Chemosphere 112 (2014) 194–202

Contents lists available at ScienceDirect

Chemosphere

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

Resistance and resilience responses of a range of soil eukaryote and bacterial taxa to fungicide application

Christopher C. Howell [a,*], Sally Hilton [a], Kirk T. Semple [b], Gary D. Bending [a]

[a] School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry, Warwickshire CV4 7AL, UK
[b] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

HIGHLIGHTS

- We studied the resistance and resilience of soil microbial communities.
- There was a significant concentration-dependent impact on dehydrogenase activity.
- Significant impacts on nematode and fungal communities were also observed.

ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form 3 February 2014
Accepted 2 March 2014

Handling Editor: A. Gies

Keywords:
Resistance
Resilience
Azoxystrobin
T-RFLP

ABSTRACT

The application of plant protection products has the potential to significantly affect soil microbial community structure and function. However, the extent to which soil microbial communities from different trophic levels exhibit resistance and resilience to such compounds remains poorly understood. The resistance and resilience responses of a range of microbial communities (bacteria, fungi, archaea, pseudomonads, and nematodes) to different concentrations of the strobilurin fungicide, azoxystrobin were studied. A significant concentration-dependent decrease, and subsequent recovery in soil dehydrogenase activity was recorded, but no significant impact on total microbial biomass was observed. Impacts on specific microbial communities were studied using small subunit (SSU) rRNA terminal restriction fragment length polymorphism (T-RFLP) profiling using soil DNA and RNA. The application of azoxystrobin significantly affected fungal and nematode community structure and diversity but had no impact on other communities. Community impacts were more pronounced in the RNA-derived T-RFLP profiles than in the DNA-derived profiles. qPCR confirmed that azoxystrobin application significantly reduced fungal, but not bacterial, SSU rRNA gene copy number. Azoxystrobin application reduced the prevalence of ascomycete fungi, but increased the relative abundance of zygomycetes. Azoxystrobin amendment also reduced the relative abundance of nematodes in the order Enoplia, but stimulated a large increase in the relative abundance of nematodes from the order Araeolaimida.

1. Introduction

Every community of living organisms is subjected to a range of stresses that can potentially deleteriously impact some or all of the species present, with the potential to affect community structure, function and/or diversity (Allison and Martiny, 2008). Such community responses can be considered in terms of resistance, which refers to the capacity of a community to maintain its size, composition, and function in the presence of a disturbance, and resilience, which describes the ability of an impacted community to recover its initial structure and function following a disturbance (Seybold et al., 1999; Allison and Martiny, 2008).

Giller et al. (1998) proposed two possible relationships between stress levels and microbial community diversity: an "extinction" relationship in which the diversity of a community is negatively correlated to an increase in stress levels, and a "competitive exclusion" relationship in which there is a hump-backed response to stress. In a hump-backed response, a mild stress would enhance the removal of dominant organisms, and promote an increase in diversity as other (normally less-abundant organisms) proliferate to fill the niche. However, there is limited experimental data to support these responses. It has been suggested that the resistance and/or resilience of soil communities to disturbances could be influenced by the initial biodiversity of a particular system. Girvan et al. (2005) observed greater resilience to benzene application in soil with a higher natural diversity, as demonstrated by a quicker recovery in the

* Corresponding author. Tel.: +44 2476 575145.
E-mail address: c.c.howell@warwick.ac.uk (C.C. Howell).

http://dx.doi.org/10.1016/j.chemosphere.2014.03.031
0045-6535/

mineralisation rate of 2,4 dichlorophenol (2,4-DCP), than in lower diversity soil.

Some previous research has indicated the presence of "competitive exclusion" diversity responses to some stresses e.g. copper or cadmium amendment (Degens et al., 2001; Zhang et al., 2009b, 2010). However, whether such relationships apply following the addition of organic chemicals such as pesticides remains unknown.

The effects of pesticides on non-target organisms and the wider environment as a whole have been a concern for many years due to the biologically active nature of the compounds (Bending et al., 2007). Such non-target effects may result from either the direct toxicity of the compound, or as indirect impacts caused by the removal and/or increased proliferation of other species. It is thought that microbial communities may have lower natural resistance and/or resilience to pesticide impacts than plants and other larger organisms (Allison and Martiny, 2008).

Indeed, previous research using a range of broad-scale and molecular methods has shown that pesticides can significantly alter microbial community structure in different environments (Engelen et al., 1998; El Fantroussi et al., 1999; Wang et al., 2008; Zhang et al., 2009a). However, such studies have primarily been limited to bacterial and fungal communities. In particular, there is a paucity of information available about the impacts of pesticides on higher trophic level soil microorganisms such as nematodes and protozoa. Such organisms are integral members of soil food webs as both predators and prey and their activities are beneficial to nutrient cycling within the soil (Mikola et al., 2002), with the potential to impact plant growth (Bonkowski, 2004). Culture-dependent methods have previously shown impacts of pesticides on non-target eukaryotic microorganisms in soils (Ekelund, 1999; Ekelund et al., 2000; Boucard et al., 2004). However, such studies are limited by the fact that only a small percentage of soil microorganisms are culturable (Janssen et al., 2002).

There has been limited use of culture-independent molecular methods to investigate the non-target effects of pesticides on eukaryotic soil microorganisms. Bending et al. (2007) showed that three fungicides each had specific effects on eukaryote communities, apparently reducing the abundance of specific taxa. However, these effects occurred in the absence of impacts on broad-scale measurements such as microbial biomass. Similarly, Adetutu et al. (2008) found that the fungicide azoxystrobin altered the structure of soil fungal communities with impacts still observed up to 84 d after application, by which time over 60% of the applied compound had been degraded.

The current study investigated the impacts of pesticide application on the resistance and resilience responses of soil microbial communities from different trophic levels (bacteria, fungi, archaea, pseudomonads, and nematodes) using the strobilurin fungicide azoxystrobin as a model compound.

The strobilurin group of fungicides represent one of the most important groups of pesticides currently in use worldwide for the control of fungal crop pathogens. In 1999, sales of strobilurins totalled US$620 million worldwide (Bartlett et al., 2002) and this had increased to US$1.636 billion by 2007 (Stanley Alliance Info-Tech, 2011). Their structures are based on those of natural products secreted by wood-degrading basidiomycete fungi such as *Oudemansiella mucida* and *Strobilurus tenacellus* and can be either fungicidal or fungistatic. Azoxystrobin acts by binding to the ubiquinone (Qo) site of cytochrome b which forms part of the cytochrome bc_1 complex in the fungal mitochondrial membrane. This binding disrupts the transfer of electrons from the cytochrome b portion of the complex, to the c_1 portion, which stops the mitochondria producing ATP for the cell (Bartlett et al., 2002). Despite their widespread use, little is known about the effects of azoxystrobin and other strobilurin compounds on soil microbial communities, particularly with reference to non-target organisms.

Soil biomass-N and dehydrogenase activity analyses were performed to give an indication of broad-scale impacts, whilst molecular methods were used to determine the impacts of azoxystrobin concentration on the structure and diversity of specific microbial groups from different trophic levels. HPLC analysis was used to monitor azoxystrobin degradation/dissipation over the course of the experiment.

2. Materials and methods

2.1. Soil collection and preparation

Soil was collected from Hunts Mill field at the Wellesbourne Campus of the University of Warwick School of Life Sciences, UK, during January 2008. The soil is a sandy loam of the Wick series with a composition of 73.4% sand, 12.3% silt, and 14.3% clay (Bending et al., 2007). The field had been managed as set-aside for over a decade and thus had received no pesticide applications. Soil was collected from the top 20 cm to comply with OECD guidelines for soil sampling in agricultural soils (OECD, 2011). Prior to azoxystrobin application, the soil was re-wetted to a matric potential of −33 kPa (Bending et al., 2007). This equated to a soil moisture content of 13.5%.

2.2. Azoxystrobin addition to soil

Azoxystrobin (Greyhound Chromatography, Birkenhead, UK) was dissolved in acetone and added to the soil at a solvent:soil ratio of 1:20 (Northcott and Jones, 2000), giving concentrations of 1, 5, 10 and 25 mg kg^{-1} soil, with 5 mg kg^{-1} representing the UK maximum recommended dose of azoxystrobin in the top cm of soil (Bending et al., 2007) and therefore the maximum dose which could reach the soil either directly, such as from spraying prior to canopy closure, or indirectly, following residue wash-off from the canopy. A total of 2.4 kg of soil was required for each pesticide concentration. The azoxystrobin solution was initially applied to one quarter of the soil and mixed with a sterile stainless steel spoon. The soil was then stored at room temperature in a fume hood for 2 h to allow evaporation of the acetone. The remaining three quarters were then mixed in gradually over a 10 min period to ensure an even distribution of the compound throughout the soil (Doick et al., 2003). Control soils were amended in the same way as the treated soils, but without azoxystrobin. 120 g Portions were then transferred to sterile 250 mL glass Duran bottles, wrapped in aluminium foil and stored at 15 °C in the dark. 4 Replicates of each treatment were destructively sampled at time 0, and then on a monthly basis for 4 months.

2.3. Effects on broad-scale microbial properties

Soil biomass-N was measured using the $CHCl_3$ fumigation method of Joergensen and Brookes (1990). Obtained ninhydrin-N values were converted to biomass-N using a conversion factor of 3.1 (Amato and Ladd, 1988). Dehydrogenase activity was monitored as detailed by Tabatabai (1994).

2.4. Azoxystrobin extraction and analysis

10 g of azoxystrobin-amended soil was added to 50 mL centrifuge tubes and mixed with 20 mL of HPLC-grade acetonitrile (Fisher Scientific, UK). The tubes were shaken by hand and placed on a shaker for 1 h. Following shaking, the samples were left for 30 min to settle and then centrifuged at 4000 rpm for 2 min. 2 mL Of the supernatant was transferred into a 2 mL screw-top glass HPLC vial (Chromacol Ltd., UK). Samples were analysed using