Evaluation of Impacts of 2,4-dichlorophenoxyacetic acid (2,4-D) on Microbial Community of an Agricultural Soil

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Introduction

The herbicide 2,4-D is used to control broad-leaved weeds in cereal crops and on pastures. Although 2,4-D is an analogue of natural auxins, it does not occur naturally in the environment. Despite this, 2,4-D is readily used as a carbon source by environmental microorganisms. It has been reported that 2,4-D can drive shifts in the microbial community of soils (Chinalia and Kilham, 2006; Macur et al., 2007). Although much is known about the physiological activity, mechanism of action, and environmental fate of this herbicide, information about its impact on biological properties in soils of Argentina is limited (Frioni, 1981; Merini et al., 2007).

Alternative approaches for monitoring the impacts of pollutants on microbial communities in functionally relevant terms are needed. The BD Oxygen Biosensor System™ (BD OBS) is a microplate with a fluorescent dye, which is quenched by the presence of O_2, enabling the rapid measurement of O_2 consumption by respiring microbes. It has been used for measuring the utilization of substrates and media amendments by whole communities in community-level physiological profiling (CLPP) studies as well as the toxicity responses of various microorganisms (Birmele et al, 2006).

Objectives

- To determine how short-term exposure to 2,4-D impacts the function and structure of the microbial community of an agricultural soil from the Pampas region, Argentina.
- To study the long-term effects of the herbicide by assessing the pollution-induced-community tolerance (PICT) in agricultural and pristine forest soils.

Materials and Methods

- The study area is located in the southern Pampas region, Argentina **(Fig. 1)**. The soil is a sandy loam (Typic Argiudoll). Samples from the upper layer (0-10 cm) were taken from a plot with reported history of 2,4-D applications.
- Soil microcosms and inoculum preparation **(Fig. 2)**
- CLPP assay: *C* sources→ acetate, casein, mannose, sucrose, succinate, coumaric acid and 2,4-D (50 mg l^{-1}), and no *C* supplementation. Half of the wells were added with fungal inhibitor cycloheximide (I) at a final concentration of 11 mg ml^{-1} to assess the relative substrate utilization of the overall microbial community and the bacterial component alone, in the C and L microcosms.
- Utilization of 2,4-D as a *C* source (50 mg ml^{-1}) in soils with L and H doses of 2,4-D (3 and 14 days after treatment).
- PICT assay: response to coumaric acid (50 mg ml^{-1}) in presence of increasing concentrations of 2,4-D (0- 50 mg l^{-1}). Soils sampled at the end (*C* and L only) were compared to a pristine, hardwood forest (F) soil (Harvard Forest, MA), in presence of five doses of herbicide (0- 250 mg l^{-1}).
- Incubation at 30°C and fluorescence readings (every 15 minutes for up to 48 h) on a Synergy HT microplate reader (BIO-TEK). The data were reported as normalized relative fluorescence units (NRFU). Various response parameters were calculated for comparison of treatments.

Fig 1. – a) Map of Argentina, b) study area

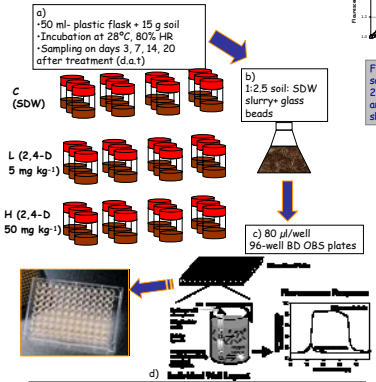

Fig.2.- a) Microcosms assay design, b) inoculum preparation, c) plate inoculation and d) configuration of the BD OBS system

Results

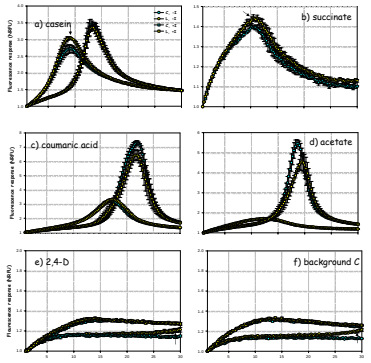

Fig.3.- Fluorescence response of agricultural (50 mg l^{-1}) soil to various *C* sources 7 d.a.t.- a) casein, b) succinate, c) coumaric acid, d) acetate, e) 2,4-D, and f) background *C*. Microcosms were previously exposed to 0 and 5 mg kg^{-1} 2,4-D (*C* and L). Error bars represent ±1 SE (n=3). Arrows show increased response in L microcosms.

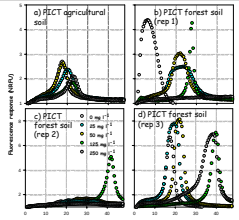

Fig.6.- Fluorescence response to coumaric acid in agricultural (a) and replicate samples of forest soils (b-d) exposed to increasing doses of 2,4-D (0-250 mg l^{-1}). The 12-h response, coincident with the onset of peak in response, was selected as the parameter to evaluate the PICT, after visual inspection of the curves. High variability is evident in F soil.

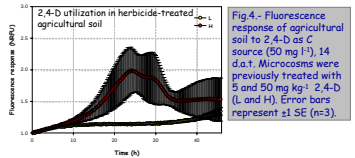

Fig.4.- Fluorescence response of agricultural soil to 2,4-D as *C* source (50 mg l^{-1}), 14 d.a.t. Microcosms were previously treated with 5 and 50 mg kg^{-1} 2,4-D (L and H). Error bars represent ±1 SE (n=3).

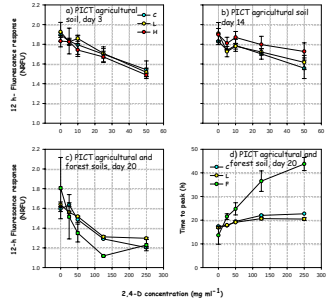

Fig.5.- 12 h-Fluorescence response to coumaric acid, a) 3 b) 14 d.a.t in herbicide-treated agricultural soil, exposed to 0-50 mg l^{-1} 2,4-D; c) 12-h flouorescence response and d) time to peak 20 d.a.t in agricultural and forest soil. Error bars represent ±1 SE (n=3).

Soil	TI_{50}	TI_{125}
C	0.93(0.018) a	0.81(0.002) a
L	0.93(0.004) a	0.80(0.007) a
F	0.75(0.05) b	0.62(0.002) b

Table 1.- Tolerance Index (TI= response to given dose/response in control) at dose of 50 and 125 mg l^{-1} 2,4-D calculated for the artificially spiked agricultural soil microcosms (*C* and L) and the forest soil (F). Results are means ±1 SE (n=3). Values followed by different letters are significantly different (p<0.05, LSD).

Summary

- Only minor changes in substrate utilization profiles were induced in soil microcosms by treatment with 2,4-D at 5 mg kg^{-1} (L). The peak in fluorescence response to casein was higher for L microcosms during the entire assay (Fig.3 a), while the response to succinate was higher in L than in *C* microcosms only on day 7 after treatment (Fig.3 b). The maximum fluorescence response to 2,4-D as *C* source was higher in L until day 14 (Fig.3 e), whereas the response to the background *C* was higher in L soils than in *C* microcosms on day 7 (Fig.3 f).
- Cycloheximide inhibited the 8 h-response of casein (43.6%), acetate (9.6%) and coumaric acid (9.7%) during the entire assay (Fig.3 a, c, d). In contrast, the response to 2,4-D and background *C* response were stimulated by the inhibitor (6.1% and 9.4%, respectively) (Fig.3 e, f).
- Two weeks after treatment H microcosms (2,4-D 50 mg kg^{-1}) showed a significantly higher peak for 2,4-D as substrate than L microcosms and the peak appeared 14 h earlier in the former (Fig.4).
- The L and H microcosms artificially spiked with 2,4-D did not differ from the *C* in their tolerance to increasing doses of herbicide (0-50 mg l^{-1}) (Fig.5 a, b). Again, *C* and L microcosms were similar in their tolerance response to 2,4-D at a range of 0-250 mg l^{-1}, whereas the F soil tended to be less tolerant to the herbicide (Fig.5 c, d, Table 1).

Conclusion

- The CLPP results indicate that agriculturally relevant levels of 2,4-D leads to enrichment of 2,4-D degraders, but has little effect on overall heterotrophic activity.
- The herbicide had no distinct effect on the fungal component of the microbial community in comparison to the control (untreated) soil.
- The agricultural soil used in this study, although not from a site with regular 2,4-D use, contained a community tolerant to 2,4-D exposure, suggesting that intermittent previous exposure to the herbicide either led to a persistently tolerant community, or that the agricultural soils are more generally resistant to potential stressors.

References

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