



Rational dose of insecticide chlorantraniliprole displays a transient impact on the microbial metabolic functions and bacterial community in a silty-loam paddy soil



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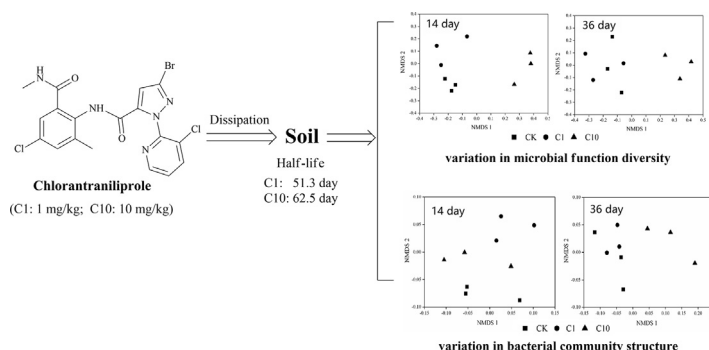
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HIGHLIGHTS

- The study examine the impact of different doses of chlorantraniliprole on soil microbial functional diversity and bacterial community structure.
- Chlorantraniliprole dissipation rates varied with application doses, and higher application dose showed lower dissipation rate.
- High chlorantraniliprole application significantly changed soil microbial features for a longer time than low chlorantraniliprole treatment.
- Response ratio (RR) was used to quantify significant responses of OTUs to different doses of chlorantraniliprole.

GRAPHICAL ABSTRACT



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ABSTRACT

Chlorantraniliprole (CAP) is a newly developed insecticide widely used in rice fields in China. There have been few studies regarding its effects on soil microbial functional diversity and bacterial community composition. An 85-day microcosm experiment was performed to reveal the dissipation dynamics of CAP under different application doses in a silty-loam paddy soil in subtropical China. The half-life of CAP was 51.3 and 62.5 d for low (1 mg kg⁻¹) and high (10 mg kg⁻¹) application dose, respectively. We used a combination of community level physiological profile (CLPP) and 16S rRNA gene sequencing analysis to get insights into the soil microbial features responded to CAP during the experiment. Non-metric multidimensional scaling (NMDS) performed on CLPP and the sequence results indicated that the soil microbial functional diversity and bacterial community composition were significantly changed by CAP application at day 14, and recovered to the similar level as no CAP treatment (CK) under low dose of CAP at day 36. However, high dose of CAP imposed longer effect on these soil microbial features, and was still significantly different from CK at day 36. Microbial taxa analysis at phylum level showed that high dose of CAP decreased the relative abundance of *Nitrospirae* at day 14, while increased *Bacteroidetes* and decreased *Actinobacteria*, *Nitrospirae*, and *Firmicutes* at day 36 in relative to CK. Low dose of CAP only increased *Crenarchaeota* and decreased *Nitrospirae* at day 14. The response ratio (RR) analysis was used to quantify significant responses of OTUs to different doses of CAP and found that CAP significantly affected the microbes involving the N transformation. This study provides a basic information to aid in the development of application regulations regarding the safe use of CAP in soil and inspire us to apply CAP at rational dose to minimize its ecotoxicity on soil microbes.

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1. Introduction

Synthetically produced pesticides are widely applied in agriculture to control pest and improve the yield of crops, mainly for economic reasons. An ideal pesticide could dissipate quickly after performing their expected roles. However, most of the pesticide residues remain into soil, and thus, soil acts as a large sink of applied pesticides in the final procedure. During pesticides dissipation, microbiological transformations are predominant (Cork and Krueger, 1991). Divergent research results indicated that pesticide residues could be a carbon source for microbe, or they could be harmful to soil microbe (Hussain et al., 2009). These nontarget effects may change soil microbial community and affect soil health. As microbe serve as an important indicator of the changes in soil health (Pimentel et al., 2005), it is important to investigate the effect of pesticide on soil microbe. Recently, with the development of modern molecular biology technology, especially the 16S rRNA amplicon sequencing technology, many studies focus on the impacts of pesticide on soil microbial community structure. For example, metam-sodium was found to decrease soil bacterial diversity and change bacterial community structure (Li et al., 2017), iprodione exerted negative effects on soil bacterial biomass and bacterial community diversity (Zhang et al., 2017), and the fumigant dazomet significantly modified both bacterial and fungal communities in soil (Nicola et al., 2017). Some biopesticides such as cantharidin and norcantharidin could induce adverse effects on soil microbial structure. But as cantharidin and norcantharidin degraded rapidly in soil, those influences were transient and microbial structure could recovered quickly (Shao and Zhang, 2017).

Chlorantraniliprole (CAP), an anthranilic diamide insecticide (Fig. 1), is developed by DuPont Crop Protection as its high efficacy on *Laodelphax striatellus* and *Cnaphalocrocis medinalis* (Lahm et al., 2005). A commercial formulation of CAP named “Rynaxypyr” has been registered and started to be widely used in paddy fields in China (Zhang et al., 2012; Zheng et al., 2011). At present, studies of CAP mainly focus on insecticidal activities, mode of action in pests, and analytical methods for the residues (Cordova et al., 2006; Teixeira et al., 2009). CAP presents low toxicity to nontarget organisms such as honeybees, birds, fishes, mammals (Lahm et al., 2007), and soil invertebrates (Lavitzar et al., 2016). In our previous research (Wu et al., 2017), CAP, under field dose, only showed a transient impact on soil respiration, enzyme activity and microbial phospholipid fatty acid (PLFA) profiles in paddy soils. However, little data is available on the influence of CAP on soil microbial functional diversity and bacterial community composition until now.

In China, farmers sometimes spray much more pesticide than recommended dose and the pesticide may also spray unevenly, thus the real initial concentration of the pesticide in certain soils may be much higher than expected. Many previous works also studied the behavior of pesticide with much higher concentration than recommended dose in soil (Mukherjee et al., 2016; Pose-Juan et al., 2015; Telo et al., 2015). The ten-fold field recommended dose (10 FR) is suggested for ecotoxicological

experiments in laboratory incubation study to evaluate the side effect of pesticide on soil microbe (Cycon et al., 2013; Sommerville, 1987). Based on previous reports, CAP spray application in the field has an initial concentration of 0.30–0.88 mg kg⁻¹ soil dry weight (a.i./dw) (Zhang et al., 2012; Sharma et al., 2014). For convenience, an initial concentration of CAP set at 1 mg kg⁻¹ a.i./dw (with reference to the field tests) was treated as the recommended field dose (FR, low application dose) in a laboratory microcosm system, and 10 mg kg⁻¹ a.i./dw was treated as 10 FR (high application dose). In this work, we mainly focus on the impact of CAP with different application doses (FR and 10 FR) on soil microbial metabolic function and bacterial community composition, to evaluate the ecotoxicity of CAP (especially with high dose) in paddy soil.

The aims of this work were to evaluate the following: (1) the dissipation dynamic of different doses of CAP in paddy soil under laboratory condition, (2) the dynamic change of the soil microbial metabolic function which is characterized by the community level physiological profiles (CLPPs) and (3) the impacts of different doses of CAP on soil bacterial community composition during its dissipation. These findings are useful to understand the interactions between CAP and soil microbes in paddy soils.

2. Materials and methods

2.1. Chemicals and reagents

The insecticide CAP (98% purity) was purchased from J&K Scientific, China. Anhydrous Sodium sulfate, primary secondary amine (PSA, 40–60 μm) sorbent and C18 bondesil (40–60 μm) were heated at 130 °C for 7 h before CAP extraction.

2.2. Soil sample collection

A silty-loam paddy soil developed from Jurassic purple shale and sandstone was collected in Beibei District, Chongqing City (29°50' N, 106°25' E) in subtropical China. The topsoils (0–20 cm) were taken in triplicate from a paddy field. The soils were air-dried and sieved (<2 mm) to remove extraneous material prior to further experiment. The physicochemical parameters (Table 1) of the soil was determined by standard procedures (Lu, 1999).

pH measured in the ratio soil:water = 1:2.5. SOC: soil organic carbon. TN, TP and TK: soil total nitrogen, total phosphorus, and total potassium.

2.3. Experimental design and CAP treatments

Incubation experiments were conducted with a completely randomized block design with independent replicates. The dissipation experiments were carried out in 100 mL polyethylene bottles covered with sterile culture sealing membranes (polytetrafluoroethylene membrane) which allow air exchange. Soil samples were aliquoted into 15.0 g and weighed into the bottles and pre-incubated for a week. The effects of CAP on soil microbes were studied using three different doses: 0 (CK, as the control), 1 (C1) and 10 (C10) mg kg⁻¹ a.i./dw. CAP was dissolved in methanol (HPLC grade) to obtain a stock solution of 1000 mg L⁻¹. 15 and 150 μL of the CAP stock solution were added to soils to give a final concentration of 1 and 10 mg kg⁻¹ a.i./dw, respectively. Control samples (CK) were prepared by adding 150 μL methanol without CAP. All samples were placed in fume hood for 2 h to remove methanol and then thoroughly mixed. The samples were placed in an incubator at 25 °C in the dark with 50% maximum soil waterholding capacity for

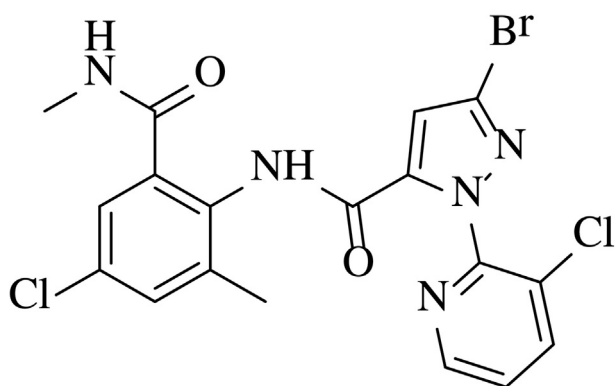


Fig. 1. The chemical structure of chlorantraniliprole (CAP).

Table 1
Physicochemical properties the paddy soil.

Sand (%)	Silt (%)	Clay (%)	pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	TK (g kg ⁻¹)
26.87	53.95	19.18	7.46	15.1	1.33	0.63	25.0

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