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No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration

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ABSTRACT

Non-target effects of pesticides are widespread and potentially modify essential ecosystem processes. Since microorganisms are major soil decomposers driving key processes such as organic matter decomposition, nutrient cycling and, thereby, plant productivity, effects of pesticides on soil microorganisms need to be considered. In the context of the biodiversity–ecosystem functioning debate, the question arises if pesticide effects are modulated by the diversity of the plant community. We applied pesticides (dimethoate, chlorpyrifos and fosthiazate) to plots of the Jena Experiment field site differing in plant diversity, and measured basal respiration, biomass and specific respiration of soil microorganisms. Pesticide applications either increased (chlorpyrifos and fosthiazate) or decreased (dimethoate) soil microbial parameters, but the effects neither varied with plant species nor with plant functional group richness.

1. Introduction

Microorganisms are the primary soil decomposers driving key ecosystem processes such as organic matter decomposition, nutrient cycling and, thereby, plant productivity (Paul and Clark, 1989; Pandey and Singh, 2004; Devare et al., 2007). Thus, agricultural practices affecting soil microorganisms are of particular interest. Modern agriculture worldwide uses a variety of pesticides including insecticides, nematicides, herbicides and fungicides to optimize crop production (Das and Mukherjee, 2000; López et al., 2002; Cycon et al., 2006). However, continuous application of pesticides may result in soil pollution threatening processes driven by soil microorganisms and, thereby, affecting soil fertility (Sturz and Kimpinski, 1999; López et al., 2002; Cycon et al., 2006). Studies investigating effects of pesticides on soil microorganisms reported conflicting results (Martikainen et al., 1998; Das and Mukherjee, 2000; López et al., 2002; Pandey and Singh, 2004; Cycon et al., 2006; Devare et al., 2007). Non-target effects of pesticides on soil microorganisms were shown to depend on soil abiotic factors (Beulke and Malkomes, 2001; Monkiedje and Spiteller, 2002), however, interacting effects between pesticides and biotic factors received little attention. One of the most prominent biotic factors governing ecosystem functioning and stability of ecosystem processes is plant diversity (Naeem et al., 1999; Tilman, 1999; Loreau et al., 2001). Several previous studies highlighted a positive biodiversity–ecosystem stability relationship by showing that resilience after disturbances (Griffiths et al., 2000; Mulder et al., 2001), resistance against invasions (Hector et al., 2001; Fargione and Tilman, 2005; Eisenhauer et al., 2008; Roscher et al., 2008) and spatial stability in aboveground biomass production (Weigelt et al., 2008) increase with diversity. Thus, in the context of the biodiversity–ecosystem functioning debate, the question arises if microorganisms are affected by pesticides and if these effects are modulated by the diversity of the plant community. We used the framework of the Jena Experiment to study the effects of three widely used pesticides (the insecticides dimethoate and chlorpyrifos and the nematicide fosthiazate) on soil microorganisms in plant communities varying in plant species and plant functional group richness. The response of soil microorganisms was investigated by analyzing microbial respiration, biomass and specific respiration. We hypothesized that effects of insecticide and nematicide applications on soil microorganisms, if they exist, are attenuated by plant diversity.

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Table 1
The design of the Jena Experiment. Combinations of plant species richness and plant functional group richness levels and the number of replicates per diversity level (given in italics). Soil samples from insecticide subplots were taken on all experimental plots (n = 82). Soil samples from nematicide subplots were taken on plots with 1, 4 and 16 plant species (n = 46; given in bold). For more details on the experimental design see Roscher et al. (2004).

Plant functional group richness	Plant species richness						Replicates
	1	2	**4**	8	**16**	60	
1	**16**	*8*	**4**	*4*	**2**	–	34
2	–	*8*	**4**	*4*	**4**	–	20
3	–	–	**4**	*4*	**4**	–	12
4	–	–	**4**	*4*	**4**	*4*	16
Replicates	**16**	*16*	**16**	*16*	**14**	*4*	82 plots

2. Materials and methods

2.1. Experimental setup

The study was carried out on the field site of the Jena Experiment investigating the role of biodiversity in element cycling and trophic interactions in grassland communities (Roscher et al., 2004). The study site is located on the floodplain of the Saale river at the northern edge of Jena (Thuringia, Germany); the soil is characterised as Eutric Fluvisol (FOA-Unesco, 1997). In May 2002, a pool of 60 native plant species was used to establish a gradient of plant species richness (1, 2, 4, 8, 16 and 60) and plant functional group richness (1, 2, 3 and 4) in a total of 82 plots of 20 m × 20 m (Table 1). Plant species were aggregated into four plant functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species) by using (1) above- and belowground morphological traits, (2) phenological traits, and (3) the ability to fix N_2 as attribute classes. Plots were arranged in four blocks following a gradient in soil characteristics (Roscher et al., 2004). Experimental plots were mown twice a year (June and September), as is typical for hay meadows, and weeded twice a year (April and July) to maintain the target species composition.

2.2. Pesticide treatments

Starting in April 2003, project-specific subplots (2 m × 4 m) were established on all plots to manipulate herbivore (dimethoate), collembolan (chlorpyrifos), herbivore and collembolan (dimethoate and chlorpyrifos), and nematode densities (fosthiazate) by applying pesticides (Fig. 1). Dimethoate subplots were sprayed with an aqueous solution of the organothiophosphate insecticide dimethoate (30 ml m^{-2}; BASF, Ludwigshafen, Germany) at 4-week intervals between April and August using a backpack sprayer (Birchmeier Senior 20 l; operating pressure 6×10^5 Pa). Chlorpyrifos subplots were sprayed monthly from April to November with an aqueous solution of the organothiophosphate insecticide chlorpyrifos (2%, w/w; 40 g in 1 l water, 125 ml m^{-2}; Celaflor, Dow AgroSciences LCC, USA) using a backpack sprayer (Birchmeier Senior 20 l; operating pressure 2×10^5 Pa). Starting in April 2005, the nematicide fosthiazate (Syngenta Agro GmbH, Maintal, Germany) was applied to nematode subplots (1 m × 1 m) as granules three times a year using a sieve (3 g m^{-2} mixed with 97 g Jena soil). The control subplots received 100 g Jena soil per application to reduce effects of nutrient addition. The amount of dimethoate and chlorpyrifos was lower than the suppliers' recommendations and the amount of fosthiazate applied is commonly used in agricultural systems to reduce nematode pest species.

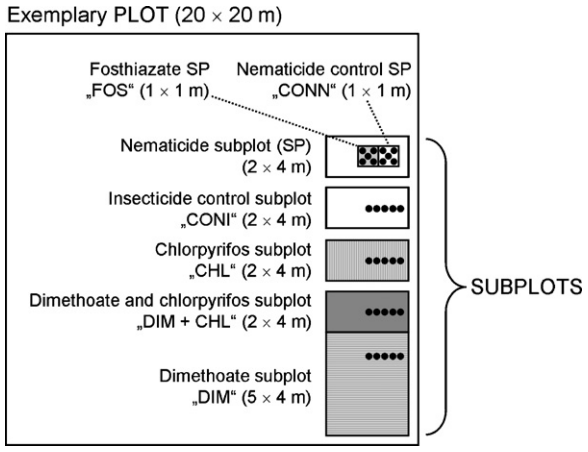

Fig. 1. Scheme of an exemplary experimental plot with project-specific subplots. The location of subplots within a plot was randomized. Black dots indicate soil samples taken on each insecticide (CONI, DIM, CHL and DIM + CHL) and nematicide subplot (CONN and FOS).

2.3. Sampling and measurements

Soil samples from insecticide subplots (insecticide control, dimethoate, chlorpyrifos, and dimethoate and chlorpyrifos; Fig. 1) were taken in May 2006 on all experimental plots (n = 82; Table 1). Soil samples from nematicide subplots (nematicide control, fosthiazate; Fig. 1) were taken in May 2007 on the 1, 4 and 16 plant species richness plots (n = 46; Table 1). Since application duration before measurements and sampling times differed between insecticides (2003–2006) and nematicide (2005–2007), these pesticide treatments are not comparable and were analyzed with separate statistical models (see Section 2.4). At each sampling, five soil samples were taken per subplot (including control subplots) using a metal corer (inner diameter 5 cm) to a depth of 5 cm, pooled and stored at 5 °C. Before measurement soil samples were homogenized and sieved (2 mm) to remove macrofauna, roots and stones. In insecticide samples the gravimetric soil water content was determined and used as covariate in the statistical analysis. Nematicide samples were adjusted to a gravimetric soil water content of 25% before measurement.

Basal respiration (BR) and microbial biomass (C_{mic}) were measured using an O_2-microcompensation apparatus (Scheu, 1992). The microbial respiratory response was measured at hourly intervals for 24 h at 22 °C. BR (μl O_2 h^{-1} g^{-1} soil dry weight) was determined without addition of substrate and measured as mean of the O_2 consumption rates of hours 12–24 after the start of the measurements. Substrate induced respiration was calculated from the respiratory response to D-glucose (Anderson and Domsch, 1978). Glucose was added in appropriate amounts (according to preliminary studies) to saturate the catabolic enzymes of the microorganisms (4 mg g^{-1} dw dissolved in 400 μl deionized water). The mean of the lowest three readings within the first 10 h was taken as maximum initial respiratory response (MIRR; μl O_2 h^{-1} g^{-1} soil dw) and C_{mic} (μg C_{mic} g^{-1} soil dw) was calculated as 38 × MIRR (Beck et al., 1997). The specific respiration (qO_2; μl O_2 mg C_{mic}^{-1} h^{-1}) was calculated as a measure of the metabolic efficiency of the microbial community by dividing BR by C_{mic}.

2.4. Statistical analyses

All datasets were log-transformed to meet requirements of ANOVA (normality and heteroscedasticity of errors). Split plot ANCOVA as part of the general linear models (GLM, type I sum of squares) was used to analyze effects of gravimetric soil water content (H_2O; for insecticide samples only), block (BL), plant species richness (S), plant functional group richness (FG), presence/absence of grasses (GR), small herbs (SH), tall herbs (TH) and