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A tiered assessment approach based on standardized methods to estimate the impact of nicosulfuron on the abundance and function of the soil microbial community

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ABSTRACT

Pesticides impact soil microorganisms in various ways. Despite the pivotal role of the latter in ecosystem functioning, the assessment of pesticides soil microbial toxicity is lagging behind the recent methodological advances in microbial ecology. We investigated the impact of nicosulfuron, a low dose sulfonylurea herbicide, on the structure, abundance and function of the soil microbial community using standardized methodologies (PLFAs, taxa-specific qPCR and enzyme activities). For this purpose a Tiered approach involving assessment i) at extreme, long term (five repeated application cycles) exposure schemes in a microcosm experiment conducted under greenhouse conditions (x0, x10, x100 and x1000 the recommended dose, Tier I) and ii) at realistic field exposure scenarios (x0, x1, x2 and x5, Tier II) was followed. Significant reductions in the abundance of Gram negative (β-proteobacteria, planctomycetes) and Gram positive bacteria (actinobacteria) were indicated by both PLFA and qPCR analyses at low soil concentrations of nicosulfuron (0.25–1 μg g^{-1}), while a reduction of fungi at equally low levels of nicosulfuron in soil was found only by qPCR analysis. C- and P-cycling enzymes were particularly sensitive even at low soil concentration of the herbicide (0–1 μg g^{-1}). In contrast, no inhibitory effects of nicosufluron at field conditions were found. The only exception was cellobiohydrolase which were impaired at herbicide rates higher than the recommended. We suggest that the use of a tiered microcosm-to-field experimentation combined with the application of standardized methodologies could provide a comprehensive assessment of the soil microbial toxicity of pesticides.

1. Introduction

Pesticides constitute an integral part of modern agriculture. Upon their release in the soil environment pesticides interact with soil microorganisms in various ways ranging from microbial stimulation, in cases where pesticides serve as energy or nutrient source, to inhibition, when the pesticide is toxic to microorganisms. Despite the key role of microorganisms for ecosystem services (Falkowski et al., 2008) the regulatory assessment of pesticides soil microbial toxicity is far from comprehensive. It currently relies solely on simple C and N mineralization tests (OECD 216, 217) which largely ignore effects on other key microbial functions and on microbial diversity. Based on the above weaknesses and in light of the recent methodological advances in soil microbial ecology, a radical revision of the relevant regulatory framework is needed (Martin-Laurent et al., 2013).

Several studies have looked into the effects of pesticides on the structure and function of soil microbial communities (Bending

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et al., 2007; Niemi et al., 2009; Crouzet et al., 2010). Recent papers have reviewed the relevant studies but overall no clear conclusions could be drawn (Imfeld and Vuilleumier, 2012). This is probably due to the lack of well-established experimental protocols and the use of not standardized methods. Regarding the first shortcoming, most of the studies have been conducted in soil microcosms spiked with pesticides at concentrations often much higher than the recommended representing solely a worst-case exposure scenario, while complementary field studies investigating the soil microbial toxicity of pesticides applied at a more realistic exposure scheme are generally scarce. Thus there is a need for establishment and testing of well-defined experimental protocols which will extend the soil microbial ecotoxicity assessment of pesticides from laboratory to field providing a comprehensive estimation of the toxicity of pesticides on soil microbes.

Standardization of the advanced methodologies available in soil microbial ecology is a necessary step towards harmonization of datasets and is a prerequisite for their integration in the regulatory framework of pesticide soil microbial toxicity assessment (Philippot et al., 2012). Standards for a number of methods have been already developed and others are under development at the International Standard Organisation (ISO) by TC190/SC4/WG4 and a list of those is presented in Table 1. Among those, PLFAs extracted from soil have been used as reliable biomarkers in studies looking at the effects of pesticides on the viable microbial biomass, community structure, nutritional and physiological status of soil microbes (Demoling et al., 2009; Ditterich et al., 2013). The impact of pesticides on the population dynamics of the most abundant microbial taxa like actinobacteria (C cycling; Goodfellow and Williams, 1983), planctomycetes (N and C1 cycling; Chistoserdova et al., 2004; Fuerst and Sagulenko, 2011) and α-, β- or γ-proteobacteria (transformation of organic pollutants and N cycling; Lorenzo, 2000; Liu et al., 2011) involved in key ecosystem services could be accurately determined by quantitative PCR using soil DNA as template (Fierer et al., 2005). Potential effects of pesticides on nutrient cycling have been extensively studied by measurement of the activity of key enzymes controlling rate-limiting steps in C, N, P and S cycling (Gianfreda and Rao, 2011). Up to now most studies dealing with pesticides soil microbial toxicity were performed using not well standardized methods, which did not allow their comparative meta-analysis, and focused on the independent assessment of effects on population, diversity or functional endpoints which did not provide a comprehensive view of the toxicity of the pesticide. The combined utilization of the above standardized molecular and biochemical methods which provide data of different resolution level guarantee an accurate estimation of pesticide-driven effects on soil microbes. Widenfalk et al. (2008) showed that the use of low resolution endpoints (H^3 leucine incorporation to assess microbial activity, and total PLFAs content to evaluate microbial biomass) overlooked the effects of pesticides on soil microbes. Effects were only identified when structural PLFAs analysis and T-RFLP fingerprinting were applied.

In the past decades, agrochemical companies have developed new pesticide classes which are characterized by selectivity, high potency and low application doses. Sulfonylureas constitute the most prominent representatives of modern pesticides encompassing all the desirable characteristics mentioned above (Sarmah and Sabadie, 2002). Nicosulfuron, a main member of this group, is used for post-emergence weed control in corn (Tapia et al., 1997). It acts by inhibiting acetohydroxyacid synthase (AHAS) which catalyzes the first common step in the biosynthesis of the branched-chain amino acids (Babczinski and Zelinski, 1991). The fact that AHASs are highly conserved and are also found in bacteria and fungi (Duggleby and Pang, 2000) increases the likelihood for direct impact of nicosulfuron on soil microbes. Little is known regarding the impact of sulfonylurea herbicides on soil microbes. The only available studies regarded the effect of bensulfuron-methyl (Allievi and Gigliotti, 2001; Saeki and Toyota, 2004) on soil microbes while little is known for nicosulfuron. In the only study available, Seghers et al. (2005) showed that nicosulfuron induced only transient effects on the community structure of soil methanotrophs. Thus all previous studies followed either a specific microbial function or a specific group of soil microorganisms, and there is a need for studies covering multiple functions and diversity of soil microbial communities after application of nicosulfuron under lab and field conditions.

Assessing the toxicity of pesticides on soil microbial communities is a pre-requisite to improve pesticide regulation in the near future. This should be based on multi-scale (lab-to-field) and well-defined experimental protocols and the implementation of advanced and at the same time well-standardized experimental protocols. Based on the identified knowledge gaps, this study aimed to establish and test a tiered microcosm-to-field experimental approach to assess the soil microbial toxicity of pesticides, using a low-dose herbicide like nicosulfuron as a target compound. We used a range of standardized methods having different resolution level to monitor the impact of nicosulfuron on the structure, abundance and function of the soil microbial community. This systematic and standardized approach could provide a multi-level, comprehensive assessment of the soil microbial toxicity of this herbicide.

Table 1
ISO standardized methods relevant to our study.

Year	Method	ISO reference
2010	Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates	ISO/TS 22939
2010	Determination of soil microbial diversity—part 1: method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis	ISO/TS 22843 part 1
2011	Determination of soil microbial diversity—part 2: method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	ISO/TS 22843 part 2
2012	Method to directly extract DNA from soil samples	ISO11063
2013	Estimation of abundance of selected microbial gene sequences by quantitative realtime PCR from DNA directly extracted from soil	ISO/DIS 17601[a]

[a] Accepted, under preparation.

2. Materials and methods

2.1. Experimental set up – microcosm experiment

A microcosm experiment conducted under controlled greenhouse conditions was employed to assess the impact of nicosulfuron on soil microbes under long-term and extreme exposure conditions. The soil used was obtained from the experimental farm of the Faculty of Agriculture of the University of Novi Sad (Serbia) where the field experiment described below was carried out. It was characterized as loamy clay (36.8% sand; 35.8% silt; 27.4% clay) with pH 6.75, organic carbon content 2.59%, total N 0.192%, P_2O_5 84 mg kg^{-1} and K_2O 273 mg kg^{-1}. Sieved soil (100 kg) was further homogenized thoroughly to eliminate possible heterogeneity between replicates and were distributed into 20 plastic microcosms (4.5 kg of soil dry weight per microcosm). The soil was watered to adjust moisture content to 70% of its water holding capacity and left to equilibrate for a week. The microcosms were subsequently seeded with maize (*Zea mays* variety NS640) and placed into a