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Effects of myclobutanil on soil microbial biomass, respiration, and soil nitrogen transformations

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ABSTRACT

A 3-month-long experiment was conducted to ascertain the effects of different concentrations of myclobutanil (0.4 mg kg^{-1} soil [T1]; 1.2 mg kg^{-1} soil [T3]; and 4 mg kg^{-1} soil [T10]) on soil microbial biomass, respiration, and soil nitrogen transformations using two typical agricultural soils (Henan fluvo-aquic soil and Shanxi cinnamon soil). Soil was sampled after 7, 15, 30, 60, and 90 days of incubation to determine myclobutanil concentration and microbial parameters: soil basal respiration (R_B), microbial biomass carbon (MBC) and nitrogen (MBN), NO^-_3–N and NH^+_4–N concentrations, and gene abundance of total bacteria, N_2-fixing bacteria, fungi, ammonia-oxidizing archaea (AOA), and ammonia-oxidizing bacteria (AOB). The half-lives of the different doses of myclobutanil varied from 20.3 to 69.3 d in the Henan soil and from 99 to 138.6 d in the Shanxi soil. In the Henan soil, the three treatments caused different degrees of short-term inhibition of R_B and MBC, NH^+_4–N, and gene abundance of total bacteria, fungi, N_2-fixing bacteria, AOA, and AOB, with the exception of a brief increase in NO^-_3–N content during the T10 treatment. The MBN (immobilized nitrogen) was not affected. In the Shanxi soil, MBC, the populations of total bacteria, fungi, and N_2-fixing bacteria, and NH^+_4–N concentration were not significantly affected by myclobutanil. The R_B and MBN were decreased transitorily in the T10 treatment. The NO^-_3–N concentrations and the abundance of both AOA and AOB were erratically stimulated by myclobutanil. Regardless of whether stimulation or suppression occurred, the effects of myclobutanil on the two soil types were short term. In summary, myclobutanil had no long-term negative effects on the soil microbial biomass, respiration, and soil nitrogen transformations in the two types of soil, even at 10-fold the recommended dosage.

1. Introduction

Myclobutanil (RS)-2-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)hexanenitrile, is an efficient broad-spectrum triazole fungicide that is widely used for protection against powdery mildew and scab in cereals and vegetables by treating the seeds and spraying the foliage (Kemmitt et al., 2008; Dong et al., 2012). Myclobutanil acts by inhibiting ergosterol biosynthesis, which is a crucial membrane component in fungi. Only in China, there are 148 commercial products of myclobutanil currently registered (China Pesticide Information Network) used for their excellent antifungal activity. Sustained and extensive application of the fungicide has caused widespread soil pollution via spray drift, foliar wash-off, and contamination from treated seeds (Cycoń et al., 2013; Karpouzas et al., 2014). This in turn can have harmful effects on soil microorganisms that dominate soil nutrient cycles and play key roles in normal functioning of agroecosystems (Widenfalk et al., 2008; Pose-Juan et al., 2015). The negative effects on soil microorganisms can also indirectly endanger soil nitrogen transformations and therefore compromise soil quality and agricultural production (Cycoń et al., 2006; Wan et al., 2014). Additionally, a highly efficient pesticide does not indicate low toxicity to non-target organisms. Cheng et al. (2013) reported that myclobutanil had medium acute toxicity to *Scenedesmus obliquus*, a microorganism that typically consists of four cells. Moreover, myclobutanil has a relatively long persistence in the field with a dissipation time (DT_{50}) of more than 35 days (Pesticide Properties DataBase, University of Hertfordshire, 2013). Thus, the persistent myclobutanil residues in soils might pose a potential threat to soil microorganisms.

Soil microbes are sensitive indicators of the potential threat of pesticides, because microbes often respond rapidly to any ecosystem perturbation (Schloter et al., 2003; Muñoz-Leoz et al., 2013; Pose-Juan et al., 2015). Soil microbial properties, such as

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microbial biomass and respiration, are commonly used as "biomarkers" to evaluate the influences of pesticides on soil quality (Cycoń et al., 2013; Silva et al., 2013). The primary transformations of nitrogen in soils, such as nitrogen immobilization, ammonification, nitrification, and microbial nitrogen fixation, are significant to maintain soil nitrogen supply capacity (Dar and Mishra, 1994; Hart et al., 1994; Levy-Booth et al., 2014; Wang et al., 2015). Some key functional groups of microbes such as the N_2-fixing bacteria, ammonia-oxidizing archaea (AOA), and ammonia-oxidizing bacteria (AOB) dominate the primary processes of nitrogen transformations (Levy-Booth et al., 2014; Wang et al., 2015). The N_2-fixing bacteria transform gaseous nitrogen to biologically available ammonium, which is an important source of nitrogen in soil ecosystems (Hayden et al., 2010). Both AOA and AOB play key roles in the regulation of soil nitrogen dynamics by controlling the ammonia oxidation process, the first and rate-limiting step in soil nitrification, although the relative contribution of each group to soil nitrification remains controversial (Wang et al., 2014). The analysis of gene abundance of these functional groups of microbes could provide a good estimation of the effects of pesticides on soil nitrogen transformations (Chen et al., 2015). However, to date, only a few studies have examined the effects of triazole fungicides on soil microbes (Hart and Brookes, 1996; Strickland et al., 2004; White et al., 2010; Yang et al., 2011; Marinozzi et al., 2013). Additionally, the information on the effects of triazole fungicides on soil nitrogen transformations is limited, with the exceptions of tebuconazole and penconazole (Cycoń et al., 2006; Puglisi et al., 2012). To the best of our knowledge, the effects of the intensive use of myclobutanil on soil microorganisms and soil nitrogen transformations have not been evaluated.

Thus, the aim of this research was to determine the following: I. The effect of myclobutanil on soil microbial biomass, respiration, and recovery of soil health after myclobutanil treatment. II. The effect of myclobutanil on soil nitrogen transformations and relative contributions of AOA and AOB in test soils. Considering the high complexity and biodiversity of soil microbial communities and for a better analysis of the overall potential hazards of myclobutanil to soil microorganisms, we designed a 3-month-long experiment using two typical soils in China (Henan [HN] fluvo-aquic soil and Shanxi [SX] cinnamon soil), and combined it with multiple measurement parameters to achieve these goals. The biochemical parameters included: soil basal respiration (R_B), microbial biomass carbon (MBC) and nitrogen (MBN), as well as the change in NH^+_4 and NO^-_3 concentrations and gene abundance of total bacteria and fungi. Special attention was given to functional microbes (N_2-fixation bacteria, AOA, and AOB) using real-time quantitative PCR (qPCR). Besides, the degradation dynamics of myclobutanil in the two soils was also ascertained.

2. Materials and methods

2.1. Soil samples

Two types of wheat field soil (0–15 cm) that were not previously exposed to myclobutanil were collected from the experimental plots of the Henan Institute of Science and Technology (HN fluvo-aquic soil) and the Shanxi Academy of Agricultural Sciences (SX cinnamon soil). Both HN and SX soils are typical soils in China with the following detailed soil physicochemical properties. HN soil: 25.12% sand, 61.11% silt, 13.77% clay, 19.52 g organic matter kg^{-1} soil, 77.33 mg available P kg^{-1} soil, 512.5 mg available K kg^{-1} soil, 5.13 mg NH^+_4 kg^{-1} soil, 74.94 mg NO^-_3 kg^{-1} soil, pH 7.76, 340 μs cm^{-1} electrical conductivity; SX soil: 42.07% sand, 45.87% silt, 12.09% clay, 32.28 g organic matter kg^{-1} soil, 67.23 mg available P kg^{-1} soil, 460 mg available K kg^{-1} soil, 7.77 mg NH^+_4 kg^{-1} soil, 59.59 mg NO^-_3 kg^{-1} soil, pH 7.8, 522 μs cm^{-1} electrical conductivity. After collection, the soil samples were mixed, sieved through a <2-mm sieve to remove plant tissue, and preincubated for 10 days under the experimental conditions described in Section 2.2 (OECD, 2000).

2.2. Experimental design and treatments

The analytical, standard myclobutanil (99.2%) was purchased from the Beijing Qinchengyixin Technology Development Co., Ltd., (Beijing, China). The preparation was applied in three different doses: 0.4 mg kg^{-1} (active ingredient per soil dry weight; T1), 1.2 mg kg^{-1} (T3), and 4 mg kg^{-1} (T10). The amount used in T1 treatment was the recommended field rate for myclobutanil in a wheat paddock (60 g active ingredient ha^{-1}), with the assumption that the soil weight was 1.5×10^5 kg ha^{-1} at the effective soil depth of 10 cm (GB/T 31270.1-2014). The T3 and T10 treatments corresponded to a 3- and 10-fold increase of the recommended dose, respectively. Each soil sample was divided into four portions of equal weight (4 kg), which were placed into separate PVC tanks. Three portions of each soil sample were treated with the three different levels of fungicide, and the fourth portion (control) received an equal volume of pure acetone. The subsamples were thoroughly mixed with a rotary mixer (ACA, AHM-P125B) to ensure uniform distribution of the pesticide. Then, each freshly treated soil subsample was distributed equally (200 g) among brown, wide-mouthed bottles after solvent evaporation. Water content of the soils was adjusted to 60% of the maximum water holding capacity, and the pots were covered with porous plastic film. The pots were placed in an environmental chamber at 25 °C and 50% humidity for 90 d. Each experiment was conducted in triplicate. Throughout the incubation period, deionized water was added to the soil to compensate for any water loss that exceeded 5% of the initial amount added. The soil samples were periodically removed from the environmental chamber (on day 7, 15, 30, 60, and 90) and analyzed for the concentration of myclobutanil and soil biochemical parameters. The remaining soils were stored at −80 °C for DNA analysis.

2.3. Determination of myclobutanil concentration in soil

Myclobutanil was extracted and its concentration was determined as previously described by Dong et al. (2012) with some modifications. Briefly, 1.5 g samples were extracted with 30 mL acetonitrile and the extracts were dehydrated and layered with 2 g anhydrous $MgSO_4$ and 1 g NaCl. By centrifugation (4000 rpm for 5 min), the supernatant (1.5 mL) was then purified in a 2.0-mL dispersive SPE tubes containing 50 mg of primary and secondary amines and 150 mg $MgSO_4$. The resulting supernatants were filtered through 0.22-μm nylon syringe filters for ultrahigh performance liquid-chromatography tandem mass spectrometry (UHPLC–MS/MS) analysis. Concentration of myclobutanil was determined by a triple-quadrupole mass spectrometer (TQD, Waters Crop., Milford, MA, USA) using the multiple reaction monitoring (MRM) mode and positive ESI mode. Precursor ion with the mass of 289.2 (m/z) was selected when the cone energy was 35 V, and its quantitative and qualitative product ions were 70 (m/z) and 125 (m/z), respectively, when the collision energies were both 20 V. The external matrix-matched standard solutions were prepared at 0.01, 0.02, 0.05, 0.1, 0.2, and 0.4 mg kg^{-1} as standard curve for correct quantification.

2.4. Soil biochemical parameters

Soil basal respiration (R_B; an indicator of the overall microbial