Resumen

El uso intensivo de herbicidas en suelos de la región Pampeana plantea una preocupante situación ambiental. Sin embargo, no hay información sobre los efectos adversos de estos agroquímicos sobre los procesos biológicos del suelo en los agroecosistemas de la región. En esta tesis se evaluaron los impactos de tres herbicidas de uso extendido (glifosato, ácido 2,4-diclorofenoxiacético [2,4-D] y metsulfurón-metil) sobre las comunidades microbianas de suelos, con un enfoque metodológico integrador. Se recolectaron tres diferentes tipos de suelos (Haplustol típico, Paleustol petrocálcico, Argiudol típico) en establecimientos agrícolas ubicados en el sur de la provincia de Buenos Aires, con historia de exposición a los herbicidas. Se estudiaron los efectos de corto plazo sobre la microbiota tras la aplicación de diferentes dosis de herbicidas en microcosmos de suelo. La actividad microbiana se evaluó midiendo la liberación de CO_2 del suelo y actividades enzimáticas (deshidrogenasas [DHA] e hidrólisis de fluoresceína [FDA]). También se determinó número de bacterias (bacterias aerobias heterótrofas cultivables [AHB]), biomasa activa (respiración inducida por sustrato [SIR]) y riqueza funcional (utilización de sustratos carbonados). El metsulfurón-metil tuvo efectos mínimos sobre la comunidad microbiana, afectando sólo la respiración en un suelo con pH ligeramente alcalino. Con las dosis más bajas utilizadas de glifosato y 2,4-D se observaron efectos agudos, transitorios y reversibles, sobre la actividad microbiana, la biomasa activa, la abundancia de bacterias y la riqueza metabólica. Las técnicas actuales para obtener un rápido patrón de utilización de sustratos carbonados de las comunidades microbianas de suelos (i.e., *community-level physiological profiling* o CLPP) dan una visión limitada y parcial de la estructura de las comunidades, con escasa relación con las actividades que éstas llevan a cabo *in situ*. En esta Tesis se optimizó un método alternativo de CLPP, basado en la detección fluorométrica de consumo de oxígeno en el sistema de microplaca BD Oxygen Biosensor System (BDOBS). Este dispositivo permite la incubación de suspensiones de suelo densas y ofrece la capacidad de manipular los factores experimentales (i.e. adición de nutrientes y sustratos, agregado de inhibidores selectivos, etc.). El análisis BDOBS-CLPP de microcosmos de suelos tratados con 2,4-D y glifosato mostró cambios mínimos en la utilización de sustratos en las comunidades microbianas. Con el sistema BDOBS se detectó la degradación aeróbica del 2,4-D en un suelo tratado experimentalmente, mientras que en los microcosmos tratados con glifosato no se verificó degradación. El análisis de tolerancia de la comunidad microbiana inducida por polución (PICT) en un suelo con historia de exposición al 2,4-D reveló que este suelo alberga una microbiota tolerante al herbicida. En consecuencia, la población degradadora aumentó luego de la adición del herbicida al suelo y se aislaron bacterias degradadoras capaces de utilizar el herbicida como única fuente de carbono y energía. Estos aislamientos se identificaron como *Cupriavidus necator*, portadores de genes de degradación posiblemente homólogos a los encontrados en *C. necator* JMP134, el organismo en el que más se ha estudiado la degradación del 2,4-D. En conclusión, estos herbicidas no tuvieron efectos considerables sobre la microbiota de suelos agrícolas que han tenido una exposición previa a los agroquímicos.

Abstract

The intensive use of herbicides in agricultural soils of the Pampas region is a matter of environmental concern. However, information on the side-effects of agrochemicals on biological processes is scarce. This thesis dealt with the impacts of three widely used, post-emergence herbicides (glyphosate, 2,4–dichlorophenoxyacetic acid (2,4–D) and metsulfuron-methyl) on soil microbial communities, by an integrated methodological approach. Three different types of soils (Typic Haplustoll, Petrocalcic Paleustoll, Typic Argiudoll) were collected from agricultural fields located in the south of the province of Buenos Aires, with reported history of herbicide application. Short-term effects on microbial communities after treatment with different doses of the herbicides were studied in soil microcosms. Microbial activity was examined by CO_2-evolution and enzyme activities (dehydrogenase activity [DHA] and fluorescein diacetate [FDA] hydrolysis); bacterial numbers (culturable aerobic heterotrophic bacterial [AHB]), active biomass (substrate-induced respiration (SIR)) and functional richness (carbon sources utilization) were also assessed. Metsulfuron-methyl had the least pronounced effects on soil microbial community, with only minor detectable effects on CO_2 evolution in a soil with relatively high pH. Short-term, reversible effects on microbial activity, active biomass, bacterial abundance and functional richness were observed with low rates of 2,4–D and glyphosate. Current approaches for rapid assessment of carbon source utilization by whole soil communities (i.e., community-level physiological profiling or CLPP) provide a limited, biased view of microbial community structure with little connection to *in situ* activities. In this Thesis an alternative CLPP approach based upon fluorometric detection of oxygen consumption in the microtiter platform BD Oxygen Biosensor System (BDOBS) was optimized. This tool allows the incubation of thick soil suspensions together with flexible manipulation of experimental factors (i.e. nutrient and substrates amendments, selective inhibitors, etc.). The BDOBS-CLPP of soil microcosms treated with low rates of 2,4-D and glyphosate showed minor changes in substrates utilization by microbial communities. Significant aerobic degradation was detected in 2,4-D-treated soils with the BDOBS, but not in glyphosate-amended microcosms. Pollution-induced community tolerance (PICT) testing of a soil with history of exposure to 2,4-D revealed that this soil harbors an herbicide-tolerant microbial community. Accordingly, the 2,4-D-degrader population increased after herbicide addition to soil, and degrading bacteria were isolated that used it as the only C and energy source. These isolates were identified as *Cupriavidus necator*, carrying degrading genes most likely homologous to the ones found in *C. necator* JMP134, the model organism for 2,4-D degradation. To conclude, these herbicides exerted no major effects on microbial communities of agricultural soils that had been previously exposed to these agrochemicals.

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