Science of the Total Environment 599–600 (2017) 254–263

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of fungicide iprodione and nitrification inhibitor 3, 4-dimethylpyrazole phosphate on soil enzyme and bacterial properties

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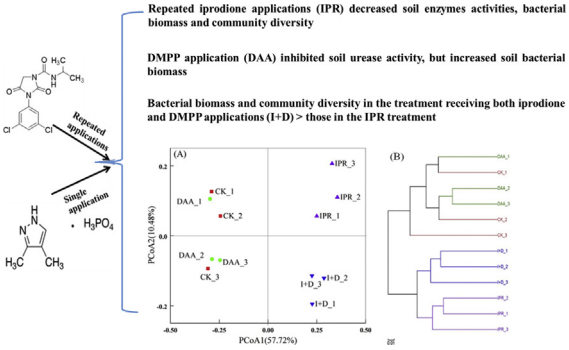
HIGHLIGHTS

- Iprodione applications decreased soil enzyme activities and bacterial biomass.
- Iprodione applications increased the relative abundance of *Proteobacteria*.
- DMPP application inhibited activities of urease, but increased bacterial biomass.
- Iprodione, alone or together with DMPP, changed bacterial community structure.

GRAPHICAL ABSTRACT



Repeated iprodione applications decreased soil enzyme activities, bacterial biomass and community diversity. DMPP application increased soil bacterial biomass, and relative to iprodione applications alone, extra DMPP application alleviated the toxic effects of iprodione applications on soil bacterial biomass and community diversity. Moreover, bacterial community structure was changed by repeated iprodione applications, alone or together with the DMPP.

ARTICLE INFO

Article history:
Received 8 January 2017
Received in revised form 1 May 2017
Accepted 1 May 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Agrochemicals
Soil enzyme
16S rRNA gene
Bacterial community diversity and structure

ABSTRACT

Agrochemical applications may have unintended detrimental effects on soil microorganisms and soil health. However, limited studies have been conducted to evaluate the effects of repeated fungicide applications and interactive effects of different agrochemical applications on soil microorganisms. In this study, an incubation experiment was established to evaluate the potential influences of the fungicide iprodione and the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) on soil enzyme activities and bacterial properties. Weekly iprodione applications decreased the activities of all enzymes tested, and DMPP application inhibited soil urease activity. Compared with the blank control, bacterial 16S rRNA gene abundance decreased following repeated iprodione applications, but increased after DMPP application. After 28 days of incubation, the treatment receiving both iprodione and DMPP application had higher bacterial 16S rRNA gene abundance and Shannon diversity index than the treatment with iprodione applications alone. Repeated iprodione applications significantly increased the relative abundance of *Proteobacteria*, but decreased the relative abundances of *Chloroflexi* and *Acidobacteria*. Simultaneously, bacterial community structure was changed by repeated iprodione applications, alone or together with DMPP. These results showed that repeated iprodione applications exerted negative effects on soil enzyme activities, bacterial biomass and community diversity. Moreover, relative to iprodione

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http://dx.doi.org/10.1016/j.scitotenv.2017.05.011

applications alone, additional DMPP application could alleviate the toxic effects of iprodione applications on bacterial biomass and community diversity.

1. Introduction

Fungicides play important roles in protecting crop quality and yield in modern agriculture (Maltby et al., 2009; Sabatier et al., 2014). The infections and phytopathies caused by fungi are the major problems and threats in agricultural production, which leads to the intensified fungicide applications in recent decades (O'Maille, 2015). Previous research has shown that in some developing countries, fungicide application rate was as high as 8 kg ha^{-1} y^{-1} (Liu et al., 2015). Iprodione, as a broad-spectrum fungicide, has been widely used in intensive agriculture to control the phytopathies of crops, and iprodione residues have already been detected in water (Goewie and Hogendoorn, 1985; Sauret et al., 2006), soil (Leistra and Matser, 2004) and farm products (Picó et al., 2004; Juan-García et al., 2005; Angioni et al., 2012). According to a report by U.S. Department of Agriculture (2014), the iprodione was the most frequently detected agrochemical in the imported fruit, and it was even detected in baby food.

Besides the crop phytopathies, low utilization efficiency of nitrogen (N) fertilizer and nitrous oxide (N_2O) emission are also worldwide problems in agricultural production (Clough et al., 2007; Menéndez et al., 2012). As a result, nitrification inhibitors are increasingly applied to reduce fertilizer N loss, and one the most widely used nitrification inhibitors in recent years is 3, 4-dimethylpyrazole phosphate (DMPP) (Menéndez et al., 2012; Florio et al., 2014).

Fungicides are designed to control fungal pathogens, but their lethal effects are not constrained to the fungi only (Muñoz-Leoz et al., 2013; Schnug et al., 2015; Fang et al., 2016). Once entering into agricultural soils, fungicides and their degradation metabolites may have detrimental effects on soil bacteria and, hence, the overall soil environment. There have been increasing research interests in the impacts of iprodione on environmental safety, because of its wide and repeated applications in agricultures (Leistra and Matser, 2004; Verdenelli et al., 2012; Morales et al., 2013). Previous studies generally focused on the impacts of a single iprodione application, whereas few studies paid attention to the effects of repeated iprodione applications which occur in intensively managed cropping systems. Furthermore, the iprodione and other agrochemicals (such as DMPP) may be applied into agriculture soils simultaneously. To the best of our knowledge, few studies have been conducted to evaluate the interactive effects of these different agrochemicals. Researches are, therefore, required to better understand the effects of combined iprodione and DMPP applications on soil enzyme and bacterial properties.

In this study, the iprodione and DMPP were applied into an agricultural soil. Soil enzyme activity, bacterial 16S rRNA gene abundance and community structure were determined. The objectives of this study were to (1) assess the effects of iprodione and DMPP applications on soil enzyme activities; (2) evaluate the impacts of these two agrochemicals on soil bacterial biomass (16S rRNA gene abundance); (3) reveal the responses of soil bacteria at different taxa to the agrochemical applications; and (4) compare the potential impacts of iprodione and DMPP applications on soil bacterial community structure. This study would improve our current understandings of the ecological risks of iprodione and DMPP applications, alone or together, to soil nutrient cycling and bacterial population.

2. Materials and methods

2.1. The agrochemicals and test soil

A commercial wettable powder formulation of iprodione (Bayer Crop Science, Hangzhou, China) and a chemical reagent DMPP (purity >97.0%; CIVI-CHEM, Shanghai, China) were used for soil treatments. Soil samples were taken from a vegetable farmland (36.78′ N, 118.67′ E) located in Shandong Province, China. The surface soils (0–20 cm) were collected, air-dried, mixed thoroughly and ground to pass through a 2 mm sieve. Selected physical and chemical properties of the soil were as follows: sand (50–2000 μm), 31.4 ± 1.4%; silt (2–50 μm), 36.9 ± 0.8%; clay (< 2 μm), 31.7 ± 0.6%; soil pH (in water), 7.19 ± 0.05; organic carbon (C) content, 10.0 ± 0.1 g kg^{-1}; total N content, 0.93 ± 0.01 g kg^{-1}; Olsen-P, 28.8 ± 0.2 mg kg^{-1}; NH_4OAc-K, 69.9 ± 1.5 mg kg^{-1}; and cationic exchange capacity, 16.9 ± 0.4 cmol kg^{-1}. All treatments were added with urea at 200 mg N kg^{-1} dry soil before the iprodione or DMPP application so that enough substrate (NH_4^+-N) was available for soil nitrification.

2.2. Experimental design

Four treatments were used in this study: Treatment 1 (CK), without any iprodione or DMPP applications; Treatment 2 (IPR), weekly iprodione applications and each application at 1.5 mg kg^{-1} dry soil (the frequency followed the instruction); Treatment 3 (DAA), nitrification inhibitor DMPP application at 2 mg kg^{-1} dry soil (equivalent to 1% of applied urea-N) at commencement; and Treatment 4 (I + D), weekly iprodione and initial DMPP applications as described in the Treatments 2 and 3. Each treatment was prepared in triplicates. The iprodione and DMPP were dissolved in double distilled H_2O (ddH_2O) and then applied into the test soils. Sixty glass jars (4 treatments × 5 sampling time × 3 replications) were filled with the treated soils at 150 g dry weight equivalent per bottle. Soil water content was adjusted to 60% water-holding capacity and was maintained with ddH_2O addition. The treated soils were then incubated at 28 °C in darkness. After 0, 7, 14, 21 and 28 days of incubation, three jars per treatment were sampled for the analyses of soil enzyme and bacterial properties.

2.3. Determinations of soil enzyme activities

Soil β-glucosidase activity was determined using a soil enzyme assay kit (Catalogue No. HK000218, Toyongbio Company, Shanghai, China), and the procedure followed manufacturer's protocol. The soil samples were treated with toluene and then incubated with the *p*-nitrophenyl-β-d-glucoside and citrate-phosphate buffer (pH = 6.0) for 1 h at 37 °C. Concentrations of the reaction product (p-nitrophenol) were determined with a spectrophotometer at 410 nm, and the β-glucosidase activity was expressed as μg p-nitrophenol g^{-1} dry soil d^{-1}. Potential urease, acid phosphatase and alkaline phosphatase activities were determined with the commercially available quantitative analytical kits supplied by the Jiancheng Bioengineering Institute (Nanjing, China). Soil samples were previously treated with the toluene to avoid microbial proliferation during enzyme assays. Urease activity assay (Catalogue No. T017) consisted of a 24 h incubation of test soil at 37 °C in the presence of urea (100 g L^{-1}) and citrate buffer (pH = 6.7). The formed NH_4^+-N was quantified by the indophenol blue method, and the urease activity was expressed as μg NH_4^+-N g^{-1} dry soil d^{-1}. The determinations of soil acid and alkaline phosphatases followed the analytical kits (Catalogue No. T008 and T009). Hydrolyses of disodium phenyl phosphate were performed at pH = 5.0 (acetate buffer) and pH = 9.4 (borate buffer) for 24 h at 37 °C to determine the activities of acid and alkaline phosphatases, respectively. The formed phenol was determined at 660 nm, and the phosphate activity was expressed as μg phenol g^{-1} dry soil d^{-1}.