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Does altered rainfall regime change pesticide effects in soil? A terrestrial model ecosystem study from Mediterranean Portugal on the effects of pyrimethanil to soil microbial communities under extremes in rainfall

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ABSTRACT

In this century, agroecosystems are subjected to multiple global change stressors acting in concert such as alterations in rainfall regimes and pesticide use. Alterations in rainfall regimes, characterised by more extreme intra-annual rainfall regimes, have been forecasted for the Mediterranean region. At the same time, the use of pesticides continues to rise. Here, we report the responses of soil microbial community to a model pesticide, i.e., fungicide pyrimethanil (PYR) under altered rainfall regimes (i.e., drought and heavy rainfall) two and eight weeks after PYR application. We measured the functional responses as enzyme activities, potential nitrification and BIOLOG carbon substrate utilisation. We also characterised the soil bacterial communities using polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) method. After two weeks, enzyme activities were mainly responsive to PYR and kinetic parameters, calculated from BIOLOG carbon substrate utilisation, indicated interaction effects from PYR and rain treatments. Bacterial band richness increased with PYR treatment under normal rain and drought regimes, but bacterial band richness was higher at 1X than 5X PYR under heavy rainfall. Bacterial community structure was also different with the PYR and rainfall treatments. By week eight, PYR treated soils remained functionally different from untreated soils. Bacterial band richness was consistent across PYR treatment regardless of rain regime. However, the bacterial community structure remained significantly different among the PYR treatments under different rain regimes. We conclude that rainfall extremes can alter the effect of PYR on the soil microbial community structure without altering PYR effects on soil functions (measured as enzyme activities, potential nitrification and BIOLOG carbon substrate utilisation).

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1. Introduction

Under the current scenarios of climate change, extreme rainfall regimes are expected to increase in number and intensity. The Intergovernmental Panel on Climate Change (IPCC) suggested that the magnitude and variability in drought and heavy rainfall events will increase in the Mediterranean region (Alcamo et al., 2007). Increase in such extreme rainfall regimes will amplify fluctuations in soil water content spatially and temporally with consequences for resource availability, and may cascade onto the biota over time (Knapp et al., 2008; Weltzin et al., 2003).

Soil microbial community living in the shallow soil layer will experience and respond rapidly to these fluctuations in its environment (Borken and Matzner, 2009; Clark et al., 2009; Insam, 1990). As soil microorganisms contribute to key soil functions including carbon and nutrient cycling, alteration in soil microbial community structure and function may further exacerbate climate change (Knapp et al., 2008; Singh et al., 2010).

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Pesticide use is one of the three main agricultural practices that impose major disturbances to soil, alongside land use change and erosion (Dale and Polasky, 2007; Lavelle et al., 2004). Soil microbial community response to pesticide are highly variable. The respond can be positive, negative and neutral manner depending on the level of biological organisation examined, e.g., species to populations to communities (Chowdhury et al., 2008; Gianfreda and Rao, 2008; Ng, 2010). This is due to several interacting factors – physicochemical characteristics of the pesticide, soil properties, climatic conditions and prevailing soil community (Fig. 1). For example, species level response ranges from mortality to utilisation of the pesticide, which at ecosystem level could translate to diminished diversity or changes in soil community composition.

This study aims to elucidate the influence of altered rainfall regimes (i.e., drought and heavy rainfall) on pesticide effects to soil microorganisms using pyrimethanil as a model fungicide. This is part of a wider study using terrestrial model ecosystem (TME) to examine soil biota (including microbial community, nematodes, enchytraeids, arthropods and earthworms) responses to pyrimethanil under global environmental change. We hypothesised that the effects of the pesticide on soil microbial community change due to its direct and/or indirect interactions with the rainfall regimes (Fig. 1).

2. Materials and methods

2.1. Soil sampling and experimental design

Intact soil cores (TMEs – 16.5 cm diameter × 40 cm depth) were collected from a field site that belongs to the Coimbra Agricultural School (40°21′N, 8°45′W; Table 1 for soil characterization). The site has been kept under biological production with corn, beans, yellow lupin, faba beans and triticale since 2004. As such, it has not received prior pyrimethanil (PYR) application. At the time of soil extraction on February 19, 2010, the site was sowed with yellow lupin as cover crop. The cores were maintained in temperature-regulated carts located in a greenhouse (see Knacker et al., 2004 for details of TME approach). The methods of soil preparation and TME extraction were similar to those described in Sousa et al. (2004).

We used a fully factorial design with three PYR application rates and three rain regimes. Site-specific rainfall was obtained from the Institute of Meteorology of Portugal. Average rainfall in the area is 2.5 mm/day (denominated as normal rain and used as control rain regime (C)) based on average over 1971–2000. Drought (D) and heavy rainfall (F) regimes were designed based on a drought occurring in 2005, which was one of the worst in 140 years in Portugal (0.3 mm/day over May–Aug 2005) and abnormally high rainfall in 2006 (8.25 mm/day over Oct–Nov 2006). Rain was simulated using artificial rainwater modified after Velthorst (1993). The different rain regimes were maintained throughout the eight-week experiment. Prior to the start of the experiment, all TMEs were acclimated and equilibrated for three weeks by watering all cores with the same amount of rainwater as the normal rainfall. After this period, vegetation was trimmed to 2 cm height. Plant biomass was collected and weighed (fresh and dry). The fungicide pyrimethanil (CAS no. 53112-28-0, log K_{OW} = 2.84, water solubility at 20 °C = 210 mg/L, DT50 field = 23–54 d, DT50 lab 20 °C = 27–82 d) was applied as the commercial formulation Scala at 0.0021 g/TME and 0.0105 g/TME corresponding to the recommended dose of 1 kg a.i./ha (1X PYR) and 5-fold the recommended dose (5X PYR), respectively. One third of TMEs did not receive pesticide an acted as control (0X PYR). All cores received the same volume of 50 ml test solution (water in the case of controls) followed by 50 ml rainwater to incorporate the pesticide. The differentiated rain treatment started the following day. Each core was randomly assigned to a treatment, a combination of PYR dose (0X PYR, 1X PYR and 5X PYR) and rain regime (C, D and F). Each treatment had four replicates which were destructively sampled at two and eight weeks after the application of PYR. Soil samples were collected from the upper 10 cm. Stones and larger roots were removed and the soil samples were homogenised by hand. All samples were stored at 4 °C until analysis for microbial analysis and at −20 °C for chemical analysis.

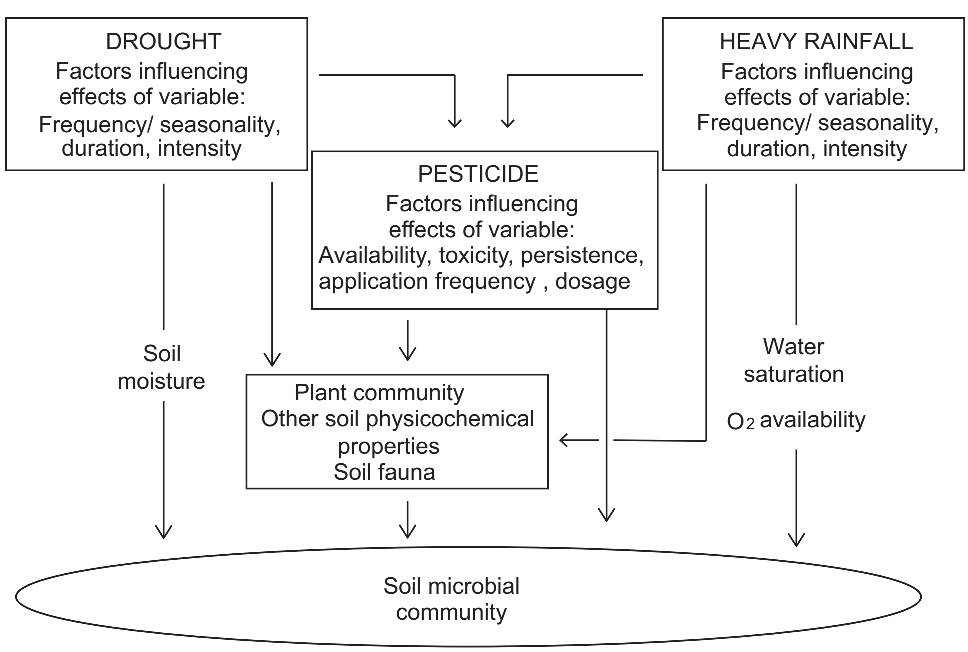

Fig. 1. Conceptual relationship between climate extremes and pesticides with microbial communities. Drought and heavy rainfall directly affects soil microbial community through changes in soil water content and O_2 availability. Pesticides directly affect microbial community, for example through mortality or changes in resource supply. Drought and heavy rainfall indirectly affects pesticide availability and persistence. All three factors can also indirectly affect soil microbial community through their impact on plant community, soil physicochemical properties and soil fauna.

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