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Tebuconazole application decreases soil microbial biomass and activity

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ABSTRACT

A short-term mesocosm experiment was conducted to ascertain the impact of tebuconazole on soil microbial communities. Tebuconazole was applied to soil samples with no previous pesticide history at three rates: 5, 50 and 500 mg kg^{-1} DW soil. Soil sampling was carried out after 0, 7, 30, 60 and 90 days of incubation to determine tebuconazole concentration and microbial properties with potential as bio-indicators of soil health [i.e., basal respiration, substrate-induced respiration, microbial biomass C, enzyme activities (urease, arylsulfatase, β-glucosidase, alkaline phosphatase, dehydrogenase), nitrification rate, and functional community profiling]. Tebuconazole degradation was accurately described by a bi-exponential model (degradation half-lives varied from 9 to 263 days depending on the concentration tested). Basal respiration, substrate-induced respiration, microbial biomass C and enzyme activities were inhibited by tebuconazole. Nitrification rate was also inhibited but only during the first 30 days. Different functional community profiles were observed depending on the tebuconazole concentration used. It was concluded that tebuconazole application decreases soil microbial biomass and activity.

1. Introduction

The intensive use of pesticides is a common practice in modern agriculture all around the world (Yang et al., 2007). Several studies have estimated that less than 0.3% of the pesticide reaches its target pest (Pimentel, 1995); the remaining 99.7% is released to the environment, representing a potential hazard for non-target organisms including humans. A significant proportion of the pesticide frequently ends up in the soil where it undergoes biological and physicochemical transformations (Bending et al., 2006). In this respect, microbial degradation is the main route of pesticide removal in soils, conditioning its persistence and susceptibility to leaching (Aislabie and Lloyd-Jones, 1995; Bending et al., 2006). However, pesticides can exert non-target effects on soil microbial communities, negatively affecting soil health (Monkiedje et al., 2002; Lupwayi et al., 2010). Some specific soil microorganisms, such as nitrifying bacteria, are very sensitive to pesticides (Ahtiainen et al., 2003). On the other hand, soil microbial properties, particularly those related to the biomass, activity and diversity of soil microbial communities, can be most useful indicators of the impact of disturbances on soil health (Hernández-Allica et al., 2006; Mijangos et al., 2006, 2009).

Tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl) pentan-3-ol], a broad-spectrum triazole fungicide used agriculturally for disease control in fruit, nut, cereal and vegetable crops worldwide, has a relatively high soil organic carbon-water binding coefficient and a half-life in soil of 49–610 days under aerobic conditions (Strickland et al., 2004). Although this relatively new fungicide is being widely used due to its effectiveness against soilborne and foliar fungal diseases, few studies on its degradation in soil (Bromilow et al., 1999; White et al., 2010) and, in particular, its non-target effects on soil microbial communities (Strickland et al., 2004; Cycoń et al., 2006; Bending et al., 2007) have been published. To prevent potential tebuconazole-induced adverse effects on the soil ecosystem, more research is needed for a better understanding of its degradation in soil and a more accurate assessment of its impact on soil microbial communities.

The main goal of this study was (i) to quantify dissipation kinetics of tebuconazole in a soil with no previous known history of pesticide application when added at three different rates (5, 50 and

Abbreviations: DW, dry weight; T5, sample with 5 mg tebuconazole kg^{-1} DW soil; T50, sample with 50 mg tebuconazole kg^{-1} DW soil; T500, sample with 500 mg tebuconazole kg^{-1} DW soil; R_B, basal respiration; SIR, substrate-induced respiration; C_{mic}, microbial biomass carbon; qCO_2, microbial metabolic quotient; Q_R, microbial respiratory quotient; URE, urease activity; ARYL, arylsulfatase activity; GLU, β-glucosidase activity; PHO, alkaline phosphatase activity; DEH, dehydrogenase activity; AWCD, average well color development; S, species richness; H′, Shannon's diversity.

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500 mg kg^{-1}), (ii) to assess tebuconazole impact on soil microbial communities, and (iii) to monitor the recovery of soil health after tebuconazole application. To evaluate tebuconazole-induced non-target effects on soil microbial communities and soil health, we determined a variety of soil microbial properties which provide information on the biomass, activity and diversity of soil microbial communities simultaneously (i.e., basal and substrate-induced respiration, microbial biomass C, ecophysiological indices, enzyme activities, nitrification rate, and functional community profiling). To our knowledge, no studies have evaluated the impact of tebuconazole on soil microbial communities and soil health using measures of microbial biomass, activity and diversity simultaneously.

2. Materials and methods

2.1. Soil characterization

Soil (top 0–25 cm) was collected from the riparian zone of the Salburua wetland (Vitoria-Gasteiz, northern Spain), an area with no previous known history of pesticide application. Immediately after collection, soil samples were taken to the laboratory in dark plastic bags, homogenized, air-dried at 25 °C during 48 h, sieved to <2 mm, and subjected to physicochemical characterization according to Sparks et al. (1996). The soil is of a Chernozem calcic character (FAO) with a clay–sandy texture, a pH of 8.3 (1:2.5 w/v in water), 17.0 g organic C kg^{-1} dry weight (DW), 2.3 g total N kg^{-1} DW, a C/N ratio of 7.8, and an electrical conductivity of 0.18 dS m^{-1}.

2.2. Experimental design

A three-month mesocosm study was carried out following a modification of the method by Monkiedje et al. (2002). For each tebuconazole concentration, a set of three replicated mesocosms was prepared by transferring subsamples of 8 kg DW soil to 10 L plastic trays, resulting in a soil layer of approximately 10 cm depth. Each subsample was artificially contaminated by spiking 100 mL of a tebuconazole (purity ≥99%, DTS Olabe, Spain) solution (in methanol) at calculated concentrations to give a final concentration of 5, 50 and 500 mg tebuconazole kg^{-1} DW soil (T5, T50 and T500, respectively). T5 corresponds approximately to the highest recommended field application dose for wheat crops (0.5 kg active ingredient ha^{-1}), assuming a soil bulk density of 1 g cm^{-3} and an effective soil depth of 1 cm (Strickland et al., 2004). T50 and T500 were chosen following standard ecotoxicological practice for establishing possible negative effects of a substance in the environment (Chen and Edwards, 2001; Cycoń et al., 2006). Subsamples were thoroughly mixed with a rotary mixer (Philips handmixer, HR1570) to assure uniform fungicide distribution and then kept for 24 h in a well aerated dark room to allow evaporation of the methanol. An equal volume of pure methanol (100 mL) was added to tebuconazole-free controls (C). Soil moisture content was adjusted to 60% water holding capacity (WHC). To avoid tebuconazole photodegradation and evaporative losses of water from soil, trays were covered with perforated polypropylene sheets and incubated in the dark at 22 ± 1 °C. Throughout the incubation, water content was held constant by daily addition of deionized water.

From each mesocosm, a subsample of 250 g FW soil was taken at 0, 7, 30, 60 and 90 days of incubation, sieved to <2 mm, and stored at 4 °C until analysis.

2.3. Soil microbial parameters

Soil basal respiration (R_B: an indicator of soil microbial activity) and substrate-induced respiration (SIR: an indicator of potentially active microbial biomass) were determined following ISO 16072 Norm (2002) and ISO 17155 Norm (2002), respectively. For basal respiration, the CO_2 released by soil samples incubated in airtight jars for 3 days at 30 °C was trapped in vials containing 0.2 N NaOH and titrated with 0.1 N HCl. Substrate-induced respiration was determined by adding 10,000 mg C (as glucose) kg^{-1} DW soil to soil samples and then measuring CO_2 evolution after 6 h of incubation as abovementioned for basal respiration. Substrate-induced respiration, developed to measure the response of the 'metabolically active' component of the soil microbial community (Anderson and Domsch, 1985), reflects the size of the potentially active microbial biomass since it evaluates the maximum potential respiratory activity, not the actual activity (Schomberg and Steiner, 1997). Microbial biomass C (C_{mic}: an indicator of the overall size of the soil microbial community) was quantified by the fumigation-incubation method (Vance et al., 1987) using a conversion factor of 2.64 (Voroney et al., 1991). From these three parameters (basal respiration, substrate-induced respiration, microbial biomass C), ecophysiological indices which reflect environmental stress in soil microbial populations and communities (Anderson and Domsch, 1985) and, concomitantly, soil health were calculated, i.e., microbial metabolic quotient (qCO_2), or the ratio of basal respiration to microbial biomass C ($qCO_2 = R_B/C_{mic}$) and respiratory quotient Q_R, or the ratio of basal respiration to substrate-induced respiration ($Q_R = R_B/SIR$).

Urease activity was determined according to Kandeler and Gerber (1988) as described in Rodríguez-Loinaz et al. (2008). Arylsulfatase, β-glucosidase, alkaline phosphatase and dehydrogenase activities were determined according to Dick (1997) and Taylor et al. (2002) as described in Epelde et al. (2008) and Rodríguez-Loinaz et al. (2008). For the study of nitrifying communities, nitrate ($N{-}NO_3^-$) and ammonium ($N{-}NH_4^+$) concentrations were determined following Sparks et al. (1996).

Functional community profiling was obtained with Biolog Ecoplates™ according to Epelde et al. (2008). Average well color development (AWCD) was determined by calculating the mean of every well's absorbance value after 48 h of incubation, which corresponded to the time of maximal microbial growth in the Biolog EcoPlates™. The number of utilized substrates (i.e., the number of substrates with an absorbance value >0.25; this value marked the beginning of the exponential phase in the Biolog EcoPlates™), equivalent to species richness, S, was calculated at 48 h incubation time. Shannon's diversity index ($H' = -\sum p_i \log_2 p_i$), where p_i is the ratio of the absorbance of a particular well to the sum of absorbances of all microplate wells, was calculated, considering absorbance values at each well as equivalent to species abundance.

2.4. Tebuconazole concentration

Tebuconazole concentration in soil was determined using a validated analytical method based on an ultrasonic assisted extraction procedure according to EPA method 3550C (EPA, 2007) with subsequent analytical determination by GC–MS. Chromatographic analyses were carried out in an Agilent 6890N Gas Chromatograph interfaced to an Agilent 5973 Mass Selective Detector. The column used was a HP-5MS (30 m length × 0.25 mm i.d. × 0.25 μm film thickness). The carrier gas was helium at a flow of 1.6 mL min^{-1}. Temperature was applied as follows: 100 °C as initial temperature for 2 min, ramped at 20 °C min^{-1} to 150 °C, then raised at 30 °C min^{-1} to 250 °C and, finally, ramped at 20 °C min^{-1} to 290 °C (this temperature was kept for 5 min). The injection (2 μL) was achieved in splitless mode, setting the injector and ion source at 250 °C, the transfer temperature at 300 °C, and the detector voltage at 1200 V. Acquisition was made in time scheduled SIM mode.