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Changes in the microbial activity in a soil amended with oak and pine residues and treated with linuron herbicide

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ABSTRACT

This work studies the effects of wood amendments on soil microbial community functioning and on the potential of this community for linuron degradation. For this purpose, soil dehydrogenase activity and the number of live bacteria, which represent broad scale measurements of the activity and viability of soil organisms, were assessed in soil treated with linuron and either amended with pine or oak wood or unamended (sterilized and non-sterilized). The overall results show that the microbial community had a significant role in linuron degradation. The linuron half-life values indicated a slower degradation rate in pine and oak amended soils than in unamended ones. This is attributed both to the higher sorption of linuron by these soils compared to the unamended ones and a consequent lower bioavailability of the herbicide for microbial degradation, and to the use of the pine and oak as an alternative carbon source by degrading microorganisms. Linuron did not affect the microbial community in terms of dehydrogenase activity and number of live bacteria, presumably because it had adapted to the herbicide. However, the dehydrogenase activity was significantly higher in the soils amended with pine or oak than in the non-amended ones, indicating that the presence of a carbon source favoured the overall bacterial community.

1. Introduction

Linuron (*N*′-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea) is a substituted phenylurea herbicide used widely to selectively control newly established broadleaf weeds and grasses in fruit and field crops, cereals and shelter belts.

Chemical degradation of phenylurea herbicides is of minor importance in most agricultural soils compared to biodegradation (Caux et al., 1998; Sørensen et al., 2003). Linuron and some of its major metabolites are suspected of being endocrine disruptors (Lintelmann et al., 2003) and of exerting toxic effects on aquatic and soil organisms (Caux et al., 1998). Microorganisms capable of degrading linuron through metabolic and co-metabolic pathways have been isolated (Caux et al., 1998; El-Fantroussi et al., 2000; Dejonghe et al., 2003; Sørensen et al., 2005; Breugelmans et al., 2007). However, degradation beyond the aniline-based metabolites is not frequently found and the mineralization process is reported to occur slowly in soil (Rasmussen et al., 2005). Several studies suggest the involvement in degradation of a bacterial consortium rather than a single strain (El-Fantroussi et al., 2000; Sørensen et al., 2003). Dejonghe et al. (2003) isolated a single strain capable of degrading linuron, but it was stimulated by a synergistic interaction with other strains.

The degradation data reported are quite variable, with DT_{50} values in the range of 38–135 days in laboratory studies and 13–82 days in field ones (Caux et al., 1998; Rodríguez-Cruz et al., 2001; Rasmussen et al., 2005; FOOTPRINT, 2007), and indicate

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linuron can be moderately persistent and moderately mobile. Linuron has been categorized as a transitional herbicide between potential leachers and non-leachers (Caux et al., 1998), and it raises concerns about the possibility of leaching widespread contamination from soil to groundwater. Organic matter has pointed out as the main soil parameter controlling linuron mobility (Sánchez-Camazano et al., 2000).

Recently, point sources of pesticides, such as spills from the devices used to apply them and uncontrolled disposal in soil of waste and equipment-washing water, have been identified as causes of soil and water pollution which can be more significant than that due to agricultural practice (Fait et al., 2007). The use of organic materials has been proposed to prevent the mobility of pesticides coming from these point sources of contamination (Rodríguez-Cruz et al., 2007a). In particular, adsorbent wood residues, such as oak and pine, have recently been investigated as biomaterials for the immobilization of several pesticides in soil, including linuron (Rodríguez-Cruz et al., 2007b). However, any organic matter and nutrients added to soil can strongly affect the structure and activity of bacterial and fungal populations as a result of their increased metabolism of these readily available nutrients (Briceño et al., 2007) and can consequently affect pesticide biodegradation. Some organic amendments may stimulate biodegradation, but others can reduce it (Moorman et al., 2001; Briceño et al., 2007). However, the effects of wood amendments on the soil microbial community and on the potential of this community for linuron degradation were not investigated in the past. As a result, the aim of this work was to assess how an agricultural soil bacterial community was influenced by the presence of pine and oak amendments both in its general functions, such as dehydrogenase activity and viability (broad scale processes, Bending et al., 2007), and in its specific linuron degradation capability (narrow niche soil process, Girvan et al., 2005).

For this purpose, the number of live bacteria and soil dehydrogenase activity were assessed in soil treated with linuron and either amended with pine or oak residues or unamended. The ability of microbiologically active soils to degrade the herbicide linuron was evaluated by comparing the half-lives ($t_{1/2}$) in the various scenarios to those in sterile soil.

2. Materials and methods

2.1. Soil and wood samples

Soil samples were collected from the surface layer (0–15 cm depth) of an agricultural field located in Aldearrubia (Salamanca, Spain) cropped with corn and potato, in which several pesticides had been intensively used for several years. Soil was left to dry at room temperature and then sieved (<2 mm). The soil was sandy-loam (11.8% clay, 13.6% silt and 74.5% sand), with 0.72% of organic carbon content, a pH of 6.3, and a cation exchange capacity of 4.8 cmol kg^{-1} (Rodríguez-Cruz et al., 2007a).

Wood samples consisting of pine and oak residues were selected because of their different Freundlich adsorption constant (K_f) values (74.4 in the case of oak and 96.2 in that of pine, which are related to their lignin content of 18.2% and 24.4%, respectively) found in a previous work (Rodríguez-Cruz et al., 2007b).

Table 1 – Total organic carbon (TOC %), soluble carbon (%) and pH of unamended (S) and amended soils with pine (SP) or oak (SO)

Sample	TOC (%)	Soluble C (%)	pH
S	0.72	0.008	6.3
SP	2.89	0.047	6.6
SO	2.79	0.037	5.9

The pine and oak residues were obtained from a local company in Salamanca (Spain), the <1 mm fraction was selected as the organic soil amendment as described in Rodríguez-Cruz et al. (2007b).

The amended soils were prepared by uniformly mixing soil with oak or pine (5%, w/w). Sub-samples were analyzed to assess both the total organic carbon (TOC) content, by using an elemental carbon analyzer (Wosthoff Carmograph 12 H Omega, Bochum, Germany), and the soluble carbon, by using a Shimadzu 5050 Carbon analyzer (Shimadzu, Columbia, MD). The carbon content in the soil amended with pine (SP) or oak (SO) sawdust was about 4-fold greater than that in unamended soil (S). Moreover, the most soluble carbon content was found in SP (Table 1).

2.2. Chemicals

Linuron (*N*′-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea) was supplied by Riëdel de Haën (Hannover, Germany) (>99% purity). The linuron metabolites (99.5% purity) *N*-(3,4-dichlorophenyl)-*N*′-methylurea, *N*-(3,4-dichlorophenyl)-*N*′-methoxyurea, *N*-(3,4-dichlorophenyl)urea and 3,4-dichloroaniline were supplied by Höechst AG (Germany).

2.3. Laboratory degradation experiments with amended and unamended soils

The herbicide degradation experiment was conducted in duplicate in accordance with SETAC guidelines (Lynch, 1995) and previous experiments (Barra Caracciolo et al., 2005a,b,c). A linuron stock solution (1 mg ml^{-1}) was prepared by dissolving the standard compound in acetone and was then diluted in sterile water. The water solution was added to soil (200 g) to obtain a final herbicide concentration of 1 mg kg^{-1}, which corresponds to an agricultural rate. Some soil samples were first sterilized (autoclaved 120 ± 2 °C, 20 min on 2 consecutive days) and then treated with linuron (SSL); other samples of soils were only treated with linuron (SL); others were treated with both linuron and pine (SPL) or oak (SOL) sawdust; and, finally, microbiological control soils were prepared with only water (S), with water and with pine sawdust (SP) and with water and oak sawdust (SO). All soils were thoroughly stirred with a sterilized spatula and the water added was in all cases sterilized by filtration (0.22 μm). The final moisture content was adjusted to 60% of the maximum soil water holding capacity.

Soils were maintained in beakers closed with a sterilized cotton plug wrapped in gauze to allow air exchange. The soil moisture was kept constant during the entire period of the experiments by periodically weighing and replacing any losses