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Temporal dynamics of the compositions and activities of soil microbial communities post-application of the insecticide chlorantraniliprole in paddy soils

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ABSTRACT

Chlorantraniliprole (CAP) is a newly developed insecticide widely used in rice fields in China. There has been few studies evaluating the toxicological effects of CAP on soil-associated microbes. An 85-day microcosm experiment was performed to reveal the dissipation dynamics of CAP in three types of paddy soils in subtropical China. The effects of CAP on microbial activities (microbial biomass carbon-MBC, basal soil respiration-BSR, microbial metabolic quotient-qCO_2, acid phosphatase and sucrose invertase activities) in the soils were periodically evaluated. Microbial phospholipid fatty acid (PLFA) analysis was used to evaluate the change of soil microbial community composition on day 14 and 50 of the experiment. CAP residues were extracted using the quick, easy, cheap, effective, rugged, and safe (QuChERS) method and quantification was measured by high performance liquid chromatography (HPLC). The half-lives (DT_{50}) of CAP were in the range of 41.0–53.0 days in the three soils. The results showed that CAP did not impart negative effects on MBC during the incubation. CAP inhibited BSR, qCO_2, acid phosphatase and sucrose invertase activities in the first 14 days of incubation in all the soils. After day 14, the soil microbial parameters of CAP-treated soils became statistically at par with their controls. Principal component analysis (PCA) determining abundance of biomarker PLFAs indicated that the application of CAP significantly changed the compositions of microbial communities in all three paddy soils on day 14 but the compositions of soil microbial communities recovered by day 50. This study indicates that CAP does not ultimately impair microbial activities and microbial compositions of these three paddy soil types.

1. Introduction

Synthetically produced pesticides are widely applied to control plant diseases and maintain high agricultural productivity. Ideally, pesticide application would result in effective disease control followed by full dissipation when its use is no longer desired. However, pesticide residues remain in the soil creating a large sink of chemical contamination. Soils, varying with their physicochemical and microbiological properties, have different profound influences in the transformation of pesticides (Bending et al., 2006). In the transformation, these molecules can impact nutrient turnover rates and soil quality by altering activities of soil-associated microbes (Cycón et al., 2012; Imfeld and Vuilleumier, 2012; Muñoz, Leoz et al., 2013; Subhadeep et al., 2016).

Chlorantraniliprole (CAP) is an anthranilic diamide insecticide developed by DuPont Crop Protection for its high efficacy control of the small brown planthopper, *Laodelphax striatellus,* and the rice leaf folder moth, *Cnaphalocrocis medinalis* (Lahm et al., 2005). CAP presents low toxicity to non-target organisms such as honeybees, birds, fishes, and mammals (Lahm et al., 2007), and has been widely used as a pesticide in agriculture (Vijayasree et al., 2015; Zheng et al., 2011). Recently, a commercial formulation of CAP under the name Rynaxypyr® has been registered and widely used in rice fields in China (Zhang et al., 2012). At present, studies of CAP mainly focus on chemical synthesis, insecticidal efficiency, mode of action in pests, and analytical methods for residues (Cordova et al., 2006; Lahm et al., 2007; Teixeira et al., 2009). Studies on the behavior of CAP in the soil are limited to persistence and dissipation in specific cropping systems (Malhat et al., 2012; Ramasubramanian et al., 2016). One study evaluated its ecotoxicity to paddy field biological communities (Kasai et al., 2015) while another study has focused on non-target soil invertebrates (Lavtizar et al., 2016). Understanding of the impact of CAP on soil microbial activity and community composition is minimal.

Microbial biomass carbon (MBC), an indicator of soil quality

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(Tripathi et al., 2007), has been widely used as a signal for pesticide toxicity (Baboo et al., 2013; Pose, Juan et al., 2015). Three soil biological and biochemical properties are used as integrative indicators for pesticide toxicity estimation: basal soil respirations (BSR), microbial metabolic quotient (qCO_2), and soil enzymatic activities (Xiong et al., 2014; Sannino and Gianfreda, 2001; Bonfleur et al., 2015). Microbial phospholipid fatty acid (PLFA) analysis is also used as an effective biomarker to estimate the change of soil microbial community composition post-pesticide application (Newman et al., 2016; Nye et al., 2014). In this study, microbial community activity (MBC, BSR and qCO_2) dynamics were evaluated concurrently with the dissipation of CAP in three types of paddy soils. Two important soil enzymes involved in soil carbon and phosphorous cycles, sucrose invertase and acid phosphatase, were also measured to indicate the influence of CAP on microbial activities. Finally, PLFA analysis was used to determine the structural variation of the soil microbial community during CAP dissipation. This study provides basic information to aid in the development of application regulations regarding the safe use of CAP in pest management and soil.

2. Materials and methods

2.1. Chemicals and reagents

The chlorantraniliprole (purity, 98%) was procured from J & K Scientific, China. All organic solvents used were High Performance Liquid Chromatography (HPLC) grade. Anhydrous Sodium sulfate ($MgSO_4$), primary and secondary amine (PSA, 40–60 μm) and C18 (40–60 μm) bondesil were heated at 130 °C for 6 h before CAP extraction.

2.2. Soil sample collection

Three typical paddy soils developed from different parent materials were collected from varying regions in subtropical China. These soils were: (1) JS: a paddy soil derived from lacustrine deposits in Xinzhuang Town, Changsu City, Jiangsu Province (31°33′ N, 120°38′ E); (2) HN: a paddy soil developed from quaternary red clay and plate shale in Wangcheng District, Changsha City, Hunan Province (28°15′ N, 112°49′ E); and (3) CQ: a paddy soil developed from Jurassic purple shale and sandstone in Beibei District, Chongqing City (29°50′ N, 106°25′ E). The topsoils (0–25 cm depth) were taken from each field in triplicate samples. The soil samples were air-dried and sieved (< 2 mm) to remove extraneous material prior to further experimentation. The physicochemical parameters of the soils were determined by standard procedures using air dried soil samples (Lu, 1999). Physicochemical properties of the soils are described in Table 1.

2.3. Soil sample treatments

Soil samples were aliquoted into 30.0 g and weighed into a sterile polyethylene bottle and pre-incubated for a week. CAP was dissolved in methanol to obtain a stock solution of 1000 mg L^{-1}. Twenty-one samples of each soil type was prepared by treating 30 μL of CAP stock solution to give a final concentration of 1 mg kg^{-1} dry soil. The treatments were recorded under the designation JS-1, HN-1 and CQ-1. Control samples were prepared by adding 30 μL methanol without CAP and labeled JS-CK, HN-CK, and CQ-CK. All samples were placed in fume hood for 2 h to remove methanol. They were subsequently thoroughly mixed and placed in an incubator at 25 °C in the dark with 50% maximum soil waterholding capacity. Sterile Milli–Q ultrapure water was added when necessary to maintain moisture content. All the treatments were collected at 3, 7, 14, 21, 35, 50, and 85 d in triplicate for CAP residues analysis. The microbial activities were analyzed in the first 50 days. The samples collected on the day 14 and 50 were used for microbial community analysis.

Table 1
Physicochemical properties of the three types of paddy soils.

Soils	Sand (%)	Silt (%)	Clay (%)	pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (mg kg^{-1})	TK (g kg^{-1})
JS	4.75 c	75.59 a	19.67 b	7.59 a	21.8 a	2.18 b	843 a	22.2 b
HN	7.98 b	57.56 b	34.46 a	5.34 c	20.6 b	2.27 a	751 b	16.5 c
CQ	26.87 a	53.95 b	19.18 b	7.46 b	15.1 c	1.33 c	627 c	25.0 a

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. The data are the means of triplicate samples (n = 3). Different letters indicate significant differences among different soils by Turkey tests ($P < 0.05$).

2.4. CAP extraction

Quick, easy, cheap, effective, rugged, and safe method (QuEChERS) which was introduced by Anastassiades et al. (2003) has been used for the extraction and cleanup of the CAP residues in soil (Zhang et al., 2012). For each soil type at each sampling time, 10 g of soil was shaken at 25 °C for 1 h with 20 mL of acetonitrile in glass tubes followed by 0.5 h sonication. The samples were centrifuged at 4000*g* for 10 min. The above steps were repeated a second time to thoroughly extract the CAP from the soils. The combined CAP extracts were concentrated by rotary evaporation to about 5 mL and transferred to a centrifuge tube with 0.5 g anhydrous $MgSO_4$, 100 mg PSA and 100 mg C18 bondesil. After shaking vigorously for 30 s, the tube was centrifuged at 4000*g* for 2 min. The supernatant was dried with nitrogen. The residues were resuspended in 1 mL of methanol and filtered through a 0.22 μm filter (Millipore, USA) prior to the HPLC analysis.

2.5. HPLC parameters and method validation

The residues of CAP were quantified by HPLC. The HPLC (Agilent 1200) was equipped with reversed–phase C18 column and a programmable variable–wavelength UV detector, column oven and electric sample valve. The C18 column ZORBA–ODS (250 × 4.6 mm size, 5 μm) was kept at 40 °C. The flow rate of mobile phase (acetonitrile) was 0.6 mL min^{-1} and injection volume was 20 μL. Detection wavelength of CAP was set at 210 nm. The total running time was 25 min. The retention time (RT) was 10.16 min. Residues of CAP were quantified by comparison of peak area of standards with that of samples with known concentrations of CAP run under identical conditions. Before quantification, the residue of CAP was confirmed by LC–MS/MS analysis method. Electrospray (ESI) was tested for determination of CAP in positive mode. The first transition *m/z* 484.1→453.3 and second transitions *m/z* 484.1→286.0 were used for confirmatory purpose. The limit of quantification (LOQ), which was defined as the concentration that yielded a signal-to-noise ratio of approximately 10 to 1 for the least responsive analyte, was set to be 0.05 mg kg^{-1} for the three soils.

The standard solutions required for constructing a calibration curve (0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 mg L^{-1}) were prepared from stock solution by serial dilutions with methanol. Calibration curves were created from the standard solution ($r^2 = 0.9996$). The recovery of CAP was investigated by the HPLC to examine the reliability and validity of analytical method adopted. CAP recovery was performed by spiking soil samples with CAP at three different concentrations (0.1, 0.5 and 1 mg kg^{-1} dry soil) in four replicates. The recoveries of CAP in the soils were 90.3–101.3% (JS), 86.3–91.9% (HN), and 87.7–93.8% (CQ) with relative standard deviation (RSD) of 7.2–12.2% for all the three soils. Similarly, in previous research, the recoveries of CAP in soils were found to be 76.9–82.4% in rice field (Zhang et al., 2012) and 87.3–95.8% in sugarcane field (Sharma et al., 2014). An acceptable analytical methods are expected to be between 70% and 110% for the analytes, with RSD no greater than 20% (Schwarz et al., 2011).