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Biological degradation and microbial function effect of norfloxacin in a soil under different conditions

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This paper investigated the degradation kinetics of norfloxacin in a soil, and its effects on soil respiration and nitrogen transformation under different conditions. Compared to the sterile control, the degradation rates of norfloxacin in the non-sterile soil were greatly enhanced, suggesting that microorganisms played a major role in the degradation. Accelerated degradation for norfloxacin in the soil was observed with decreasing concentrations (30 mg/kg to 5 mg/kg) with its half-life decreasing from 62 days to 31 days. Amending swine manure into the soil and increasing the soil moisture level enhanced the biological degradation of norfloxacin. No obvious inhibition of norfloxacin on soil respiration was observed in the soil, while only slight effect on nitrogen transformation was found. The results suggested that norfloxacin at the reported environmental concentrations (<100 mg/kg) would have little effect on microbial activity and functions in the soils.

Keywords: Norfloxacin, biodegradation, microbial function, soil.

Introduction

Antibiotics have been widely administered to prevent and treat diseases in humans and animals.[1] Owing to incomplete metabolism in human and animal, and incomplete removal in wastewater treatment plants, antibiotics could enter into the soil environment through application of animal manure and biosolids on land, which result in soil contamination of antibiotic residues.[2] These antibiotic residues may affect microbial function and activity in the soil environment.[3,4] Kotzerke et al.[4] reported that sulfadiazine could not only reduce microbial activity, but also affect the nitrogen turnover negatively. Therefore, it is imperative to investigate the fate of antibiotics in the soil environment.

To date, there have been some studies regarding biological degradation of antibiotics in soil and the influencing factors.[5–12] The influencing factors investigated so far include chemical concentration,[5,6,11,12] moisture and temperature,[5,6] manure amendment[5,6,10] and soil properties.[10–12] Accinelli et al.[10] studied the environmental fate of two sulfonamide antibiotics (sulfamethazine and sulfachloropyridine) in soils and found that the sulfonamides dissipated more rapidly in the silt loam soil than in the sandy soil. Similar results were also found for sulfadiazine and oxytetracycline.[11,12] However, it should be emphasized that different antibiotics could have completely different degradation behaviors in soil due to their different physicochemical characteristics.[7–12] Blackwell et al.[7] found that the half-lives of sulfachloropyridazine and oxytetracycline in a sandy loam soil were 3.5 and 21.7 days, respectively. Schlusener and Bester[8] found that the half-lives of erythromycin, roxithromycin, oleandomycin, tylosin, salinomycin and tiamulin in soil ranged from 5 to 27 days. So far, little information has been available in the literature on the degradation behavior of fluoroquinolones in soil and their potential impacts on soil microbial activity.

Fluoroquinolones are an important class of antibiotics currently in use[13] and they could combine with DNA gyrase and interrupt normal DNA replication to achieve the goal of inhibiting microbes.[14,15] It is reported that less than 25 % (mass) of an administered fluoroquinolone could be metabolized in human and animals,[16] which results in contamination of soil environment via application of manure and biosolid. However, only a few studies have investigated the dissipation of fluoroquinolones in soil. Chen et al.[17] found that the degradation half-lives of danofloxacin in different soils ranged from 87 to 143 days. It has been known

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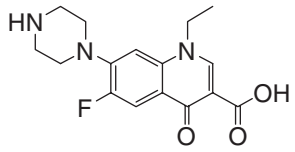

Norfloxacin

molecular formula:	$C_{16}H_{18}FN_3O_3$
molecular weight:	319.33 g/mol
pK_a:	3.11/6.10/8.6/10.56
water solubility:	0.28 mg/mL
logK_{ow}:	-0.31

Fig. 1. Chemical structure and properties of norfloxacin.

that fluoroquinolone antibiotics could be microbiologically transformed and degraded.[17,18]

The objective of this study was to investigate the degradation kinetics of fluoroquinolones in a soil under laboratory conditions, with the commonly used norfloxacin as the model compound (Fig. 1). Three potential influencing factors (initial concentration, swine manure content and soil moisture) were studied for the degradation process of norfloxacin in the soil. At the same time, the effects of norfloxacin on microbial respiration intensity and N-transformation function were also investigated in the soil.

Materials and methods

Chemicals

Chemical standard norfloxacin (98 %) was purchased from DeBioChem Company (Nanjing, Jiangsu, China). Formic acid [high performance liquid chromatography (HPLC) grade] was obtained from Tedia (Fairfield, OH, USA), whereas acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, German). Agar was obtained from Sanland-Chem International Inc. (Xiamen, Fujian, China). All reagents used during extraction and analysis were of analytical reagent grade or better grade.

Preparation of stock solution and culture medium

Norfloxacin stock solution (1000 mg/L) was prepared as follows: 0.05g norfloxacin was weighed into a 20-mL beaker, dissolved in 1mL 3M NaOH, then transferred into a 50-mL volumetric flask and diluted with methanol to the required volume. In order to determine bacterial number in soil samples, the culture medium was prepared to include 3g/L beef extract, 5g/L peptone and 1L sterile water. The final medium pH was adjusted to 7 and sterilized by autoclave for later use.

Collection of soil and manure

Surface soil (soil A, 0–20 cm) was collected from Conghua vegetable base in Guangdong province, China, in which norfloxacin has never been used. The soil is an acidic soil, which is the most common soil type in the region of southern China. After collection, the soil was passed through a 2-mm sieve and stored at 4°C prior to the use. The soil consisted of 32 % clay, 39 % silt and 29 % sand with the organic matter content of 2.4 % and total nitrogen content of 0.16 %. The soil pH value was 4.30, which was measured using soil to 0.01M $CaCl_2$ ratio of 1:5; the maximum water holding capacity (MWHC) of the soil was 48 %; and its cation exchange capacity (CEC) was 9.5 cmol(+)/kg.

Manure used in the experiment was obtained from a piggery, where antibiotics had never been administered in pigs. After being air-dried, the manure was grinded and passed through a 2-mm sieve and stored at 4°C until use. No norfloxacin reidue was detected in the collected manure and soil samples.

Degradation experiment

The degradation experiment of norfloxacin was designed with the follows conditions: three initial concentrations (5 mg/kg, 10 mg/kg and 30 mg/kg), three manure contents (3 %, 6 % and 9 % manure), and four soil moisture levels (20 %, 50 %, 80 % and 300 % MWHC). Sterile controls were also performed at the same time. All treatments were conducted in triplicate.

The procedure to set up the degradation experiment was given as follows. Firstly, 10 grams each of the soils were weighed into 50 mL glass centrifuge tubes followed by spiking norfloxacin solution into soils. Secondly, after methanol evaporated completely, equivalent hydrochloride was added to eliminate soil pH change due to the addition of norfloxacin solution. Finally, a specified volume of sterile MilliQ water was added to adjust soil moisture level. Except for the moisture experiment, the water content in the soil was adjusted to 50 % MWHC. All glass centrifuge tubes were incubated in darkness at 25°C. To keep aerobic conditions, centrifuge tubes were opened every three days with the exception of those soils with 300 % MWHC. Three tubes from each treatment were sacrificed at given time intervals, and the concentrations of norfloxacin in the soil samples were analyzed by reverse-phase high performance liquid chromatography coupled with a fluorescence detector (HPLC-FLD).

To assess microbial activities, soil samples from various treatments (sterile controls, blanks without addition of antibiotics, soils with 10 mg/kg norfloxacin and soils treated with 10 mg/kg norfloxacin and 3 % manure) were taken for bacterial counting. The detailed procedure for bacterial counting can be referred to the previous study by Yang et al.[11]

Soil respiration and nitrification

Seven treatments with norfloxacin concentrations of 0(CK), 1, 5, 10, 50, 100 and 200 mg/kg in the soil were set up to investigate the effects of norfloxacin on soil respiration and nitrification. Each treatment was performed in triplicate. The experimental procedure was described in detail as follows.

For soil respiration test, norfloxacin was spiked into 20 g soil in a 100 mL plastic cup and homogenized, then placed in a fume hood for methanol to evaporate completely. Equal hydrochloride acid was added into the soil in order to eliminate the influence of sodium hydroxide in norfloxacin stock solution. To enhance soil microbial respiration, 0.2 gram glucose was added into the soil and the soil moisture was adjusted to 50 % MWHC. Each plastic cup was put into a 1L air-tight plastic jar with a little cup holding 20 mL of 0.15 M NaOH at the bottom of the jar and incubated at 25°C in the dark. At specified time intervals, the sodium hydroxide in each jar was titrated with 0.1M hydrochloric acid and a new 20 mL of 0.15 M sodium hydroxide was placed in the jar. The calculation method of soil respiration intensity can be referred to in the previous study.[19]

For nitrification test, 10 g soil was weighed into a 250 mL glass flask and norfloxacin was spiked into the soil as above. After methanol was evaporated completely, ammonium sulfate was added into the soil up to a concentration of 100 mg/kg NH_4-N. Soil moisture was adjusted to 50 % MWHC and the soil was mixed completely. Before being incubated at 25°C in darkness, the flasks were sealed tightly with aluminum foil. In order to maintain aerobic conditions for the soils, the foil was opened every three days. When the test was over, 35 mL 1M KCl was poured into each flask and agitated for one hour. After the mixture was filtered through a qualitative filter paper, the NO_3-N content in the solution was measured using ultraviolet spectroscope method.[20]

Extraction and analysis of samples

Soil samples were extracted each with 15 mL 50 % magnesium nitrate solution plus 0.2 mL ammonia for three times using ultrasonic extraction method. Briefly, extraction solution was added into soil and mixed for 20 seconds on a homogenizer followed by ultrasonication for 15 minutes in an ultrasonic bath. The resulting extract was pooled into a volumetric flask and diluted to a constant volume of 50 mL with sterile MilliQ water with the exception for the samples with 300 % MWHC which were diluted to 100 mL. One milliliter of the extract was filtered through a 0.22-μm glass fiber membrane into a 2-mL amber vial. In order to prevent degradation, all extract samples were stored under 4°C in darkness before HPLC analysis.

Norfloxacin concentrations in the soils were determined by HPLC-FLD. Analysis of norfloxacin was conducted on a Agilent 1200 HPLC system equipped with a fluorescence detector (Agilent, Palo Alto, CA, USA). The separation was performed on Alltech Inertsil ODS-2 (4.6×150 mm, 5-μm particle size). The detection wavelengths for the target compound were set at 278nm for excitation wavelength and 440nm for emission wavelength. The mobile phase consisted of acetonitrile (A) and ammonium formate (B) which included 1 % formic acid and pH was adjusted to 3.2 by ammonia. A gradient elution program was applied as follows: 0–6.5 min, 20 % A; 7 min, 90 % A; and 9 min, 90 % A. The flow rate of the mobile phase was 0.8 mL/min and the injection volume is 20 μL. Under these conditions, the chromatographic peak shape of norfloxacin was good without any impurity peak interference (Fig. 2). The instrumental detection limit for norfloxacin (S/N = 3) was 0.021 mg/L. Concentrations of norfloxacin in the soils were quantified using the external standard method.

For recovery experiments, 10 g soils were spiked with three different concentrations of norfloxacin (1 mg/kg, 5 mg/kg and 10 mg/kg) as well as equivalent hydrochloric acid to eliminate the interference of sodium hydroxide in norfloxacin stock solution. The spiked soils were placed in a fume hood until methanol was evaporated completely, and kept in darkness at 4°C overnight before extraction. The results showed that the extraction recoveries (n = 3) of norfloxacin in the soils were 125 ± 6 % for 1 mg/kg, 95.2 ± 2.4 % for 5 mg/kg and 97.5 ± 2.4 % for 10 mg/kg, respectively. Reported concentration data in this study have not been adjusted with the extraction recoveries.

Kinetics model

Degradation kinetics in the soil environment were assessed by using the pseudo-first-order reaction model, which can be expressed as:

$$dC/dt = -kC \tag{1}$$

and

$$C_t = C_0 e^{-kt} \tag{2}$$

where t is time (d), C_0 (mg/kg) and C_t (mg/kg) are the concentrations of the target compound at time 0 and time t, k (mg/kg/d) is the first-order rate constant. The half-life of the target compound can be calculated with the following equation.

$$t_{1/2} = \ln 2/k \tag{3}$$

Data analysis

All degradation data were fitted by the first-order kinetic equation by using Origin 7.0 (OriginLab Corporation, MA, USA) at the 5 % significance level. All data from the respiration and nitrogen transformation tests were analyzed with SPSS 13.0 (SPSS Inc, IL, USA) using Duncan's new multiple range test at the 5 % significance level.

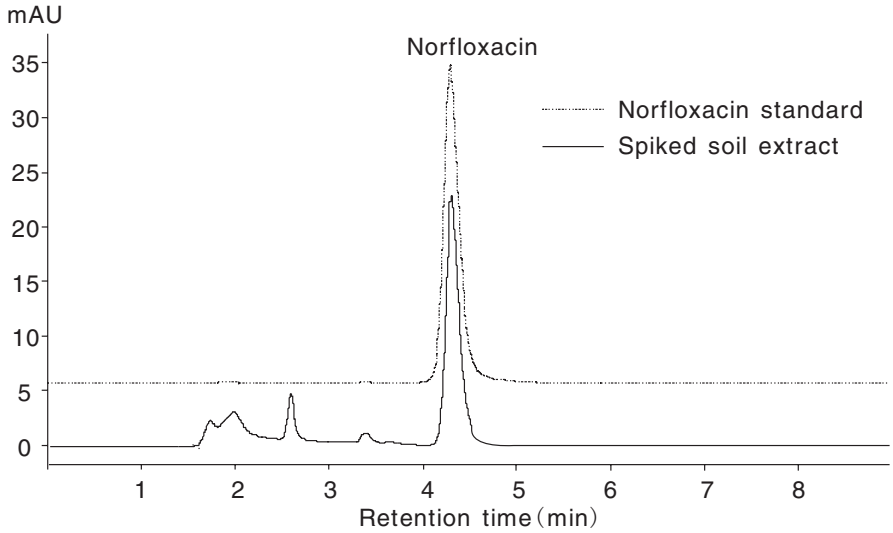

Fig. 2. Liquid chromatogram of norfloxacin in a standard solution (10mg/L) and a soil extract (10mg/kg).

Results

Effect of concentration on Norfloxacin degradation

Norfloxacin degradation in the sterile and non-sterile soils at the concentration of 10 mg/kg is shown in Figure 3 and they all followed the first-order reaction kinetics (Table 1). The degradation of norfloxacin in soil A was much slower under sterile conditions than under non-sterile conditions (Fig. 3). The degradation rate constant for norfloxacin was 0.0145 mg/kg/d in the non-sterile soil, and 0.0045 mg/kg/d in the sterile soil. The half-lives of norfloxacin at the concentration of 10 mg/kg in the non-sterile and sterile soils were 48 d and 153 d, respectively.

Effect of initial concentration on norfloxacin degradation is showed in Figure 4. The degradation of norfloxacin in the soil slowed down with increasing concentrations. Within 42 days, the percentages of the norfloxacin loss in the soils were 62.0 %, 47.7 % and 39.5 % for the soils treated at initial concentrations of 5 m/kg, 10 mg/kg and 30 mg/kg, respectively. The calculated degradation rate constants and half-lives for the degradation of norfloxacin in the non-sterile soils at the three concentrations using the first-order reaction equation (Table 1) all showed a decreasing degradation trend with its concentration increasing from 5 mg/kg to 30 mg/kg.

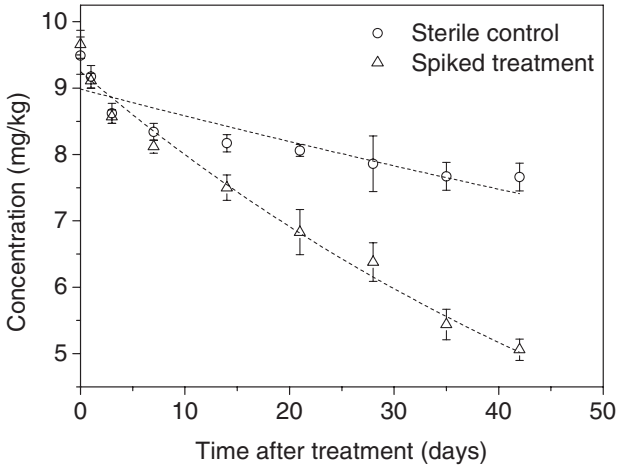

Fig. 3. Degradation of norfloxacin in soil at a concentration of 10 mg/kg under sterile and nonsterile conditions. The error bars represent the standard deviations of the measured concentrations (n = 3).

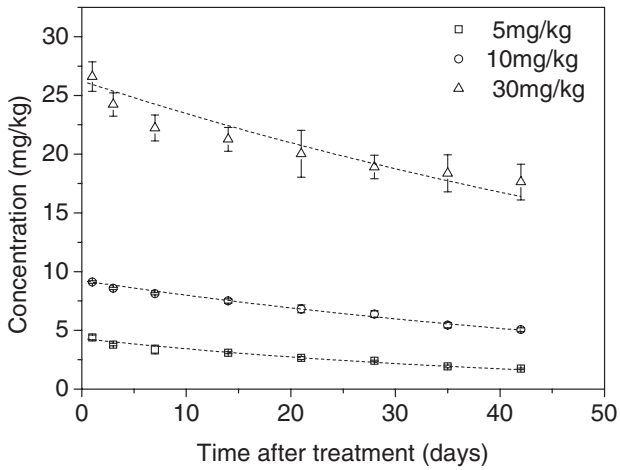

Fig. 4. Concentration effect on norfloxacin degradation in non-sterile soil. The error bars represent the standard deviations of the measured concentrations (n = 3).

Table 1. Parameters of the first-order kinetics for norfloxacin in the soil under different conditions.

Concentration	*Manure (%)*	*Moisture (% MWHC)*[a]	*k (mg/kg/d)*[b]	*Half-life (days)*	R^2 [c]
5 mg/kg	0	50 %	0.0223	31	0.97
10 mg/kg	0	50 %	0.0145	48	0.98
30 mg/kg	0	50 %	0.0112	62	0.85
10 mg/kg	3 %	50 %	0.0182	38	0.98
10 mg/kg	6 %	50 %	0.0239	29	0.99
10 mg/kg	9 %	50 %	0.0286	24	0.98
10 mg/kg	0	20 %	0.0071	98	0.86
10 mg/kg	0	50 %	0.0145	48	0.98
10 mg/kg	0	80 %	0.0161	43	0.98
10 mg/kg	0	300 %	0.0134	52	0.96
10 mg/kg(Sterile control)	0	50 %	0.0045	153	0.81

[a]MWHC: maximum water holding capacity;
[b]k: rate constant of the first-order reaction kinetics;
[c]R^2: coefficient of the fitting curve.

Effect of moisture on norfloxacin degradation

Moisture effect on norfloxacin degradation behavior in the soil is shown in Figure 5. After 42 days of incubation, the percentages of norfloxacin loss in the soils reached 27.7 %, 47.7 %, 52.6 % and 42.5 % for the treatments with soil moisture levels of 20 %, 50 %, 80 % and 300 % MWHC, respectively. Under aerobic conditions (20 % to 80 % MWHC), the degradation rate constants increased with the moisture levels, but decreased under anoxic conditions (300 % MWHC) (Table 2). The corresponding degradation half-lives for norfloxacin were 98, 48, 43 and 52 days, respectively. On the whole, norfloxacin in the soil with 20 % MWHC degraded at the lowest rate; but its degradation was enhanced when the soil moisture increased to more than 50 % MWHC.

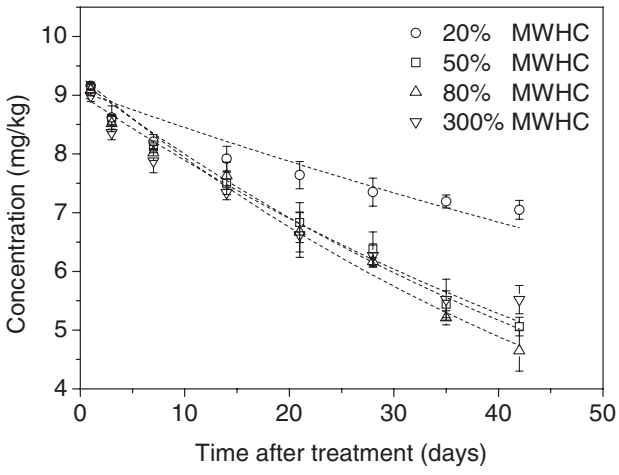

Fig. 5. Moisture effect on norfloxacin degradation in non-sterile soil. MWHC: maximum water holding capacity of the soil. The error bars represent the standard deviations of the measured concentrations (n = 3).

Effect of manure on norfloxacin degradation

The effect of manure on norfloxacin degradation is shown in Figure 6. Within 42 days of incubation, the loss percentages for norfloxacin in the soils increased with increasing manure content, and were 47.7 %, 55.4 %, 67.5 % and 74.8 % for the treatments with 0, 3, 6 and 9 % manure. The degradation of norfloxacin in the soils treated with different amounts of manure followed the first-order reaction kinetics. The calculated half-lives for norfloxacin in the soils were 48, 38, 29 and 24 days for the treatments with 0, 3, 6 and 9 % manure, respectively. Thus amendment of manure enhanced the degradation of norfloxacin in soil.

Effect of norfloxacin on soil respiration and nitrification

Bacterial counting from the soils used in the degradation experiments showed that the addition of norfloxacin did not have much effect on the microbial activity, but the addition of manure increased the bacterial numbers in the treated soils (Table 2). This suggests that amendment of manure could increase microbial activity in the soils.

The results of norfloxacin effect on soil respiration are shown in Table 3. Microbial respiration intensity decreased with time in the soil. In the first 2 days, stimulating effect on soil respiration was observed for the soil treated with norfloxacin. The effect of norfloxacin on soil respiration intensity was found not significant during the later incubation period, when comparing with the control without addition of norfloxacin.

The results from the soil nitrification test are shown in Fig. 7. Interestingly, significant differences in soil nitrification were observed in the soil. The amount of NO_3-N in the soil treated with 1 mg/kg of norfloxacin was significantly higher than that in the control; however, the amounts of NO_3-N in the soils treated with 100 mg/kg and 200 mg/kg of norfloxacin were much lower than that in the control.

Table 2. Soil bacterial numbers (colony forming units, CFU, per gram) in the soil for the three different treatments at three incubation intervals.

Treatment	*7th day*	*28th day*	*42nd day*
A	$2.88 (\pm 0.74) \times 10^6$	$1.78 (\pm 0.98) \times 10^6$	$1.75 (\pm 0.58) \times 10^6$
B	$2.51 (\pm 0.53) \times 10^6$	$1.20 (\pm 0.18) \times 10^6$	$1.93 (\pm 0.42) \times 10^6$
C	$3.52 (\pm 0.29) \times 10^7$	$1.71 (\pm 0.25) \times 10^7$	$3.30 (\pm 0.56) \times 10^7$

A: soil without any treatment;
B: soil treated with 10 mg/kg norfloxacin;
C: soil treated with 3 % manure and 10 mg/kg norfloxacin.

Discussion

The present study investigated the effects of various factors (initial concentrations, soil moisture and manure amendment) on norfloxacin degradation in soil. The results clearly demonstrated significant effects on the degradation of norfloxacin by these factors. It was also found to have time-dependent effects of norfloxacin on soil respiration and nitrification. But the results also suggest that norfloxacin at real environmental concentrations would have little effect on soil microbial activity and functions.

Antibiotics are designed to have antibacterial activities, which may affect the soil microbial activity and functions by exerting selective pressure on soil microorganisms. But the present study showed no effect on soil bacterial numbers in norfloxacin-treated soils when comparing with the control without addition of norfloxacin. This is probably due to the strong adsorption of this antibiotic onto soil components,[21] which can significantly reduce its bioavailability and effects on soil microbial community. The present study found little effect of norfloxacin on soil respiration in the soil, but significant inhibiting effect on soil nitrification at concentrations >100 mg/kg was found. So far the highest reported concentrations for antibiotics in manure and soils were mostly less than 100 mg/kg,[22] suggesting little adverse effect on soil microbial activity posed by this antibiotic at realistic environmental concentrations. It is well known that that nitrification process is performed by two types of autotrophic bacteria (nitrite bacteria and nitrate bacteria). The former can transform ammonia into nitrite acid and the later can transform nitrite acid into nitrate acid. The whole process could be influenced by many factors, such as soil acidity, aeration status, temperature, and humidity. To date, there are few studies about the effect of antibiotics on the nitrification process.[4,23,24] Gomez et al.[23] found that ampicillin, benzylpenicillin, novobiocine, oxytetracycline and chloramphenicol had no effect on nitrate production of a stabilized nitrifying sludge. The study by Campos et al.[24] also showed that chloramphenicol and oxytetracycline did not inhibit the nitrification of biofilm. However, Kotzerke et al.[4] found that high concentrations of sulfadiazine in the manure led to a reduction of the potential nitrification rates in soils.

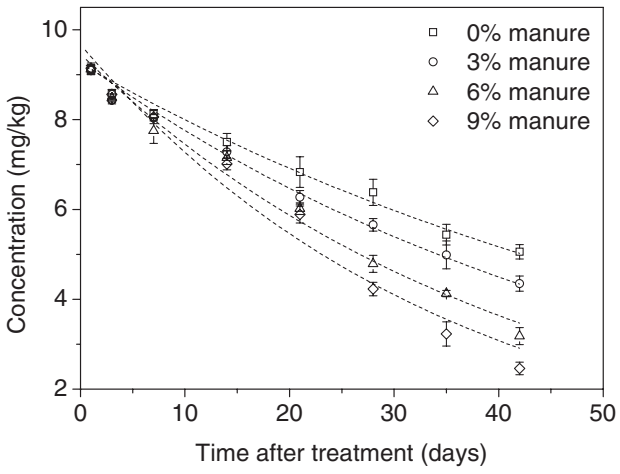

Fig. 6. Effect of swine manure on norfloxacin degradation in nonsterile soil. The error bars represent the standard deviations of the measured concentrations (n = 3).

As for soil respiration, Thiele-Bruhn and Beck[25] also observed time-dependent effect of sulfapyridine and oxytetracycline on the substrate-induced respiration of two soils (sandy Cambisol and loamy Luvisol). Liu et al.[19] found significant effects of two sulfonamides (sulfamethoxazole and sulfamethazine) on soil, but no obvious effects of tetracycline, chlortetracycline and tylosin on soil respiration. They attributed the difference between these antibiotics to their difference in solubility, sorption and bioavailability.

Degradation of an antibiotic could be influenced by various factors such as initial concentrations, moisture and nutrients. The present study also found that norfloxacin was degraded in the sterile soil, suggesting abiotic factors were responsible for the loss in the soil. But in the non-sterile soils, microbial degradation was the dominant process which accounted for the majority loss of norfloxacin. Similar dissipation behavior was also observed for other antibiotics such as sulfadiazine and oxytetracycline in soils.[5,6,11,12] Sulfadiazine was reported to bind irreversibly with soil organic matter.[12,26] Yang et al.[11] also suggested that strong sorption of oxytetracycline onto soil during the incubation period could lead to a decrease in its recovery from the soil.

In theory, the first-order rate constants for the degradation of a chemical are independent on the initial

Table 3. Effect of norfloxacin on soil respiration (CO_2 mg/100 g dry soil).

	Time (days)				
Concentration (mg/kg)	*0–2*	*2–4*	*4–8*	*8–14*	*14–21*
0 (CK)	87.5c	78.8bc	36.1a	19.1a	10.3a
1	101a	81.2ab	28.2b	14.9a	9.53a
5	98.1ab	67.7d	32.5ab	15.1a	10.1a
10	92.6bc	69.9d	28.5b	16.7a	10.3a
50	97.2ab	77.7bc	29.6ab	15.7a	9.90a
100	93.5bc	86.2a	31.4ab	17.5a	9.21a
200	95.2ab	73.9cd	28.6b	14.7a	9.06a

The alphabets behind the respiration data in the table represent the statistical results by using Duncan's test at the significance level of 0.05. CK: control without norfloxacin.

concentration,[27] but in reality, increasing initial concentration could reduce the degradation rate of the chemical in soil. The present study clearly demonstrated that the higher the initial concentration of norfloxacin in the soil, the lower the degradation rate constants. The similar results have also been reported by the previous studies on some pesticides such as fipronil and chlorpyrifos, and they attributed this phenomenon to inhibition of microbial activity by the chemicals.[28,29] The activity of microorganisms could be inhibited in the presence of antibiotics since antibiotics are designed to inhibit or kill certain species of microorganisms.[30] However, the present study only found little or slight effect of norfloxacin on soil microbial activity and function. More future studies may explain the observed effect of initial concentration on degradation of this antibiotic and other chemicals in soils.

As expected, increasing moisture level from 20 % to 80 % MWHC in the soil enhanced the degradation of norfloxacin, but decreased the degradation with a further increase in moisture content to 300 % (flooded soil). Soil moisture can affect microbial activity and redox potential in the soil. Aerobic conditions were maintained in the soils with 20 % to 80 % MWHC, whereas anoxic conditions were created with 300 % MWHC. Yang et al.[11,12] found sulfadiazine and oxytetracycline degraded faster in soils under aerobic conditions than under anoxic conditions. Heberer et al.[31] also found that the degradation of some antibiotics (clarithromycin, roxithromycin, clindamycin and sulfamethoxazole) is closely related to redox potential.

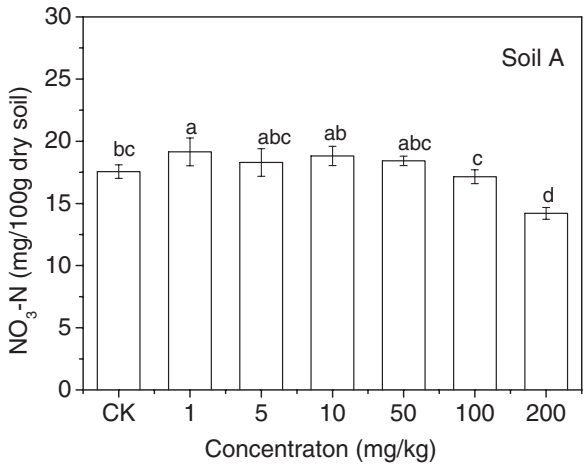

Fig. 7. Effects of norfloxacin on soil nitrogen transformation. Soil A is the soil used in the investigation. The error bars are the standard deviations of the measured values (n = 3). The alphabets on top of the bars of the graphs indicate significant differences from the controls (1-tailed, $P \leq 0.05$) by using Duncan's multiple comparison test.

Amendment of manure into soil increased microbial activity as demonstrated in the present study (Table 3) and in the previous study.[4] Manure has a stimulating effect on microbial activity by supplying nutrients required by microbes.[4] As shown in Table 3, the bacterial number in the soil with 3 % manure was one order of magnitude higher than that in the soil without manure amendment. As a result, norfloxacin degraded faster with increasing manure contents. Similar results were also found for sulfadimethoxine degradation in the soils with manure amendment.[5] Therefore, degradation of antibiotic residues in soil could be enhanced by application of manure.

The observed degradation for norfloxacin was investigated in only one soil with a pH of 4.3. Different soil pH values could lead to formation of different ion species (pK_a 3.11, 6.10, 8.6 and 10.56) of norfloxacin, which may have different effects. For norfloxacin, at pH below 6.10 the positively charged form of the molecule is dominant, while at pH between 6.2 and 8.5, the zwitterionic form becomes dominant.[32] Soil pH could change the species composition of norfloxacin, resulting in different behavior and effects. The effect of pH value on degradation of norfloxacin should also be considered in future studies.

Conclusion

The results showed that the degradation of norfloxacin in the treated soils followed the first-order reaction kinetics. Microbial degradation played a dominant role in

the dissipation of norfloxacin in soil. The degradation of norfloxacin in the soil was influenced by the initial concentrations, moisture levels and manure amendment. Manure amendment enhanced norfloxacin degradation in soil through increasing microbial activity. The degradation rates for norfloxacin increased with soil moisture levels (20 to 80 % MWHC), but decreased with 300 % MWHC and increasing initial norfloxacin concentrations. Application of norfloxacin could have little or slight effect on soil microbial activity and functions, but further research is needed to understand more about the effects of the antibiotic on soil microbial community and functions.

Acknowledgments

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