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Combined effects of chlortetracycline and dissolved organic matter extracted from pig manure on the functional diversity of soil microbial community

Bei Liu, Yanxia Li*, Xuelian Zhang, Jing Wang, Min Gao

State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, 100875 Beijing, China

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A B S T R A C T

Large amounts of tetracyclines (TCs), the most widely used veterinary antibiotics (VAs), can enter agricultural soil via the application of animal manure to land. The effects of VAs on the soil microbial community function are not well understood. Particularly, few works have examined the combinational effects of VAs and dissolved organic matter (DOM) from animal wastes. In this study, DOM was extracted from pig manure and spiked into soils in combination with chlortetracycline (CTC) at three levels (0, 10, 100 mg kg^{-1} soil). The control soil received only deionized water, and all treatments were incubated for 1, 6, 12 and 45 days. The microbial community function was characterized by investigating the community level physiological profiles (CLPP) using Biolog EcoPlates™ and the activities of several soil enzymes using the spectrophotometry method. The dynamic dissipation of CTC during the incubation period was determined using HPLC. The extractable concentration of CTC was shown to exponentially decrease, and the residual concentration after 45 days was ≤10.8% of the spiking concentration. DOM and CTC had contrary influences on the soil microbial community. The functional diversity indicated by the CLPP and soil enzyme activities were all clearly enhanced by DOM but tended to be inhibited by the co-application of CTC compared to DOM application alone. The significant effect of CTC was deduced to be primarily caused by DOM, which might facilitate bioavailability of CTC and stimulate the activity of microorganisms as additional carbon in the soil. Moreover, all of the microbial parameters were more affected by the incubation time than they were by the DOM and/or CTC treatments. The study demonstrated that the addition of CTC could have a measurable effect on the function of the soil microbial community in the presence of DOM.

1. Introduction

Veterinary antibiotics (VAs) are widely used in livestock farming to treat infectious diseases or promote growth (Boxall et al., 2003; Aust et al., 2008). After being fed to livestock, VAs are poorly absorbed in the animal gut, and approximately 40–90% are excreted as parent compounds or metabolites (Phillips et al., 2004; Kumar et al., 2005; Li et al., 2013). They reach the soil either directly or indirectly when the slurry or manure is applied to agricultural soil. Many investigations have reported that agricultural soils were contaminated by antibiotic residues in the order of several μg kg^{-1} because of the long-term use of animal wastes (Song et al., 2010; Toth and Feng, 2011). In some hot spots, such as soils adjacent to feedlots, concentrations even reached 16.77 mg kg^{-1} soil (Ji et al., 2012). Such high concentrations far exceed the recommended trigger value of 100 μg kg^{-1} soil (EMEA, 2008). Hence, VAs can have detrimental effects on the function of a microbial community (e.g., basal respiration), especially for compounds that are known to persist in soils, such as tetracyclines (Hamscher et al., 2002), fluoroquinolones (Golet et al., 2003) and sulfonamides (Hammesfahr et al., 2008). Studies have shown that VAs influenced the general and potential microbial activities, the bacterial community functions and reduced the ratio of bacteria:fungi (Thiele-Bruhn and Beck, 2005; Zielezny et al., 2006; Liu et al., 2011).

Tetracyclines (TCs) are the most important type of VAs used in animal production, with an estimated consumption of 3230 t y^{-1} in the USA and mainly used to fatten or cure pigs of disease (Benbrook, 2002). It is suspected that the residual TCs will influence the soil

* Corresponding author. State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, No. 19, Xinwai Str., Beijing 100875, China. Tel./fax: +86 10 58807743.
E-mail addresses: liubei0102@126.com (B. Liu), liyxbnu@bnu.edu.cn (Y. Li).

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microbial community due to their broad-spectrum and high antimicrobial activity (Martinez, 2009). Dose–response relationship of TCs' toxicity on microorganisms has been obtained through short-term test, and the effect concentration was in the range of expected environmental level (Thiele-Bruhn, 2005; Schmitt et al., 2006). However, in some simulations, TCs often less affected the microflora than the expected degree because of its high affinity to soils (Hund-Rinke et al., 2004; Zielezny et al., 2006), leading to the effects might be underestimated. On the other hand, detection of microbial influences from VAs needs a prior activation of microbial growth in some cases (Thiele-Bruhn and Beck, 2005). The effect of antibiotics on microorganisms is essentially influenced by their bioavailability and availability of nutrients (Thiele-Bruhn, 2003). Therefore, the actual influences of TCs on soil microbial community was probably affected by coexistent matters that have potential to affect both bioavailability of TCs and activity of microorganisms.

Dissolved organic matter (DOM) is often introduced into the soil as a result of the agricultural disposal of animal manure, which could act as a substrate resource for soil microorganism. Moreover, many studies showed that manure-derived DOM could play an important role in enhancing the mobility of organic contaminants like VAs and thus increase their bioavailability in soil (Thiele-Bruhn and Aust, 2004; Lee et al., 2007). Besides, DOM from wastewater treatment plants was proven to have assisted the transportation of organic pollutants in soil (Ilani et al., 2005; Haham et al., 2012). Based on these literatures, we hypothesized that the adverse effects of TCs on the microbial community will occur in the presence of DOM. In contrast to intensive studies with manure (Nelson et al., 2011) or glucose (Thiele-Bruhn and Beck, 2005; Zielezny et al., 2006), the combined effects of manure-derived DOM and TCs on soil microflora are still largely unknown, which is of importance due to the extensive application of pig manure (containing both TCs and DOM) to agricultural soils.

In this study, we selected chlortetracycline (CTC), one of the most common TCs used in livestock, and aimed to 1) quantify the dissipation of CTC in soil at two spiking levels (10 and 100 mg kg^{-1}) with the supplementation of additional carbon resource; 2) assess the combined effect of CTC and DOM on the microbial community; and 3) highlight the non-target effect of CTC on the functional diversity of microbial communities and its ecological risk on soil health. To evaluate the shifts of microbial communities, we investigated the community-level physiological profiles (CLPP) using Biolog-ECO plates and the activities of four soil enzymes (dehydrogenase, acidic and alkaline phosphatase, urease) using the spectrophotometry method, which have previously been shown to be very sensitive for studying microbial community changes.

2. Materials and methods

2.1. Chemicals, soil samples and preparation of DOM

Chlortetracycline (CTC, 99%) was purchased from Sigma Company (Milan, Italy). All organic reagents used in the experiments were of HPLC-grade and inorganic reagents were of analytical grade.

The soil was sampled from the agricultural experimental plots of the Chinese Academy of Agricultural Sciences (CAAS, China), which had never received antibiotic-containing manure before. After the soil was transported to the laboratory, it was homogenized, sieved to <2 mm and then divided into two parts. One part was stored at 4 °C until being used for the microcosm experiment as described below, another part was air-dried for the physicochemical properties analysis. Soil pH value was measured in 0.01 M $CaCl_2$ solution. The particle-size distribution and organic C content of the soil was determined using the particle size analyzer (Malvern Mastersizer 2000, UK) and TOC analyzer (Liqui, Elementar, Germany), respectively. Properties of the soil are presented in Table 1.

DOM was extracted from pig manure, which was collected from a pig farm in the rural area of Beijing. The treatment of animals with VAs was stopped two weeks before the manure was sampled to obtain the pig manure in which none VAs could be detected. After being air dried and sterilized three times at 121 °C for 20 min, the manure was extracted with distilled water using a solid to water ratio of 1:10 (w/v, dry weight basis) on a reciprocal shaker at 200 rpm for 16 h at 4 °C. The suspension was centrifuged at 12 000 g for 20 min and filtered through a 0.45-μm sterilized membrane. The filtrate was stored at 4 °C and analyzed for dissolved organic matter content using a TOC autoanalyzer (Liqui, Elementar, Germany). Properties of the DOM are described in Table 1.

2.2. Experimental setup

The study was carried out by a 45-day microcosm experiment. The DOM solution was diluted and then spiked to 4 kg of soil (dry weight equivalent) at a concentration of 40 mg C kg^{-1} soil, which increased the C content of soil by 0.2%. Two hundred grams of the subsample were weighed out and amended with 2 ml of distilled water or aqueous CTC solution at calculated concentration to give a final concentration of 0, 10 or 100 mg CTC kg^{-1} soil, corresponding the DOM + C0, DOM + C10 and DOM + C100 treatments, respectively. The control soil did not receive CTC or DOM, but an equivalent amount of deionized water. Each treatment had three replicates. Subsequently, all subsamples were artificially mixed and incubated in darkness at 25 ± 0.1 °C. The moisture content was maintained at 50% of the maximum water holding capacity during the incubation. The mixtures were sampled after 1, 6, 12 and 45 days of incubation and stored individually at 4 °C until further analysis.

2.3. Determination of antibiotic concentration

The CTC concentrations in the soil were determined using a validated analytical method based on an ultrasonic assisted extraction procedure with subsequent analytical determination by HPLC according to Blackwell et al. (2004). The extraction consisted of the following steps: 1 g of soil was weighed into a 40 ml centrifuge tube and 20 ml of extraction buffer (MeOH: 0.1 M Na_2EDTA: Mcllvaine buffer, 50:25:25) was added. The tubes were vortex mixed for 30 s and then placed into an ultrasonic bath for 30 min before being centrifuged at 1200 *g* for 15 min. The extraction was repeated two more times and a total of 60 ml of supernatant was pooled. Subsequently, the extracts were diluted to approximately 400 ml with distilled water to reduce the methanol content below 10% and concentrated using an Oasis® HLB cartridge (6 cc/500 mg, Waters, USA). The cartridges were activated in advance by successively adding 5 ml of MeOH and 5 ml of 0.1 M Na_2EDTA-Mcllvaine buffer. Ten milliliters of dichloromethane were applied to wash the objective analytes after being prewashed with 10 ml of a 5% methanol solution and 10 ml of ultrapure water. The eluents were concentrated under a

Table 1
Physical and chemical characterization of the soil and DOM.

Property	Clay%	Sand%	Silt%	pH	Organic C
Soil	13.65	42.95	43.40	7.59[a]	20.66 g/kg
DOM	—	—	—	6.85	67.1 g/L

[a] 7.59: measured in 0.01 M $CaCl_2$.