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Effects of hexaconazole application on soil microbes community and nitrogen transformations in paddy soils

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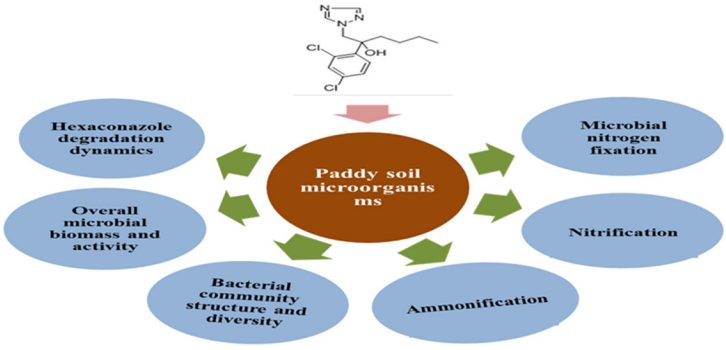
HIGHLIGHTS

- The impacts of hexaconazole on the non-target bacterial community and soil nitrogen transformations were first investigated.
- T10 negatively affected the overall soil microbial biomass and respiratory activity.
- T10 did not change the bacterial diversity and community structure but decrease the populations of bacteria.
- T10 did not affect the soil ammonification and microbial nitrogen fixation but stimulate the nitrification process.
- The relative contributions of AOA and AOB to the nitrification in test soils were studied.

GRAPHICAL ABSTRACT



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ABSTRACT

The ecological risks of widely used hexaconazole on soil microbes remain obscure. Thus, a 3-month-long experiment using two typical paddy soils in China (red soil and black soil) was conducted to assess the effects of hexaconazole (0.6 (T1) and 6 (T10) mg kg^{-1} soil) on the overall microbial biomass, respiratory activity, bacterial abundance and community structure, and nitrogen transformations. Soil was sampled after 7, 15, 30, 60, and 90 days of incubation. The half-lives of the two doses of hexaconazole varied from 122 to 135 d in the black soil and from 270 to 845 d in the red soil. Both dosages of hexaconazole did not affect NH^+_4-N content, N_2-fixing bacterial populations, total bacterial diversity, and community structure, but transitorily decreased the populations of total bacteria in both soil types. In the black soil, T10 negatively affected microbial biomass carbon (MBC) and soil basal respiration (R_B), but transitorily increased NO^-_3–N concentration and ammonia-oxidizing bacteria populations, while T1 had almost no effect on most of the indicators. As for red soil, both concentrations of fungicide significantly, but transitorily, inhibited MBC and R_B, while only T10 had a relatively long stimulatory effect on NO^-_3–N concentration and ammonia-oxidizing archaea populations. This study showed that over application of hexaconazole is indeed harmful to soil microorganisms and may reduce soil quality and increase the risk of nitrogen loss in paddy soils.

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1. Introduction

Maintenance of soil quality and crop yields relies on the functions of soil microorganisms for organic matter decomposition, residue degradation, and nutrient transformations (Álvarez-Martín et al., 2016; Crouzet et al., 2016; Ling et al., 2016; Zheng et al., 2016). However, modern agriculture has led to an upswing in reliance on the use of pesticides, which has caused nearly 70–80% of pesticides to eventually end up in the soil (Muñoz-Leoz et al., 2011; Nguyen et al., 2016; Zhu et al., 2003). Pesticide residue may negatively affect overall soil microbial biomass and activity, as well as the biodiversity of non-target microorganisms. Thus, it may generate harmful impacts on microbially mediated nitrogen-transformation processes, such as ammonification, nitrification (driven by the ammonia-oxidizing archaea [AOA] and ammonia-oxidizing bacteria [AOB]), and microbial nitrogen-fixation processes (driven by N_2-fixing bacteria), compromising the normal nitrogen supplying capacity of the soil (Nettles et al., 2016; Zhang et al., 2015). Several studies have already found that pesticide applications indeed generate harmful effects on soil microbes and nitrogen transformations (Nettles et al., 2016; Tan et al., 2013; Wu et al., 2014; Zhang et al., 2010); it is critical to enhance the evaluation of these effects.

One of the most commonly used fungicides in rice is hexaconazole, (*RS*)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol, a highly active broad-spectrum triazole fungicide that was introduced in 1986 and registered as a systemic foliar fungicide (Han et al., 2013). It is mainly used to control diseases that arise from basidiomycetes and ascomycetes by inhibiting ergosterol synthesis to deter fungal mycelium development (Li et al., 2013). Owing to its high antifungal activity and comparatively low risk of resistance, the usage of hexaconazole has increased annually (Liang et al., 2012). There are already 86 kinds of commercially available hexaconazole products registered in China, of which 64 are applied to rice (China Pesticide Information Network). However, it has led to a considerable proportion of hexaconazole to miss the target crop and enter the soil, not only by spray drift but also by foliar wash-off, which constitutes a potential hazard to non-target soil microorganisms and nitrogen transformations (Álvarez-Martín et al., 2016; Cycoń et al., 2013a; Zhang et al., 2015). Unfortunately, hexaconazole has a long persistence in soil (DT_{50} = 225 d) because of its high octanol-water partition coefficient (Log P = 3.9) and low mobility (Liang et al., 2013). Moreover, hexaconazole has a moderately acute toxicity to aquatic invertebrates (Pesticide Properties DataBase, University of Hertfordshire, 2013). Liang et al. (2013) found it could produce oxidative stress and induce apoptosis in zebrafish. The United States Environmental Protection Agency has classified it as a Group C (Possibly Carcinogenic to Humans) carcinogen (Yao et al., 2015). Moreover, hexaconazole has been designated as a Class II Chemical Substance by the Pollutant Release and Transfer Register in Japan (Tsukatani et al., 2008). Considering its long persistence and ecological toxicity, there is an increasing necessity to explore the effects of extensive hexaconazole application on soil microbes and nitrogen transformations. However, to date, only Kalam and Mukherjee (2001) have examined the short-term (35 d) effects of hexaconazole on the biomass of culturable soil microbes and soil enzyme activity. Nevertheless, culturable microbes do not represent and reflect the actual effect of hexaconazole on the overall soil microbes (Lo, 2010). Besides, the experimental period should be extended appropriately when considering the long persistence of hexaconazole. Furthermore, the changes in non-target microbial community structure after hexaconazole application have not been studied yet and, more importantly, no literature could be acquired about the effects of hexaconazole on soil nitrogen transformations.

Therefore, a 3-month-long experiment using two typical paddy soils in China (Hunan red soil and Heilongjiang black soil), combined with real-time quantitative PCR (*q*PCR) and terminal restriction fragment length polymorphism (T-RFLP) technologies, was conducted for the present study. The aim of this study was to assess (i) the degradation dynamics of different concentrations of hexaconazole; (ii) the extended effects of different concentrations of hexaconazole on overall microbial biomass and activity; (iii) the non-target effects of different concentrations of hexaconazole on the abundance, diversity, and community structure of bacteria; and (iv) the effects of different concentrations of hexaconazole on nitrogen transformations in two typical paddy soils. To ascertain these impacts, we evaluated some sensitive microbial parameters, including microbial biomass carbon (MBC), soil basal respiration (R_B), microbial metabolic quotient (qCO_2), and changes in bacterial gene abundance, diversity, and community structure. Additionally, we measured the changes in ammonium (NH^+_4-N) and nitrate (NO^-_3-N) concentrations and the variations in gene abundance of special functional microbes involved in soil nitrogen transformations (AOA, AOB, and N_2-fixing bacteria). During the incubation period, the degradation of residual hexaconazole in the two paddy soils was also detected. Thus, this experiment broadens our knowledge about the effects of hexaconazole on overall soil microbial biomass and activity. Notably, this is the first report about the effects of hexaconazole application on the non-target bacterial community and soil nitrogen transformations.

2. Materials and methods

2.1. Soil samples

Two representative paddy soils (0–15 cm) were gathered from the Qi Xing Farm, Heilongjiang province (black soil), and the experimental field of Hunan Academy of Agricultural Sciences, Hunan province (red soil). The two soils had not been previously exposed to hexaconazole and hexaconazole was not detected in them. Black soil and red soil are classified as loam and sandy loam, respectively; their detailed physicochemical properties are provided in Supplementary material (SM) (Part 1). After collection, the soil samples were mixed, air-dried at room temperature, and sieved to <2 mm to remove stones and plant tissues, then pre-incubated for two weeks in a dark room at 25 ± 1 °C per OECD (2000).

2.2. Experimental design

Analytical standard hexaconazole (98%) was purchased from the National Pesticide Quality Inspection Center (Nanjing, China) and the standard stock solution was prepared using acetone. The experiment had a completely randomized block design with three treatments: control and two fungicide rates (0.6 and 6 mg kg^{-1} (active ingredient per soil dry weight)). The low dose reflected the maximum recommended field rate (T1) of hexaconazole in rice paddies (90 g active ingredient ha^{-1}), assuming the weight of soil was 1.5×10^5 kg ha^{-1} at the effective depth of 10 cm (GB/T 31270.1-2014). The high dose of hexaconazole corresponded to 10 times the recommended rate (T10). For each treatment, three independent replicates were prepared by transferring samples of 4 kg dry weight soil to separate PVC tanks. After being treated with the above-mentioned doses (the control soil received an equal volume of pure acetone), the soil samples were thoroughly mixed with a rotary mixer (ACA, AHM-P125B) to ensure uniform distribution of the fungicide and kept in a dark room for 24 h at 25 °C to allow the acetone to evaporate. Then, each freshly treated soil sample was distributed equally (200 g) into brown wide-mouthed bottles (15 cm × 8 cm) as independent subsamples of different sampling times. Finally, for each soil type, there were a total of 45 brown wide-mouthed bottles in the experiment (i.e. three treatments × three replications × five sampling times). Soil humidity was adjusted to 60% of the maximum water holding capacity; every bottle was then covered with porous plastic film and incubated in an environmental chamber at 25 °C and 50% humidity for 90 d. Deionized water was added to the soil to maintain a constant humidity every 2 days throughout the incubation period. Soil subsamples were periodically removed from the environmental chamber after 7, 15, 30, 60, and 90 d. Then, soil biochemical parameters and hexaconazole concentrations were analysed after