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Soil Microbial Communities and Mineralization Responses to Penicillin and Tetracycline Loads

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Research Article

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ABSTRACT

Residual effects of pharmaceutical antibiotics on soil microorganisms and turnover processes have merely been investigated. Therefore, this study explored the possible toxic effects of penicillin and tetracycline on indigenous bacterial communities and nitrogen mineralization in soil. Concentrations of 10 and 100 mg.kg^{-1} of penicillin and tetracycline antibiotics in soil affected the microbial community. The effect became apparent by a small tolerance increase and change in the phospholipids fatty acid (PLFA) pattern. The PLFA content revealed that most of the microbial groups decreased, while some specific microbial groups, e.g., 18:1ω9c, did not change in the soils even when soils were exposed to high concentrations of penicillin and tetracycline. Both antibiotics reduced the concentration of ammonium significantly, but that of nitrate was affected slightly. It was concluded that even at higher concentration, pharmaceutical antibiotics exert only a temporary pressure on soil microorganisms and selective processes in nitrogen turnover were negatively influenced.

INTRODUCTION

Antibiotics are widely used to protect the human health and to increase the growth rate of animals in livestock husbandry. Approximately 96,600 tons of antibiotics were used for livestock in all over China in 2009 [1]. However, 50-90% of these antibiotics in both animals and humans can be excreted in an unaltered state [2-5] reported that antibiotics were found in topsoil treated with manure in high concentrations (20 mg antibiotic kg^{-1} soil). Slowly degrading antibiotics can accumulate in the soil due to repeated application of manure during the vegetation period. Although various antibiotics occur naturally in soils, more and more antibiotic residues released into soil differ widely from the natural background.

Setback associated with the extensive use of antibiotics is that it is often accompanied by the rapid appearance of resistant strains, which implies ecological and public health impacts yet to be explored and fully understood. For example, resistance to penicillin, the antibiotic that originally revolutionized human health 50 years ago, is now as high as 79% in *Staphylococcus pneumoniae* isolates in South Africa [6,7]. Both penicillin and tetracycline are zoonotic agents, tetracycline is more persistent with half-life approaching to 100 d in manure; however, the half-life of penicillin in manure is only 5 d [8] for the structure of β-lactams can be opened by the widespread β-lactamase or by chemical hydrolysis [9]. It is vital to investigate the changes of microorganisms' activities and turnover processes in soils by these two kinds of broad-spectrum antibiotics with different chemical stability. Effects of antibiotics on ecological functions have also been discussed. Soil nitrification and denitrification processes may be disturbed by adding sulfadiazine (SDZ). Takeover of ammonia oxidation from ammonia-oxidizing bacteria (AOB) to the less sensitive

ammonia-oxidizing archaea (AOA) has been indicated under antibiotic stress [5,10]. The effects of pharmaceutical antibiotics on soil microorganisms and turnover processes are not well known. We are interested to figure out soil nitrogen dynamics interfered by antibiotics without additional manure substrate to magnify their effects. The concentration of antibiotics released into soils differs widely from natural concentrations [11,12]. Studied sulfonamide and tetracycline antibiotics effects on microbial structure and function.

Soil ecosystem is highly complex containing tremendous microorganisms' communities. Most of the soil microorganism species are still unidentified and knowledge of community structure and its relations to the performance of the soil are poorly understood. Phospholipid fatty acids (PLFAs) are major constituents of the membranes of all living cells. Soil phospholipid fatty acids (PLFA) profiles can help isolate specific biomarker groups of organisms without the need to culture organisms. Several studies indicate PLFA analysis as a powerful and responsive tool to investigate environmental effects on the soil microbial community [13,14]. Among veterinary drugs, the tetracycline group was ranked highest as a concern of possible adverse effects on the environment [15]. Tetracyclines inhibit protein synthesis by disrupting amino acid chain elongation at the 30S subunit of ribosomes [16]. β-Lactams, the first class of naturally produced antibiotics to be implemented clinically, and among the most extensively prescribed antibacterial in North America. Therefore, our interests in penicillin and tetracycline and their effect on soil microorganisms were provoked by the evidence that these antibiotics are the most widely used in both clinical and livestock production due to outstanding efficacy against various bacterial strains. This study sought to obtain a more detailed understanding of the dynamics of microbial communities involved in the turnover of nitrogen in soil and the disturbance caused by penicillin or tetracycline.

MATERIAL AND METHODS

Soil sampling

The experiments were performed with topsoil (0-15 cm) from a compost-treated site (Soil C) where waste compost had been mixed into the soil, and a pristine forest soil (Soil F). These sites are located near the Zhejiang University, Hangzhou, China. None of the soils had been directly treated with penicillin or tetracycline according to the survey. However, organic manure composting may have contributed some level of antibiotics to soil though penicillin and tetracycline could not be detected in the soil. From each spot, a total of 10-12 samples were collected using a sterile spade, mixed thoroughly and then placed into a plastic bag to obtain a composite sample. The composite samples were transported to the laboratory for chemical analysis **(Table 1)**, the samples were kept in ice (2-6 h) and stored at -20°C for further microbial study.

Table 1. Chemical properties of the investigated topsoil samples (<2 mm).

Soil	pH(H_2O)	Total N	Total C	CEC[a]	Available P	Available K
		------- $g.kg^{-1}$ -------		cmol+ kg^{-1}	---------- $mg.kg^{-1}$ ----------	
C	6.71	2.4	22.2	27.5	17.1	1964
F	5.54	3.1	26.3	5.3	66.7	137

[a]CEC, cation exchange capacity.

Chemical analysis of soil

Soil samples, corresponding to 100 g dry weight, were placed in 500 mL Erlenmeyer flasks stopper with cellulose plugs to allow gas exchange while minimizing water evaporation. Antibiotic standard solution treated soils were prepared by first dissolving Penicillin G (Pen) or Tetracycline hydrochloride (Tet) in water and then thoroughly mixing the different volumes of standard solution into the soil to final concentrations of 10 and 100 mg antibiotics kg^{-1} dry soil. Unamended (treatments C and F) samples served as controls. **Table 2** presents details of the treatments. After mixing, the soil moisture was adjusted to 60% of the water holding capacity (WHC) using ultrapure water. Quadruplicate samples were incubated aerobically at 10°C in the dark. Sampling was performed at 0, 1, 3, 7, 14 and 27 days.

Table 2. Antibiotics concentration ($mg.kg^{-1}$) in treated soils.

Treatments	Soil	Antibiotics	Concentration ($mg.kg^{-1}$)
C	C	none	0
CP10	C	Penicillin G	10
CP100	C	Penicillin G	100
CT10	C	Tetracycline hydrochloride	10
CT100	C	Tetracycline hydrochloride	100
F	F	none	0
FP10	F	Penicillin G	10
FP100	F	Penicillin G	100
FT10	F	Tetracycline hydrochloride	10
FT100	F	Tetracycline hydrochloride	100

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Soil microbial biomass

Soil microbial biomass was measured using the fumigation extraction method. Fifteen grams of moist soil was taken and extract was collected by shaking for 30 min with 60 mL of 0.5 mol L^{-1} K_2SO_4. Another lot of 15 g soil was fumigated for 24 h with ethanol-free chloroform and then extract was collected. Microbial biomass carbon (MBC) was determined using Shimadzu TOC-5000 analyzer. It was estimated as BC=EC/0.45, where EC (extractable carbon) is the difference between carbon extracted from fumigated and non-fumigated samples [17].

Soil microbial population

After incubation, 10 g of fresh soil samples was drawn, added to 90 mL of sterile 0.2% agar in water, vigorously stirred for 5 min and serially diluted. These diluted samples were plated on 0.1% tryptic soy agar (TSA) media for total bacterial counts. Potato dextrose agar (PDA) media with 50 mg chlortetracycline and 1 mg.L^{-1} tergitol was used for total fungal counts [18]. Water agar media with antibiotics were used for actinomycetes counts [19]. Bacterial and actinomycetes plates were incubated at 28°C for 9 days, whereas fungal plates were incubated at 25°C for 7days.

Phospholipid fatty acid (PLFA) analysis

Lipid extraction and PLFA analysis were performed according to [20] with some modifications [21]. Samples were kept frozen for 3 weeks until analysis (3 replicates). An internal standard (methyl nonadecanoate fatty acid (19:0) was added before the methylation step. Fatty acids are designated in terms of the total number of carbon atoms, with the number of double bonds given after a colon. Position of the double bond is defined by the symbol ù followed by the number of carbons from the methyl end of the fatty acid molecule. *Cis* and *trans* configurations are indicated by c and t; the *i* and a refer to*iso* and *anteiso* branching; *br* indicates an unknown branch position; and *cy* refers to cyclopropyl fatty acids. Hydroxy groups are indicated by 'OH'. 10 Me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule [22-24].

Soil NH4-N and NO3-N determination

Ten gram of soil per replicate was extracted with 40 mL KCl (1 M) for 1 h in an orbital shaker (175 rpm; 20°C). After filtration, the extracts were analyzed for ammonium-N and nitrate-N by continuous flow analysis (CFA-SAN Plus/SkalarAnalytik, Germany).

Statistical analyses

Data preparation, calculation and statistical analyses were performed using Microsoft Office Excel and SPSS 11.0 for Windows. Analysis of variance (ANOVA) was used to determine treatment effects. Means were compared using Duncan's multiple range test at $P<0.05$.

RESULTS

Soil microbial biomass C and N

Contents of microbial carbon (C) and nitrogen (N) under various treatments were significantly different at the 5% level **(Figure 1).** Relative to C and F treatments, in the first day of incubation soil microbial C decreased 15% in P10, 25% in P100, 12% in T10, and 23% in T100. However, after 27 d incubation, soil microbial C was at the highest in CP10 and FT10. Except C/F treatment, soil microbial biomass C of 27 d incubation was significantly higher than that of the first day. The soil microbial biomass N showed the same behavior and marked reduction was seen in the penicillin and tetracycline treated soils as compared to unamended soils (C, F) after 1 d incubation. The soil microbial biomass N was markedly higher after 27 d of antibiotic treatments compared with unamended soils (C, F).

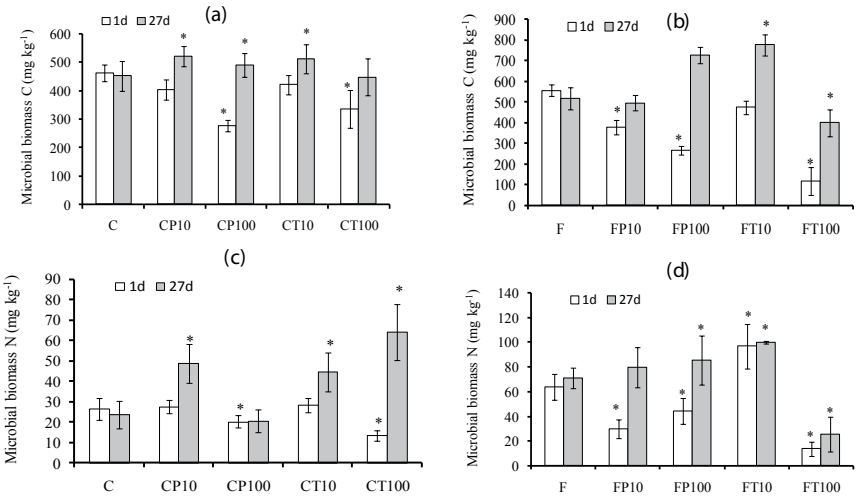

Figure 1. Soil microbial biomass carbon (a, b), and nitrogen (c, d) as influenced by different antibiotic treatments. The *sign indicates columns are statistically different from the controls C/F.

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Microbial populations

Microbial populations in soil were determined by dilution plating method on various agar media. **Table 3** shows the general microbial populations in two soils and five treatments at five different sampling times. Total microbial count of the soils without antibiotic amendment was not different between the two soils and decreased slightly over the incubation period. Microbes in two soils reacted very fast to the addition of antibiotics and showed a strong reduction in the number of culturable bacteria after 1 d incubation. Before 7 d incubation, culturable bacterial population ranked as follows: C/F>P10>T10>P100>T100. However, afterwards, the count of bacteria increased with incubation, and there were no significant differences between unamended soils and antibiotic treated soils after 27 d incubation **(Table 3).** In this study, culturable fungal population was also influenced by different antibiotic amendments, having significantly (P=0.05) higher counts with C and F than with CP100 and CT100 treatments **(Table 3).** Population of actinomycetes was less affected by antibiotics treatment throughout the incubation period.

Table 3. Populations of bacteria, fungi and actinomycetes in soil after penicillin and tetracycline treatments.

Treatment	1d	3d	7d	14d	27d
		Bacteria (10^7 cfu g^{-1} dry soil)			
C	73.5a	64.0a	65.2a	40.7a	44.0a
CP10	55.0b	48.0b	49.5b	40.5a	45.7a
CP100	33.5c	34.2c	38.5c	33.2b	42.2a
CT10	45.0b	43.7b	49.0b	23.7c	47.2a
CT100	34.7c	37.5c	41.5bc	32.0b	42.0a
F	72.5a	64.5a	55.7a	46.7a	48.5bc
FP10	41.2b	33.2b	29.0b	28.2b	60.0a
FP100	26.0c	23.7c	14.7c	25.5b	55.0ab
FT10	43.2b	39.7b	29.7b	26.7b	54.5ab
FT100	39.5b	39.2b	14.5c	20.0b	40.7c
		Fungi (10^4 cfu g^{-1} dry soil)			
C	40.0a	55.0a	47.5a	45.0a	42.5b
CP10	30.0b	45.0ab	37.5b	42.5ab	55.0a
CP100	17.5c	25.0c	40.0ab	35.0b	45.0ab
CT10	37.5a	40.0b	35.0bc	27.5c	35.0c
CT100	22.5c	20.0c	30.0c	42.5ab	45.0ab
F	35.0a	52.5a	45.0a	32.5a	20.0b
FP10	27.5b	27.5b	22.50b	25.0b	20.0b
FP100	15.0cd	9.0d	7.5b	7.5c	27.5a
FT10	20.0bc	27.5b	20.5b	27.5ab	22.5b
FT100	12.5d	20.0c	15.5b	20.0b	32.5a
		Actinomycetes (10^4 cfu g^{-1} dry soil)			
C	46.0a	40.2ab	39.0b	35.5a	45.7a
CP10	45.2a	37.0abc	44.5ab	34.5a	30.7b
CP100	50.7a	43.2a	37.7b	30.5a	23.5 c
CT10	56.5a	36.0bc	51.5a	38.0a	39.7b
CT100	51.7a	34.0c	37.0b	35.7a	35.7b
F	45.50a	40.5b	34.0a	33.2a	25.2a
FP10	35.2b	43.7ab	30.5a	32.0a	9.7c
FP100	43.2ab	52.2a	33.0a	33.0a	16.2b
FT10	24.0b	43.5ab	35.7a	26.2a	15.2b
FT100	44.7a	43.7ab	34.5a	27.2a	15.5b

It is not surprising that both soils showed the same behavior after adding the same antibiotics, and both antibiotics and antibiotic concentrations led to the reduction of culturable bacteria. Overall, a reduction occurred in bacterial number and alterations in microbial populations were observed at the beginning of incubation, but 27 days after the addition, the soil micro flora had recovered not only in soils with low antibiotic concentrations but also in those with high antibiotic concentrations. A tendency for recovery of bacteria was visible.

Microbial community structure: PLFA pattern

Both soils showed the same behavior after the addition of antibiotics. Thus, only one soil was chosen for microbial community structure analysis by PLFA. Twenty six PLFAs with chain lengths from C12 to C20 were identified from different soils **(Figure 2).** They were also tested to assess whether the observed changes in microbial composition parameters were accompanied by changes in the composition of microbial communities under different antibiotic treatments. Soil from each treatment contained a variety of PLFAs, including saturated, unsaturated, methyl branched and cyclopropane fatty acids. The PLFA profiles varied in

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response to various treatments as revealed by their relative abundance and compared to treatment C, the amount, expressed in nmole.g^{-1}, of polyunsaturated fatty acid16:1ù7c, 16:12OH, 16:00, 10Me16:0, i17:0, a17:0, 10Me17:0, cy17:0, 17:00, i18:0, 18:1ù7c, 11Me18:0, 10Me 18:0 in antibiotic treatments decreased from 10% to 50%, respectively. However, monounsaturated fatty acid18:1ù9c and 16:1ù5c contents changed slightly during the incubation period.

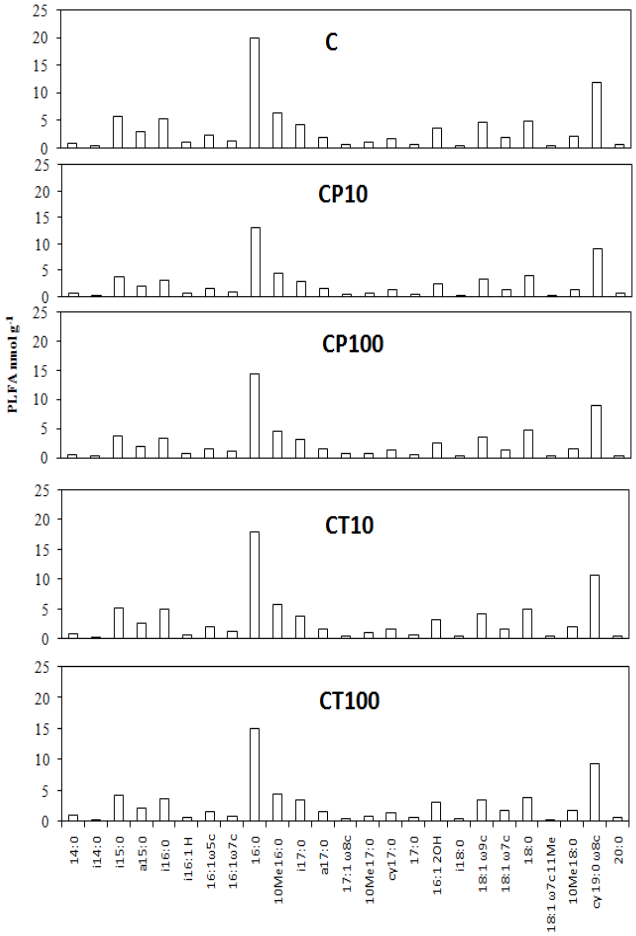

Figure 2. Content of various PLFAs (expressed in nmol.g^{-1} dry soil) as influenced by antibiotic treatments.

The PLFA concentration data were subjected to principal component analysis (PCA) as shown in **Figure 3.** Differences were identified using PCA and outlined according to proportions of structural classes, biomarkers and individual fatty acids present under each treatment. Ordination plots in **Figure 3a** illustrate the difference in PLFA composition for the five treatments. PC1 and PC2 accounted for 66.33% and 12.77%, respectively, of the total variability in the profiles. The treatments C and CT10 were found on the right side of the plot. Although the points representing CT100, CP10 and CP100 treatments were found on the left side of the plot, the CT100 treatment was in the second quadrant, and the CP10 and CP100 treatments were in the third quadrant. The plots suggested that although microbial community composition could be dissimilar with different antibiotic treatments, composition could be similar with CP10 and CP100 treatments. Principal component analysis also identified fatty acids that were important for explaining the variability in PLFA profiles **(Figure 3b).** Except only one out of 26 PLFAs with chain lengths from C12 to C20 were on the left side of the plot, while only the saturated straight chain lipid 20:0 was on the right. This finding indicated that the straight chain lipid 20:0 comprised the greatest proportion of fatty acids in soils with a higher concentration of tetracycline, and soil microbial community structure changed significantly after 27 d incubation of penicillin and tetracycline treatment.

Total amount of PLFA (expressed in nmol.g^{-1} dry soil) is an indicator of total microbial biomass present in the samples. It was significantly affected by antibiotic treatments **(Table 4)**, varying between 62.06 and 89.44 nmol.g^{-1}. Approximately 80% of PLFA is contributed by bacteria. The total amount PLFA in bacteria decreased significantly because of antibiotics addition, in the order of C>CT10>CT100>CP100>CP10, which was the opposite trend as for soil microbial biomass carbon. Actinomycetic PLFAs were also significantly ($P \leq 0.005$) affected by various treatments, having the greatest molar gram in treatment C and the lowest in CP10. Fungi were not affected by antibiotics. Ratios of Gram negative to Gram positive (G-/G+) lipids were also calculated **(Table 4).** The ratios were significantly ($P \leq 0.005$) affected by high antibiotic treatments, being greater in antibiotic treated soils than in control soils. No significant differences were detected between CP10 and CP100, as well as between CT10 and CT100 treatments.

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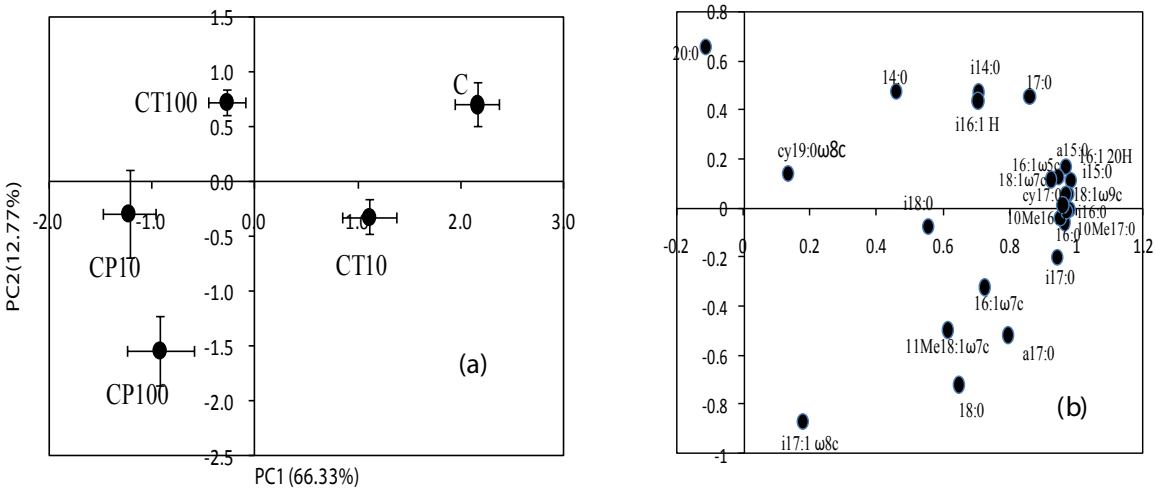

Figure 3. Effect of antibiotic treatments on variation in PLFA patterns. (a) Scores for samples treated with antibiotics. (b) PCA showing loading values for individual PLFAs.

Table 4. Content of PLFAs (nmol.g^{-1}) and the ratio of G$^-$ to G$^+$ in microbial biomass under different antibiotics treatments h^{-1}.

Treatments	PLFA (nmol.g^{-1})				G$^-$/G$^+$
	Total	Bacteria	Fungi	Actinomycetes	
C	87.44 a	70.81 a	6.99 a	9.63 a	0.86 b
CP10	63.28 b	49.17 c	6.87 a	6.48 c	0.90 ab
CP100	65.36 b	52.17c	6.45 a	6.74 bc	0.92 a
CT10	79.27 a	64.01 b	6.38 a	8.88 a	0.95 a
CT100	68.78 b	55.20 c	6.53 a	7.05 b	0.91 a

Influence of antibiotics on nitrogen turnover

Results of ammonium and nitrate measurements are presented in **Figure 4.** Both penicillin and tetracycline decreased concentrations of ammonium in all treatments over the incubation period **(Figures 4a and 4b).** Ammonium concentration decreased with rising antibiotic concentrations in the beginning of the incubation period (days 3 and 7). A sudden rise of ammonium concentration was recorded in the penicillin treated samples after day 7, especially in the sample with a penicillin concentration of 100 mg.kg^{-1}; however, concentrations were still lower than in controls (C and F). In **Figures 4c and 4d** smaller effect of penicillin and tetracycline on nitrate was noticed over the incubation period compared with ammonium; however, the nitrate concentrations decreased at high antibiotic concentrations.

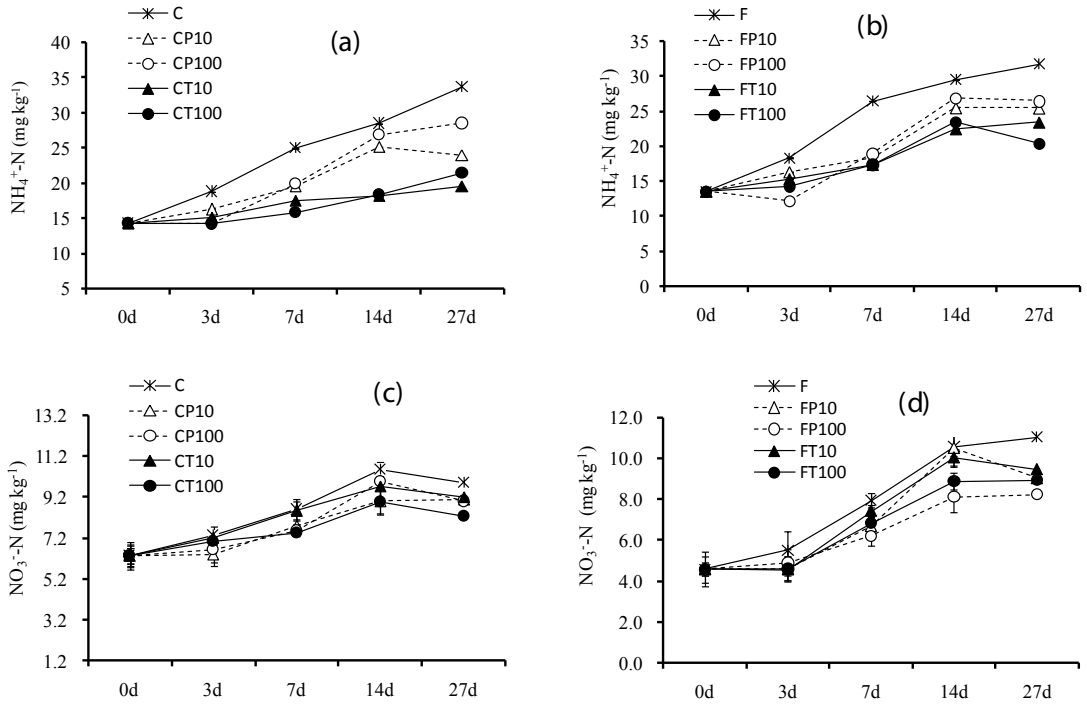

Figure 4. Ammonium (a,b) and nitrate (c,d) concentration in two soils as affected by different antibiotic concentrations at two time periods after their application.

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DISCUSSION

A general understanding is that antibiotics influence the microbial activity and functions in soil, and microbes adapt to these stressors over time. For verification, we selected an experimental setup that included two different soils, two antibiotics and several sampling times. We applied antibiotics with two concentrations in the soils. The unamended soils served as controls. Although the two soils were different in their characteristics as well as in their microbial activity, most of the results obtained were independent of the soil type.

For both soils, microbial biomass and microbial counts indicated that antibiotics inhibited bacterial activity and growth in the beginning of the incubation period; however, the counts also indicated the activating effect of penicillin and tetracycline. Overall, these effects were time dependent and showed a dose response relationship [11] also demonstrated inhibiting effect of the antibiotic sulfadiazine (SDZ) on the growth of microorganisms in soil. They also illustrated that the microbial biomass and structural composition react more sensitively to SDZ contamination than functional processes. The results reported by [12] on the impact of oxytetracycline on the structure and activity of microbial communities in wheat rhizosphere soil, indicated reduced microbial activity profiles that were not be eliminated during 30 d incubation. However, microbial activity tested as basal respiration (BR) and dehydrogenase activities (DHA) were not influenced even at antibiotic concentrations of 1000 $\mu g.g^{-1}$. These results were at par as reported by Thiele-Bruhn and Beck [25]. The BR and DHA results revealed that it could be caused by a low sensitivity of both methods as it has already been reported for the BR methods [26] and shifts in the microbial community structure that compensated for effects on single species. In our study, the effect of different antibiotic concentrations by microbial population and PLFA was visible, but smaller than expected, indicating that the lower antibiotic concentrations used were highly effective and inhibited microbial activity, but were also time dependent. Here, the antibiotics penicillin and tetracycline were added to the soil samples in doses of up to 100 $mg.kg^{-1}$, which exceeded the residual concentrations typically found in the environment by several orders of magnitude [27]. Treatments with 100 mg Pen kg^{-1} soil led, at the late sampling points, to different behaviors and a reduction of microbial activity compared to treatments with 10 mg Pen kg^{-1} soil according to the general microbial populations. Contrary results revealed the fact that most pharmaceutical antibiotics such as Pen and Tet effectively changed microorganisms' structure, for which the toxic dose is often several orders of magnitude smaller than for higher organisms.

Soils which have never been treated with antibiotics before have proved to contain the resistant bacterial strains [28]. Bacterial isolates represented a diverse set of species and multiple antibiotic resistance patterns for more antibiotics. Their findings also revealed that antibiotic-resistant genes pool, which is potentially large and diverse, may have considerable implications for ecology and health of soil. B-Lactam antibiotics such as penicillin and cephalosporin are still the main class of agents used in treating bacterial infections [1]. They share a common mode of action, inhibiting the synthesis of bacterial cell walls by covalently binding with serine residues in the nucleophilic active site of D, D-transpeptidases (called penicillin-binding proteins, PBPs) [29]. Consequently, penicillin is only selectively affecting bacterial populations. Tolerant bacteria can even profit from dead biomass, and during the incubation of soils with antibiotics, resistance mechanisms can be spread easily by the transfer of corresponding plasmids over the bacterial community [18]. The most prevalent mechanism of penicillin resistance is enzymatic drug inactivation by β-Lactamase enzymes which hydrolyze the β-Lactam ring essential for antibiotic activity. Sequences from oceanic metagenomic and environmental databases suggest the presence of these β-Lactamases in diverse environmental locations, speaking to the ubiquitous dispersion of this important resistance determinant. Some soil bacteria can degrade and grow using carbon and nutrients from antibiotic substrates, even when not previously exposed to these antibiotics [30]. These bacteria are phylogenetically diverse and it is not known how widely distributed they are in this region. Reduction in bacterial community together with alterations in microbial populations were observed at the beginning of incubation, but 27 days after the addition, the soil microflora had recovered not only in soils with low antibiotic concentrations but also in those with high antibiotic concentrations. This suggested some soil microbes would adapt to the antibiotics by the development of a resistant population as in our study. Microbial community was further assessed using PLFA, indicating that the antibiotic effect decreased in most microbial groups, while some specific microbial groups, e.g., 18:1ω9c did not change during incubation. The fatty acid 18:2ω6, 9 and 18:1ω9c are the proposed fungal molecular marker [20]. Results indicated that fungal PLFAs were suitable for antibiotic stress. Archaea are also not inhibited by the addition of antibiotics and may substitute bacterial activity in the treatments where antibiotics have been applied [31]. The PLFA results also showed that antibiotics increased the G-/G+ ratio because the bacteria resistant to tetracycline and penicillin are more likely to be Gram-negative [32,33]. This might be an interesting starting point for additional ecological studies underlying the importance of redundant microbial populations for the stability of soil ecosystems after the occurrence of stressors. This question can be answered by investigating the influence of antibiotics on selected turnover processes. Nitrogen turnover plays an important role in soils. Both penicillin and tetracycline treatments led to lower concentrations of ammonium in all treatments over the incubation period; however, a smaller antibiotic effect on the nitrate concentration was visible over the incubation period. It has been stated over the years that the first step of nitrification, the oxidation of ammonium, can only be performed by selected proteo bacteria. However, it has been shown recently that archaea are also able to oxidize ammonium and are, in many cases, more abundant in soil than ammonium oxidizing bacteria [34,35]. This finding explained that penicillin and tetracycline influence nitrate concentration less than ammonium concentration.

CONCLUSION

The mode of action of antibiotics is generally regarded as the biochemical mechanism of the inhibition of sensitive bacteria. Temporal variations in soil bacterial community composition were influenced depending on the antibiotic concentration, especially for the community structure of specific microbial groups, e.g., PLFA cy19:0W8c and i17:1w8c. Additionally, ammonification processes in nitrogen turnover were negatively influenced by adding antibiotics while having little effect on ammoxidation. Our results were based on a single application of antibiotics. It would be interesting to see how the results differ when adding antibiotics to manure or fertilization, or when applying antibiotics more often. In both soils, 27 days after the application of not only penicillin but also tetracycline, an increase in bacteria biomass occurred in comparison to the unamended soil, indicating a potential persistence as well as enduring effect of penicillin or tetracycline. It is important to ask what would happen if these persisting bacteria were broadly distributed.

Thus, in this study soil function is affected by antibiotics. It was evident that overuse of antibiotics can breed resistant bacteria in livestock, hence threatening the future success of these drugs in humans. However, an anti-microbial is an umbrella for microbe-killing products that include antibiotics. Thus, we should be rational while using the antibiotics.

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