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Repeated treatments of ciprofloxacin and kresoxim-methyl alter their dissipation rates, biological function and increase antibiotic resistance in manured soil

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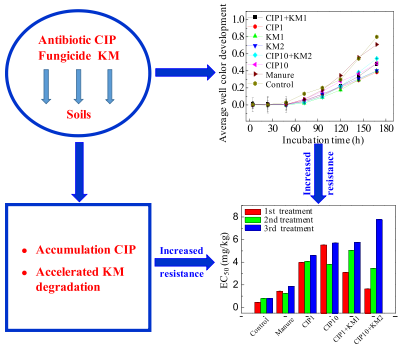
HIGHLIGHTS

- Repeated treatments led to CIP accumulation and accelerated KM degradation.
- KM and CIP could transiently stimulate CAT in the initial stage of each treatment.
- KM and CIP had an inhibitory effect on NPA and URA during repeated treatments.
- KM and CIP suppress soil microbial functional diversity during repeated treatments.
- Bacterial community resistance increased with treatment frequency and concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

The dissipation of ciprofloxacin (CIP, 1.0 and 10.0 mg/kg) and kresoxim-methyl (KM, 1.0 and 2.0 mg/kg) in manure-amended soil, the variations in soil enzyme activities and microbial functional diversities, and CIP-induced bacterial community tolerances were studied using a chromatographic analysis, enzyme colorimetric and titration analyses, and the BIOLOG EcoPlate method. Three successive treatments of individual and combined samples of CIP and KM at low and high concentrations were performed at 60 d intervals. The dissipation half-life of CIP increased, but that of KM decreased in manured soil with treatment frequency; furthermore, the combined treatment altered the dissipation rates of CIP and KM. A stronger inhibitory effect on the activities of soil neutral phosphatase and urease was observed in the individual KM treatment than in the individual CIP treatment. A similar inhibitory trend was also found in soil neutral phosphatase activity in the combined treatment at high concentration compared to that at low concentration, but the activity of soil catalase was enhanced in the early stages of the KM or CIP treatments. Meanwhile, the inhibitory trend on the overall activity and functional diversity of soil microorganisms was observed in the individual KM or CIP treatment, and the combined treatment exerted a greater suppression effect than that in the individual treatment. Bacterial community resistance to CIP increased significantly with increasing treatment frequency and concentration, and furthermore antibiotic resistance developed faster in the combined treatment than in the individual treatment. It was concluded that the repeated treatments of CIP and KM could alter their dissipation rates and soil enzyme activities, suppress microbial functional diversity, and increase bacterial community resistance to CIP in manured soil.

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1. Introduction

Substantial studies have reported that the 30%–90% of the used antibiotics in livestock and poultry breeding were excreted through animal feces, which has been considered as major reservoir of antibiotics (Martinez-Carballo et al., 2007; Hvistendahl, 2012; Van Boeckel et al., 2014). Antibiotic-contaminated manures are often repeatedly used as fertilizer in greenhouse soils, which can cause soil contamination (de la Torre et al., 2012; Fahrenfeld et al., 2014). Meanwhile, fungicides are heavily and frequently used in greenhouse cultivation. A considerable portion (50%–60%) of fungicides were deposited in soil and eventually lead to the accumulation and persistence of fungicides (Niti et al., 2013). The substantial application of antibiotic-contaminated manure and fungicides during the crop growth period has resulted in the long-term combined pollution of antibiotics and fungicides in greenhouse soil. Fungicides and antibiotics have frequently been detected in greenhouse soils, with concentrations ranging from the order of μg/kg to mg/kg (Li et al., 2015; Cheng et al., 2016; Niu et al., 2016). Some researchers have revealed that individual antibiotic or fungicide residues in soil could affect the overall soil microbial activity and induce the formation and development of bacterial community resistance, which posed a potential risk on the soil ecological environment and human health (Fang et al., 2015; Kurenbach et al., 2015; Rangasamy et al., 2017). However, the long-term combined pollution of antibiotics and fungicides may have more serious impacts compared to their individual pollution. Therefore, the repeated effects of combined pollution from antibiotics and fungicides on soil microbial ecology have attracted much attention.

Ciprofloxacin (CIP), which is a type of fluoroquinolone antibiotic, is widely used in livestock husbandry in China, because it can treat bacterial infections by inhibiting DNA and protein synthesis. Kresoxim-methyl (KM) is a highly efficient strobilurin fungicide, which encompasses a broad-spectrum of fungicidal activity against downy and powdery mildew in many vegetables and fruits. CIP residues could persist in soil for a long time, with the half-life ranging from days to months (Walters et al., 2010; Zhang et al., 2012). Some researchers have reported that CIP could change the bacterial community structure, decrease bacterial community diversity, and suppress the soil nitrogen cycle and respiration activities (Naslund et al., 2008; Girardi et al., 2011; Ma et al., 2013). KM could also affect soil enzyme activities and change soil microbial community structures and the functional diversity (Lefrancq et al., 2013; Sabale et al., 2015). However, these studies only focused on the individual effect of CIP or KM. It is worrying that the long-term combined pollution of KM and CIP may cause a succession of variations in soil microbial community structure and ecological function due to the fungicidal activity of KM and the antibacterial activity of CIP. Therefore, there is an increasing interest in the effects of repeated combined treatments of CIP and KM on their dissipation rates, soil biological functions, and bacteria community resistance.

In this study, repeated treatments of CIP and KM, both individually and combined, were conducted in manured soil. The objectives of this study were: 1) to determine the effects of repeated treatments of CIP and KM on their dissipation rates; 2) to examine the variations in the activities of soil neutral phosphatase (NPA), catalase (CAT), and urease (URA) during the repeated treatments; 3) to illustrate the changes in soil microbial functional diversity during the repeated treatments; and 4) to reveal the formation and development of bacterial community resistance to CIP with treatment frequency. This study will be useful to assess soil ecological risks due to the long-term combined pollution of fungicides and antibiotics in greenhouse cultivation.

2. Materials and methods

2.1. Chemicals

The fungicide KM standard (purity ≥ 98.0%) and the antibiotic CIP standard (purity ≥ 94.0%) were provided by Dr. Ehrenstorfer Co. (Augsburg, Germany). Acetonitrile and methanol (chromatographic grade) were provided by Merck (Darmstadt, Germany). Acetonitrile, ethyl acetate, and other reagents (analytical grade) were provided by Dafang Chemical Co. (Hangzhou, China).

2.2. Soil and manure

Surface soil samples (0–10 cm, the soil tillage layer) used in this study were collected at one time from a mulberry field located at the Huajiachi Campus of Zhejiang University (Hangzhou, China), and the residues of KM and CIP in the soil were less than the limit of detection. All soil samples were air-dried and passed through a mesh sieve (2 mm) to remove debris and stones. Meanwhile, manure samples were collected from an experimental pig farm located at the Huajiachi Campus of Zhejiang University (Hangzhou, China), which did not have detectable amounts of antibiotic and pesticide residues. The physical and chemical properties of the soil were detected as follows: sand (21.5%), silt (71.1%), clay (7.4%), organic matter content (3.05%), cationic exchange capacity (10.6 cmol/kg), total nitrogen (0.14%), and pH (6.8).

2.3. Soil treatment

One kilogram (dry weight equivalent) soil sample was divided into two parts, and one part of the soil was first mixed with pig manure at an addition level of 3% (m/m) in a plastic basin, and which was amended by standard solutions of CIP in distilled water and KM in acetone that were sprayed using a small watering can with a proper amount of water. Subsequently, the other part of the soil was added and stirred thoroughly with a glass bar to obtain final concentrations of 1.0 mg/kg and 10.0 mg/kg for CIP and 1.0 mg/kg and 2.0 mg/kg for KM. All soil samples were passed through a mesh sieve (2 mm) to ensure the uniform distribution of the added chemicals and manure, and subsequently placed in a fume hood to volatilize organic solvents. Finally, the soil samples were transferred into plastic pots covered with aluminum foil, which had five pinholes and were incubated in the dark at 25 °C. Soil moisture was maintained at 60% of the maximum water holding capacity in every supplementation by the periodic addition of sterile water using a weighting method. All treatments were performed in triplicate. The experimental treatments included the control, manure, 1.0 mg/kg CIP + manure (CIP1), 10.0 mg/kg CIP + manure (CIP10), 1.0 mg/kg KM + manure (KM1), 2.0 mg/kg KM + manure (KM2), 1.0 mg/kg CIP + 1.0 mg/kg KM + manure (CIP1 + KM1), and 10.0 mg/kg CIP + 2.0 mg/kg KM (CIP10 + KM2). The concentrations of CIP were set at 1.0 and 10.0 mg/kg based on its actual residual level in the soil, and the concentrations of KM were set at 1.0 and 2.0 mg/kg based on the recommended dose and double the recommended dose, respectively. Three successive individual and combined treatments of CIP and KM were performed at 60 d intervals. After each treatment, 30.0 g soil samples were collected at 0, 1, 3, 7, 15, 30, and 60 d for the determination of CIP and KM residues and soil enzyme activities. Soil samples were collected at 60 d after each treatment for the determination of soil microbial functional diversity and bacterial community resistance.

2.4. Extraction and determination of CIP and KM residues

CIP residues were extracted from 10.0 g soil samples (dry weight equivalent) according to the method described by Uslu et al. (2008), and KM residues were extracted from 10.0 g soil samples (dry weight equivalent) according to the method described by Manna et al. (2013). CIP was analyzed using Agilent 1200 high-performance liquid chromatography (HPLC), which was equipped with a chromatographic column (XDB-C18, 4.6 mm × 150 mm, 5 μm) and a diode-array detector (DAD). The mobile phase was comprised of a mixture of acetonitrile and 0.02 M H_3PO_4 at a ratio of 15:85 (v/v). The flow rate and the DAD