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Soil Biology & Biochemistry 37 (2005) 2017–2024

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Facilitation of pentachlorophenol degradation in the rhizosphere of ryegrass (*Lolium perenne* L.)

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Received 18 August 2004; received in revised form 5 March 2005; accepted 10 March 2005

Abstract

The phytoremediation of xenobiotics depends upon plant-microbe interactions in the rhizosphere, but the extent and intensity of these effects are currently unknown. To investigate rhizosphere effects on the biodegradation of xenobiotics, a glasshouse experiment was conducted using a specially designed rhizobox where ryegrass seedlings were grown for 53 days in a soil spiked with pentachlorophenol (PCP) at concentrations of 8.7 ± 0.5 and 18 ± 0.5 mg kg^{-1} soil. The soil in the rhizobox was divided into six separate compartments at various distances from the root surface. Changes in PCP concentrations with increasing distance from the root compartment of the rhizobox were then assessed. The largest and most rapid loss of PCP in planted soil was at 3 mm from the root zone where total PCP decreased to 0.20 and 0.65 mg kg^{-1}, respectively with the two PCP treatments. The degradation gradient followed the order: near-rhizosphere > root compartment > far-rhizosphere soil zones for both concentrations where ryegrass was grown. In contrast, there was no difference in PCP concentration with distance in the unplanted soil. The increases in both soil microbial biomass carbon and the activities of soil urease and phosphatase were accompanied by the enhanced degradation of PCP, which was higher in the near-rhizosphere than far-rhizosphere soil. The results suggest that the effect of root proximity is important in the degradation of xenobiotics such as PCP in soil.
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Keywords: Rhizosphere; Pentachlorophenol (PCP); Degradation; Microbial activity; Root exudates

1. Introduction

The rhizosphere is the soil volume surrounding a root where physical, chemical, and/or biological parameters are being modified by the presence of the root (Anderson et al., 1993). The rhizosphere is most commonly distinguished from the bulk soil by a depletion of immobile nutrients (NH_4^+, $H_2PO_4^-/HPO_4^{2-}$, and certain micronutrients) and an elevated microbial activity based on readily available carbon derived from root exudation and other root depositions (Curl and Truelove, 1986). Other soil parameters may also be altered directly or indirectly by the roots. These include pH, Eh, partial pressures of O_2/CO_2 and other gases and soil moisture (Hinsinger et al., 2003). Rhizosphere technology has been applied to bioremediation of xenobiotics through plant growth for accelerating degradation rates (Schwab and Banks, 1994), and the presence of plants has positive effects upon the degradation of a wide range of compounds including simple phenolics, aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), trichloroethylene, chlorinated aromatics, insecticides and explosives (Hsu and Bartha, 1979; Anderson et al., 1993; Günther et al., 1996; Haby and Crowley, 1996; Reilley et al.,1996). The positive effects of roots on biodegradation may be derived from the extra input of easily degradable C that induces microbial growth or cometabolic processes (Haby and Crowley, 1996). Root exudation varies both qualitatively and quantitatively between plant species, as a function of plant age, mycorrhizal infection and nutrient status (Jay and Jodi, 1995; Günther et al., 1996; Grayston et al., 1997). Other parameters that have profound influences on

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0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.03.002

phytoremediation of xenobiotics include rooting density, fertilization and soil moisture. Soil properties such as texture, structure, organic matter content, hydrophobicity and moisture content will influence the movement of root exudates into the soil and mineral nutrients toward the roots. Thus, the extension of the rhizosphere may vary for well-described parameters such as C diffusion and nutrient depletion (Nye and Tinker, 1977; Grayston et al., 1997). The question of how far a rhizosphere effect on degradation of xenobiotics may extend has, however, never been approached, but the preferential use of plants with fibrous root systems for rhizo-degradation indicates that it is rather narrow.

Rhizosphere soil is commonly separated from plant root by gentle shaking. With shaking methods, the quantity of soil released from the roots may differ with plant species, soil type and conditions such as moisture content (Rillig et al., 1997). Such methods also inevitably result in the removal of some root material (especially root hairs) with the rooting media. The presence of root material has also been reported to increase the amount of extractable carbon from chloroform-fumigated soils leading to overestimates of microbial biomass (Mueller et al., 1992), which might cause problems in assessing the role of the biomass in the removal process of xenobiotics in the rhizosphere. Washing of the plant roots, an alternative sometimes used, makes soil recovery difficult and may remove soluble material from the root (Clemensson-Lindell and Persson, 1992). In an attempt to overcome some of these problems, a rhizobox was designed where soil in close proximity to roots and then separated by mm increments could be harvested intact.

Pentachlorophenol (PCP, $C_6C_{l5}OH$) was selected as the test compound for this experiment because of its resistance to degradation in soil. PCP is a weak acid with a molecular weight of 266.5 g, solubility of 3.0 μg ml^{-1} at 25 °C, pK_a of 4.75, and log K_{ow} of 5.01 (Joel and Jerald, 1998; Chen et al., 2004). It is moderately persistent in soil, with a reported field half-life of 45 days (Augustin-Beckerst al., 1994). As a pesticide, herbicide, and antiseptic, it was once used worldwide and has been designated as a priority pollutant and is a probable human carcinogen (Banerji and Wei, 1993; Galil and Novak, 1994). Although the use of PCP has decreased in recent years, it still causes environmental problems at many locations. High concentrations of PCP have been found in soil and aquatic sediments (Maatela et al., 1990; Abrahamsson and Klick, 1991; Sung-Kil and Angela, 2003). Therefore, the toxicity risk of PCP will remain for a long time.

The aim of the present work was thus to study the rhizosphere effect on the removal process of PCP in specially designed rhizoboxes. Likewise, the interest was directed towards the effect of the increasing distance on PCP removal. We hypothesized that the differences with distance in rhizosphere effects would coincide with the degradation gradients of PCP.

2. Material and methods

2.1. Soil

The soil, a typical ferralsol under agricultural use, was collected from the surface layer (0–20 cm) of cultivated soils. It was air-dried, ground and sieved <1 mm. Precautions were taken to avoid contamination during sampling, drying, grinding and storage. The soil contained 17.2% clay, 7.4% silt and 75.4% sand, and was classified as a sandy loam and identified to be free of PCP before use. Total C, total N and pH of the soil were 6.6 g kg^{-1}, 2.9 g kg^{-1} and 4.34, respectively.

2.2. Experimental design

A rhizobox was designed, basically as described by Wang and Shan (2002) (Fig. 1). The dimension of the rhizobox was 150×140×230 (length×width×height in mm). The rhizobox was divided into three sections: a root compartment, or central zone (20 mm in width), and left and right soil compartments, or rhizosphere zones (60 mm in length). Each soil compartment was further separated into several sub-zones (1 mm thick) by nylon mesh (<25 μm), sandwiching layers of soil from either side of the root compartment as required, referred to here as the 1–5 mm near-rhizosphere zones and the >5 mm far-rhizosphere zone according to the distance from the central zone to the boundary of the rhizobox. The design successfully prevents root hairs from entering the adjacent soil zones as well as keeping the soil zones separated, while permitting

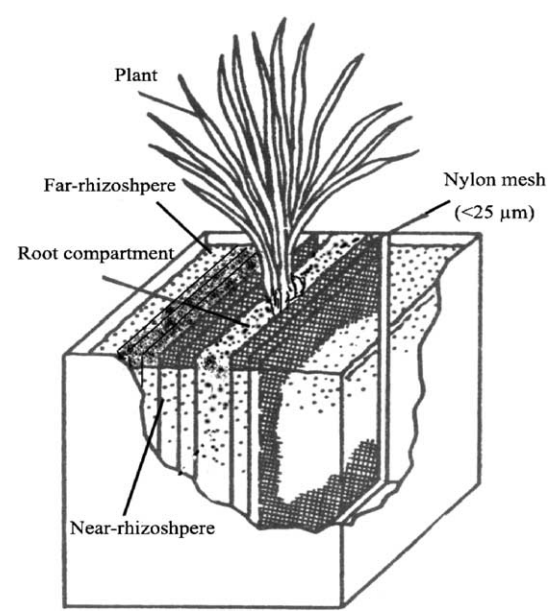

Fig. 1. Diagram of the rhizobox. Dimension: 150×140×230=length×width×height, mm; Root compartment: 20 mm in width; Rhizosphere zones: 60 mm in length, including 1–5 mm near-rhizosphere and >5 mm far-rhizosphere sub-zones (1 mm thick) separated by nylon mesh (<25 μm).

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