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Assessment of the impact of three pesticides on microbial dynamics and functions in a lab-to-field experimental approach

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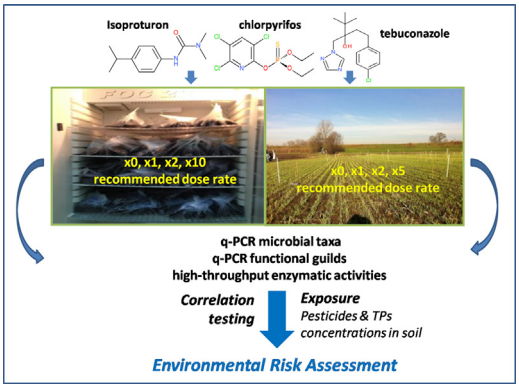
HIGHLIGHTS

- The soil microbial toxicity of CHL, IPU, TBZ and their TPs was determined in a lab-to-field assay.
- Pesticides did not induce extensive toxicity to the soil microbial community.
- Functional microbial groups were the most responsive toxicity endpoints.
- AOA and AOB were the most sensitive functional microbial group to pesticides.
- TPs of CHL and IPU induced negative effects on key soil microbial endpoints.

GRAPHICAL ABSTRACT



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ABSTRACT

The toxicity of pesticides on soil microorganisms is as an emerging area of concern. Novel and well-standardized tools could be now used to provide a robust assessment of the ecotoxicity of pesticides on soil microorganisms. We followed a tiered lab-to-field approach to assess the toxicity of three pesticides, widely used at EU level, (chlorpyrifos (CHL), isoproturon (IPU) and tebuconazole (TBZ)) on (i) the abundance of 11 microbial taxa and 8 functional microbial groups via q-PCR and (ii) the activity of enzymes involved in biogeochemical cycles via fluorometric analysis. Correlation of microbial measurements with the concentration of pesticides, and their transformation products (TPs) in soil enabled the identification of the compounds driving the effects observed. At lab tests (×1, ×2 and ×10 the recommended dose), CHL and TBZ significantly reduced the relative abundance of

Abbreviations: CHL, chlorpyrifos; IPU, isoproturon; TBZ, tebuconazole; TCP, 3,5,6-trichloro-2-pyridynol; MD-IPU, mono-demethylated isoproturon; DD-IPU, di-demethylated isoproturon; TPs, transformation products; AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea; ISO, International Standards Organization; AcP, acid phosphomonoesterase; AlkP, alkaline phosphomonoesterase; BisP, phosphodiesterase; PiroP, pyrophosphodiesterase; AryS, arylsulfatase; Chit, *N*-acetyl-b-D-glucosaminidase; Leu, leucine aminopeptidase; Bgluc, beta-glucosidase; SOB, sulfur-oxidizing bacteria; EFSA, European Food Safety Authority; MUF, 4-methyl-umbelliferyl; AMC, 7-amino-4-methyl coumarin.

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ammonia-oxidizing bacteria (AOB) and archaea (AOA) which recovered by the end of the study, while all pesticides induced a persistent reduction in the relative abundance of sulfur-oxidizing bacteria (SOB). The two demethylated metabolites of IPU (MD-IPU and DD-IPU) adversely affected P-cycling enzymes and leucine aminopeptidase (Leu). At field tests (×1, ×2 and ×5 the recommended dose), a persistent reduction on the relative abundance of AOA was induced by all pesticides, but only CHL and its hydrolysis product 3,5,6 trichloro-2-pyridynol (TCP) soil levels were negatively correlated with AOA relative abundance. Our findings suggest that ammonia-oxidizing microorganisms constitute the most responsive microbial group to pesticides and could be potential candidates for inclusion in pesticide risk assessment.

1. Introduction

Soil microorganisms constitute corner-stone organisms in ecosystem functioning (Falkowski et al., 2008). Adverse effects on soil microorganisms are expected to have a direct impact on environmental quality and human health at the global scale (Graham et al., 2016). Pesticides are considered key potential stressors of soil microorganisms (Imfeld and Vuilleumier, 2012). In response to this, the European Food Safety Authority (EFSA) identified soil microorganisms as a specific protection goal for pesticides environmental risk assessment (EFSA, 2010). Despite that the assessment of the toxicity of pesticides on soil microorganisms remains a controversial issue. This is because it is solely based on a simple N mineralization test (OECD, 2000) which fails to provide a robust assessment of the toxicity of pesticides on key soil microbial functions and on soil microbial diversity.

Recent methodological advances in soil microbial ecology enabled the in-depth analysis of the effects of pesticides on the abundance, function and diversity of the soil microbial community (Crouzet et al., 2010; Howell et al., 2014; Feld et al., 2015; Newman et al., 2016; Romdhane et al., 2016). Novel tools, which mostly focus on microbial dynamics like q-PCR determination of microbial abundance (ISO17601),and microbial function like high-throughput measurements of soil enzymatic activities (ISO/TS22939), are now standardized by the International Standards Organization (ISO) (Philippot et al., 2012; Karpouzas et al., 2016). This paves the way for their possible implementation in pesticide risk assessment. On the other hand, microbial diversity analysis tools, like high-throughput amplicon sequencing are still evolving, at both technological and data analysis level, although significant steps towards standardization have been performed by the Earth Microbiome Project (Thompson et al., 2017). Martin-Laurent et al. (2013) first proposed the use of a tiered experimental approach where the impact of pesticides on soil microorganisms is assessed at lab scale, under high exposure conditions, and at field scale under more realistic exposure scenarios, using novel and well-standardized tools. Implementation of this approach allowed an in-depth analysis of the impact of nicosulfuron on the soil microbial community (Karpouzas et al., 2014).

Chlorpyrifos (CHL), isoproturon (IPU) and tebuconazole (TBZ) are three pesticides heavily used in Europe in various crops (EFSA, 2011, 2014, 2015). Previous studies have addressed the impact of CHL (Singh et al., 2002; Supreeth et al., 2016), and TBZ (Cycon et al., 2006; Bending et al., 2007; Muñoz-Leoz et al., 2011; Wang et al., 2016) on the soil microbial community. Whereas only a few studies have assessed the toxicity of IPU on soil microorganisms and most of them were performed in the 90s using culture-dependent approaches (Harden et al., 1993; Kuriyal and Pandey, 1994). The overwhelming majority of these studies were performed at lab scale level, with the use of not standardized methods and in several cases at not agriculturally relevant exposure levels (×100 the recommended dose rate (Medo et al., 2015) or nominal soil concentrations of up to 100 and 500 mg kg^{-1} (Muñoz-Leoz et al., 2011; Wang et al., 2016)). Such experimental setups could lead to misleading interpretations.

Most of the studies investigating the toxicity of pesticides on soil microorganisms do not consider the role of transformation products (TPs) on the effects observed. It is now well documented that pesticide TPs can occasionally exert higher toxicity to non-target organisms than the parent compound (Pappola et al., 2014; Wu et al., 2014). In such a case, Papadopoulou et al. (2016b) showed that quinone imine, an oxidation product of the antioxidant ethoxyquin in soil, was responsible for the toxicity imposed to ammonia-oxidizing microorganisms. To this end, appropriate experimental and statistical approaches using both pesticide dissipation/metabolism and microbial endpoint measurements could identify the compounds that are responsible for the effects observed.

The aim of the present study was to assess the soil microbial ecotoxicity of three pesticides, CHL, IPU and TBZ, following a tiered lab-to-field experimental approach. These pesticides cover the three major pesticide classes (insecticide, herbicide and fungicide) and they were selected based on their high use at EU level and the lack of in-depth knowledge about their effects on soil microorganisms. The effects of pesticides were determined with the use of standardized biochemical and molecular methods measuring (a) the activity of soil microbial enzymes with high-throughput fluorometric tools, (b) the abundance of major microbial taxa and (c) the abundance of key functional microbial groups involved in nutrient cycling with q-PCR. Correlation testing between microbial measurements and pesticide soil dissipation and metabolism data derived from the lab and field tests (reported by Papadopoulou et al., 2016a) pointed to the compound, parent or TPs, driving the effects observed.

2. Materials and methods

2.1. Experimental setup

2.1.1. Laboratory study

For the laboratory study, soil samples were collected from an agricultural field in North Italy (45°05′20.8″N 9°45′59.4″E, Google Maps). The field was selected based on the lack of previous record of treatment with the studied pesticides (for at least the last five years) and its cultivation with cereals, where all three studied pesticides are registered for use at EU level. Soil texture analysis indicated that it is loamy sand (4.2% clay, 13.5% silt, 82.2% sand) with an organic carbon content of 1.5%, pH of 7.5 and a microbial biomass of 160.2 mg C kg^{-1} soil dwt. Topsoil samples (0–20 cm) were collected in July 2013 following the ISO10381-6 (2009) protocol for collection and handling of soil samples. The soil was homogenized, partially air-dried, sieved (2 mm) and stored at 4 °C for a week. Triplicate samples per pesticide-dose rate combination were treated with an appropriate volume of aqueous solutions of CHL, IPU or TBZ (prepared from their commercial formulations, approved at EU level, Carposan® (CHL) Sumitomo Chemical Italia; Quintil® (IPU) Phytorus S.A.; and Folicur® (TBZ), Bayer A.G.) aiming to pesticide dose rates of X1, X2 or X10 the recommended dose for agricultural use. These dose rates were selected to represent a high pesticide exposure scenario (Tier I) (Martin-Laurent et al., 2013). The application of the recommended dose of CHL, IPU and TBZ is expected to result in top-soil concentrations (0–10 cm) of 2.0, 1.9 and 0.6 mg kg^{-1} respectively. Triplicate samples were treated with water instead of pesticides to serve as untreated controls. The soil moisture content was adjusted to 40% of the water holding capacity and it was maintained throughout the