Adaptation strategies of soil biodiversity (earthworms) to pesticides: physiological mechanisms and soil ecological implications

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Introduction

In agricultural landscapes, soil biodiversity amongst them earthworm communities have to face intensive land-use such as ploughing and tillage, and application of chemical pesticides and fertilizers (Paoletti 1999). Intensive land use has been shown to dramatically reduce abundance and diversity of earthworm communities (Smith et al. 2008). Assuming that immigration rate is low, this suggests that earthworm populations living in pesticide-polluted soils are subject to a chronic chemical stress that could lead to tolerance via physiological acclimatisation or genetic adaptation.

In this study, we focused on two populations of the common endogeic earthworm \textit{Aporrectodea caliginosa} originating from two different agricultural backgrounds: a conventionally cropped agricultural field (with frequent application of herbicides, fungicides, and insecticides) and a field cropped according to organic agriculture requirements since at least 20 years. The available agricultural historical background of the conventional field (10 years) revealed the fungicide epoxiconazole as one of the most frequently applied and most persistent molecules, hence we used it as our model contaminant. We compared sub-lethal effects in both, pre-exposed and naïve populations of \textit{A. caliginosa} after 7 and 28 days of exposure to the Predicted Environmental Concentration (PEC) of epoxiconazole. We tested the hypothesis that pre-exposed earthworms inhabiting soil of conventional cropped fields have acquired tolerance to face pesticides. An adaptive mechanism was quantified in terms of the main energy resources storage (glycogen, lipids and soluble proteins) and of the metabolism (respiration and metabolites levels). If these physiological challenges lead to differences in their soil bioturbation ability, was measured as cast production.

Material and methods

Earthworms were exposed for 7 and 28 days to the commercial formulation OPUS\textsuperscript{®} at a concentration of 0.1 mg of the active ingredient epoxiconazole per kg soil in polycarbonate boxes filled with 100 g of soil originating from a long-term organic pasture moistened to 25 % H\textsubscript{2}O. Control groups consisted of worms reared in unpolluted soil in the same conditions. Metabolic and energetic endpoints were measured (energy resources as total glycogen, lipids and soluble proteins; photometric), respiration rate (CO\textsubscript{2} production, micro GC), metabolomics (22 metabolites, GC-MS) as well as cast production (burrowing activity).

Results

Epoxiconazole levels in soil decreased slowly over the course of the experiment and were still 66% of the initial day 0 value after 28 days, with more pronounced decrease in the soil containing the pre-exposed population. Fungicide treatment increased CO\textsubscript{2} production in both populations after 7 and 28 days. Glycogen content was decreased after 7 days in the naïve earthworms but only after 28 days in the pre-exposed earthworms, which also had a higher soluble protein content. Metabolite profile of the pre-exposed group at 28 days was
very separated from the control group according to a Principal Component Analysis (Figure 1). In the naïve earthworms, exposed and control groups were not well separated.

![Figure 1](image1)

**Figure 1** Scores plots (Principal Component Analysis) performed on the whole metabolomic dataset on the two populations showing the relationship between metabolite profiles and exposure. Empty symbols, dashed lines = control groups, Full symbols, full lines = exposed groups. Crosses are mean scores ± standard error of the mean (SEM).

Cast production was significantly increased in fungicide-treated group compared to control after 7 days in the pre-exposed worms, and decreased in both populations after 28 days, significantly in the naïve worms (Fig 2).

![Figure 2](image2)

**Figure 2** Mean cast production (in g dry cast weight g⁻¹ earthworm body mass day⁻¹) of pre-exposed and naïve Aporrectodea caliginosa after exposure to epoxiconazole for 7 and 28 days (n=8). * indicates significant differences between exposed and control groups (student-t-test, ° p<0.1, * p<0.05, ** p<0.01) and # between earthworm populations.

### Conclusion

The populations responded differently to epoxiconazole treatment, suggesting a possible tolerance mechanism acquired by pre-exposure on conventional fields. Pesticide tolerance could allow earthworms to maintain survival of the population at lower numbers and carry on their burrowing processes, however it seems that it is at the detriment of other physiological processes such as energy storage.

### References
