil, Environ. Sci. Technol. 37 (2003) 3243–3249.
[6] A.B.A. Boxall, D.W. Kolpin, B. Halling-Sørensen, J. Tolls, Peer reviewed: are veterinary medicines causing environmental risks? Environ. Sci. Technol 37 (2003) 286A–294A.
[7] C.G. Daughton, T.A. Ternes, Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107 (1999) 907–938.
[8] S. Thiele-Bruhn, Pharmaceutical antibiotic compounds in soils – a review, J. Plant Nutr. Soil Sci. 166 (2003) 145–167.
[9] E. Topp, S.C. Monteiro, A. Beck, B.B. Coelho, A.B.A. Boxall, P.W. Duenk, S. Kleywegt, D.R. Lapen, M. Payne, L. Sabourin, H. Li, C.D. Metcalfe, Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field, Sci. Total Environ. 396 (2008) 52–59.
[10] D.G. Larsson, C. de Pedro, N. Paxeus, Effluent from drug manufactures contains extremely high levels of pharmaceuticals, J. Hazard. Mater. 148 (2007) 751–755.
[11] E.M. Golet, A. Strehler, A.C. Alder, W. Giger, Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction, Anal. Chem. 74 (2002) 5455–5462.
[12] E. Martínez-Carballo, C. González-Barreiro, S. Scharf, O. Gans, Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria, Environ. Pollut. 148 (2007) 570–579.
[13] M. Uslu, A. Yediler, I. Balcıoğlu, S. Schulte-Hostede, Analysis and sorption behavior of fluoroquinolones in solid matrices, Water Air Soil Pollut. 190 (2008) 55–63.
[14] D. Vasudevan, G.L. Bruland, B.S. Torrance, V.G. Upchurch, A.A. MacKay, pH-dependent ciprofloxacin sorption to soils: interaction mechanisms and soil factors influencing sorption, Geoderma 151 (2009) 68.
[15] S.J. Rooklidge, Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways, Sci. Total Environ. 325 (2004) 1–13.
[16] A.L. Cordova-Kreylos, K.M. Scow, Effects of ciprofloxacin on salt marsh sediment microbial communities, ISME J. 1 (2007) 585–595.
[17] J.D. Maul, L.J. Schuler, J.B. Belden, M.R. Whiles, M.J. Lydy, Effects of the antibiotic ciprofloxacin on stream microbial communities and detritivorous macroinvertebrates, Environ. Toxicol. Chem. 25 (2006) 1598–1606.
[18] J. Näslund, J.E. Hedman, C. Agestrand, Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment, Aquat. Toxicol. 90 (2008) 223–227.
[19] B.A. Wilson, V.H. Smith, F. Denoyelles, C.K. Larive, Effects of three pharmaceutical and personal care products on natural freshwater algal assemblages, Environ. Sci. Technol. 37 (2003) 1713–1719.
[20] P.N. Gaunt, L.J.V. Piddock, Ciprofloxacin resistant Campylobacter spp. in humans: an epidemiological and laboratory study, J. Antimicrob. Chemother. 37 (1996) 747–757.
[21] A. Al-Ahmad, F.D. Daschner, K. Kümmerer, Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria, Arch. Environ. Contam. Toxicol. 37 (1999) 158–163.
[22] B. Halling-Sørensen, Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents, Arch. Environ. Contam. Toxicol. 40 (2001) 451–460.
[23] K. Xia, A. Bhandari, K. Das, G. Pillar, Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids, J. Environ. Qual. 34 (2005) 91–104.
[24] OECD, OECD Guidelines for Testing of Chemicals, Guideline 301B: Ready Biodegradability – CO_2 Evolution Test, OECD, Paris, 1992.
[25] OECD, OECD Guideline for Testing of Chemicals, OECD 307: Aerobic and Anaerobic Transformation in Soil, OECD, Paris, 2002.
[26] N. Blair, R.D. Faulkner, A.R. Till, M. Korschens, E. Schulz, Long-term management impacts on soil C, N and physical fertility: Part II: Bad Lauchstadt static and extreme FYM experiments, Soil Tillage Res. 91 (2006) 39–47.
[27] M. Weiß, R. Geyer, T. Günther, M. Kaestner, Fate and stability of 14C-labeled 2,4,6-trinitrotoluene in contaminated soil following microbial bioremediation processes, Environ. Toxicol. Chem. 23 (2004) 2049–2060.
[28] P. Sukul, M. Lamshoft, S. Kusari, S. Zuhlke, M. Spiteller, Metabolism and excretion kinetics of C-14-labeled and non-labeled difloxacin in pigs after oral administration, and antimicrobial activity of manure containing difloxacin and its metabolites, Environ. Res. 109 (2009) 225–231.
[29] H.J. Heipieper, B. Loffeld, H. Keweloh, J.A.M. de Bont, The cis/trans isomerisation of unsaturated fatty acids in Pseudomonas putida S12: an indicator for environmental stress due to organic compounds, Chemosphere 30 (1995) 1041–1051.
[30] D. Lane, 16s/23S rRNA sequencing, in: M.G.E. Stackebrandt (Ed.), Nucleic Acid Techniques in Bacterial Systematics, Wiley, Chichester, UK, 1991, pp. 11–175.
[31] H. Heuer, M. Krsek, P. Baker, K. Smalla, E. Wellington, Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients, Appl. Environ. Microbiol. 63 (1997) 3233–3241.
[32] P. Bombach, A. Chatzinotas, T.R. Neu, M. Kästner, T. Lueders, C. Vogt, Enrichment and characterization of a sulfate-reducing toluene-degrading microbial consortium by combining in situ microcosms and stable isotope probing techniques, FEMS Microbiol. Ecol. 71 (2010) 237–246.
[33] V. Cattoir, L. Poirel, V. Rotimi, C.-J. Soussy, P. Nordmann, Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates, J. Antimicrob. Chemother. 60 (2007) 394–397.

[34] G. Rees, D. Baldwin, G. Watson, S. Perryman, D. Nielsen, Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics, Antonie van Leeuwenhoek 86 (2004) 339–347.
[35] Ø. Hammer, D. Harper, P. Ryan, PAST: paleontological statistics software package for education and data analysis, Palaeontologia Electronica 4 (2001) art. 4.
[36] R Development Core Team, R A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2011.
[37] H.-G. Wetzstein, M. Stadler, H.-V. Tichy, A. Dalhoff, W. Karl, Degradation of ciprofloxacin by basidiomycetes and identification of metabolites generated by the brown rot fungus Gloeophyllum striatum, Appl. Environ. Microbiol. 65 (1999) 1556–1563.
[38] G. Welp, G.W. Brümmer, Effects of organic pollutants on soil microbial activity: the influence of sorption, solubility, and speciation, Ecotoxicol. Environ. Saf. 43 (1999) 83–90.
[39] S. Thiele, Adsorption of the antibiotic pharmaceutical compound sulfapyridine by a long-term differently fertilized loess Chernozem, J. Plant Nutr. Soil Sci. 163 (2000) 589–594.
[40] Y. Chen, J.P.N. Rosazza, C.P. Reese, H.Y. Chang, M.A. Nowakowski, J.P. Kiplinger, Microbial models of soil metabolism: biotransformations of danofloxacin, J. Ind. Microbiol. Biotechnol. 19 (1997) 378–384.
[41] J.R. Marengo, R.A. Kok, K. O'Brien, R.R. Velagaleti, J.M. Stamm, Aerobic biodegradation of (14C)-sarafloxacin hydrochloride in soil, Environ. Toxicol. Chem. 16 (1997) 462–471.
[42] P. Calza, C. Medana, F. Carbone, V. Giancotti, C. Baiocchi, Characterization of intermediate compounds formed upon photoinduced degradation of quinolones by high-performance liquid chromatography/high-resolution multiple-stage mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 1533–1552.
[43] M.W. Lam, K. Tantuco, S.A. Mabury, PhotoFate: a new approach in accounting for the contribution of indirect photolysis of pesticides and pharmaceuticals in surface waters, Environ. Sci. Technol. 37 (2003) 899–907.
[44] I.A. Parshikov, J.P. Freeman, J.O.J. Lay, R.D. Beger, A.J. Williams, J.B. Sutherland, Regioselective transformation of ciprofloxacin to N-acetylciprofloxacin by the fungus Mucor ramannianus, FEMS Microbiol. Lett. 177 (1999) 131–135.
[45] H.-G. Wetzstein, J. Schneider, W. Karl, Comparative biotransformation of fluoroquinolone antibiotics in matrices of agricultural relevance, in: Veterinary Pharmaceuticals in the Environment, American Chemical Society, Washington, DC, 2009, pp. 67–91.
[46] B. Halling-Sørensen, G. Sengeløv, F. Ingerslev, L.B. Jensen, Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazin, streptomycin, ciprofloxacin, and olaquindox due to environmental processes, Arch. Environ. Contam. Toxicol. 44 (2003) 0007–0016.
[47] C. Murphy, B. Clark, J. Amadio, Metabolism of fluoroorganic compounds in microorganisms: impacts for the environment and the production of fine chemicals, Appl. Microbiol. Biotechnol. 84 (2009) 617–629.
[48] A.J.H. Marshall, L.J.V. Piddock, Interaction of divalent cations, quinolones and bacteria, J. Antimicrob. Chemother. 34 (1994) 465–483.
[49] N. Esiobu, L. Armenta, J. Ike, Antibiotic resistance in soil and water environments, Int. J. Environ. Health Res. 12 (2002) 133–144.
[50] N.R. Krieg, J.G. Holt, Bergey's Manual of Systematic Bacteriology, 1st edn., Williams & Wilkins, Baltimore, 1984.
[51] K. Schauss, A. Focks, H. Heuer, A. Kotzerke, H. Schmitt, S. Thiele-Bruhn, K. Smalla, B.-M. Wilke, M. Matthies, W. Amelung, J. Klasmeier, M. Schloter, Analysis, fate and effects of the antibiotic sulfadiazine in soil ecosystems, Trends Anal Chem. 28 (2009) 612–618.
[52] S. Thiele-Bruhn, I.-C. Beck, Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass, Chemosphere 59 (2005) 457.
[53] R. Moore, B. Beckthold, S. Wong, A. Kureishi, L. Bryan, Nucleotide sequence of the gyrA gene and characterization of ciprofloxacin-resistant mutants of Helicobacter pylori, Antimicrob. Agents Chemother. 39 (1995) 107–111.
[54] A. Kotzerke, S. Sharma, K. Schauss, H. Heuer, S. Thiele-Bruhn, K. Smalla, B.-M. Wilke, M. Schloter, Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure, Environ. Pollut. 153 (2008) 315–322.
[55] B. Halling-Sørensen, H.-C.H. Lützhøft, H.R. Andersen, F. Ingerslev, Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin, J. Antimicrob. Chemother. 46 (2000) 53–58.
[56] R.A. Brain, D.J. Johnson, S.M. Richards, H. Sanderson, P.K. Sibley, K.R. Solomon, Effects of 25 pharmaceutical compounds to Lemna gibba using a seven-day static-renewal test, Environ. Toxicol. Chem. 23 (2004) 371–382.
[57] European Commission, Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, in Off. J. Eur. 1993.
[58] I. Muñoz, M. José Gómez, A. Molina-Díaz, M.A.J. Huijbregts, A.R. Fernández-Alba, E. García-Calvo, Ranking potential impacts of priority and emerging pollutants in urban wastewater through life cycle impact assessment, Chemosphere 74 (2008) 37–44.
[59] J.L. Martinez, Environmental pollution by antibiotics and by antibiotic resistance determinants, Environ. Pollut. 157 (2009) 2893–2902.
[60] T. Backhaus, M. Scholze, L.H. Grimme, The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri, Aquat. Toxicol. 49 (2000) 49–61.
[61] M. Cleuvers, Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects, Toxicol. Lett. 142 (2003) 185–194.