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Regulation of bacterial and fungal MCPA degradation at the soil–litter interface

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ABSTRACT

Much is known about mechanisms and regulation of phenoxy acid herbicide degradation at the organism level, whereas the effects of environmental factors on the performance of the phenoxy acid degrading communities in soils are much less clear. In a microcosm experiment we investigated the small-scale effect of litter addition on the functioning of the MCPA degrading communities. ^{14}C labelled MCPA was applied and the functional genes *tfdA* and *tfdAα* were quantified to characterise bacterial MCPA degradation. We identify the transport of litter compounds as an important process that probably regulates the activity of the MCPA degrading community at the soil–litter interface. Two possible mechanisms can explain the increased *tfdA* abundance and MCPA degradation below the litter layer: 1) transport of α-ketoglutarate or its metabolic precursors reduces the costs for regenerating this co-substrate and thereby improves growth conditions for the MCPA degrading community; 2) external supply of energy and nutrients changes the internal resource allocation towards enzyme production and/or improves the activity of bacterial consortia involved in MCPA degradation. In addition, the presence of litter compounds might have induced fungal production of litter-decaying enzymes that are able to degrade MCPA as well.

1. Introduction

Soil microbial communities play a key role in degrading xenobiotic compounds. This function is part of the filter capabilities of soils and belongs to the ecosystem services listed by the Millennium Ecosystem Assessment (2005). MCPA (4-chloro-2-methylphenoxyacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) have been two of the most heavily used phenoxy acid herbicides against dicotyledonous plants for around 50 years. They were often studied as model compounds for the environmental fate of xenobiotics. Several environmental factors govern phenoxy acid degradation in soils. One such key factor is the soil organic matter content, which influences the ratio between sorbed and dissolved 2,4-D and thereby the degradation rate (Greer and Shelton, 1992). Recently, Vieublé Gonod et al. (2003) suggested that uneven distribution of 2,4-D degradation in an arable soil at the millimetre scale might be explained by a heterogeneous distribution of the degrader community and of carbonaceous substrates required for co-metabolic 2,4-D degradation. Another study suggested that MCPA degradation is independent of the background density of the degrader community and more connected to growth of the microbial degraders (Fredslund et al., 2008). Therefore, microbial growth conditions might play an important role in MCPA degradation. This is in accordance with Cederlund et al. (2007), who suggested that MCPA mineralisation in railway embankments was N-limited, and to Duah-Yentumi and Kuwatsuka (1980), who showed that adding plant residues increased MCPA degradation. The activity of the soil microbiota is favoured in hot-spots where more nutrients are available than in other more oligotrophic habitats of soils. Previous studies have shown enhanced degradation of several xenobiotics in the rhizosphere (Piutti et al., 2002; Shaw and Burns, 2004). Similar to the rhizosphere, the detritusphere is characterized by high availability of soluble litter compounds, which stimulates microbial activity, growth and C turnover (Poll et al., 2008). However, the effect of litter addition on pesticide degradation as well as on the involved functional microbial communities at the small-scale has not yet been investigated.

Several bacteria have been identified as 2,4-D or MCPA degraders (e.g. Bell, 1960; Bollag et al., 1967; Chaudhry and Huang, 1988; Pieper et al., 1988) and much is known about the mechanisms and

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regulation of the degradation processes at the organism level. The most intensively studied is the *tfd* pathway. The *tfd* genes are often located on plasmids (Don and Pemberton, 1985) and are seldom chromosomal. Bacteria might be capable of the complete degradation pathway or only harbour genes encoding a truncated degradation pathway (Ka et al., 1994; Top et al., 1996). The initial step of this pathway encoded by the *tfdA* gene (Streber et al., 1987) is the cleavage of the acetate side chain by an α-ketoglutarate-dependent dioxygenase (Fukumori and Hausinger, 1993). Beside bacteria that harbour genes of the *tfd* pathway, several other phenoxy acid degraders have been identified. Among these are slow-growing oligotrophic bacteria of the α subdivision of the class *Proteobacteria*, which were isolated from pristine soils. They harbour the *tfdA*α gene that encodes another α-ketoglutarate-dependent dioxygenase (Itoh et al., 2002; Kamagata et al., 1997). The degradation of phenoxy acid herbicides, however, is not restricted to bacteria. Fungi have been reported to degrade these compounds as well (Castillo et al., 2001; Reddy et al., 1997). Vroumsia et al. (2005) screened ninety fungal strains and found up to 52% of added 2,4-D degraded by single fungal strains after an incubation of 5 days. In contrast to bacteria, no distinct pathway of fungal phenoxy acid degradation is known. Non-specific enzymes like lignin peroxidase or manganese peroxidase/laccase are suggested to be involved in the degradation process (Castillo et al., 2001).

The direct effects of environmental factors on the performance of the phenoxy acid degrading community in soils, however, are poorly known. Small-scale studies provide the unique opportunity to directly relate environmental factors (e.g. substrate availability or soil texture) to the functioning of the microbial degrader community. We studied the effect of litter addition on MCPA degradation as well as on the MCPA degrading community at the small-scale. We hypothesise that i) MCPA degradation is enhanced due to increased substrate availability within the detritusphere and that ii) this is accompanied by an increase in the genetic potential for MCPA degradation. Since bacteria and fungi show different substrate utilization strategies within the detritusphere (Poll et al., 2006), we further expected that iii) these two microbial groups differentially respond to the addition of MCPA and litter. For this purpose, we studied the degradation of ^{14}C labelled MCPA in the detritusphere in a microcosm experiment over 20 days. The abundance of 16S rDNA and 18S rDNA sequences was quantified to detect the response of the bacterial and fungal components of the soil microbiota to MCPA and litter addition. Finally, we quantified *tfdA* and *tfdA*α sequences as indicators for the abundance of bacterial MCPA degraders. Our results elucidate the regulation of microbial MCPA degradation by the properties of the soil habitat.

2. Material and methods

2.1. Soil and plant residues

Soil was sampled from an agricultural field located at an experimental farm in Scheyern, north of Munich (Germany, 48°30′N, 11°21′E) in July 2007. Samples were taken from the loamy topsoil of a Luvisol (World Reference Base for Soil Resources) [pH ($CaCl_2$) 5.3, total C content 13.6 g kg^{-1}, total N content 1.32 g kg^{-1}]. After sampling, the soil was sieved (<2 mm) and stored at −20 °C to minimise disturbance by soil faunal activity during the experiments. For the incubation, maize (C/N ratio 48) residues were chosen. Maize leaf litter and stems were shredded into pieces of 2–10 mm length and stored air-dried until the start of the experiment. The soil was pre-incubated for 6 weeks at 20 °C with an MCPA addition of 20 mg kg^{-1} to increase the initial number of MCPA degrading microorganisms (Baelum et al., 2008). Pre-experiments indicated that, after 6 weeks, MCPA is totally dissipated in this soil.

2.2. Experimental design

The experiment consisted of the following four treatments: (i) no MCPA and no litter addition, (ii) addition of litter, (iii) addition of MCPA, and (iv) addition of litter and MCPA. After thawing, the soil was homogenised and half of the soil was spiked with ^{14}C ring labelled MCPA (radioactive purity 91%, specific activity 60 MBq $mmole^{-1}$) at a concentration of 50 mg kg^{-1}. Labelled MCPA was mixed and dissolved in water with unlabelled MCPA to give a final activity of 26.4 kBq $microcosm^{-1}$. Soil moisture was adjusted to a volumetric water content of 35.2%, corresponding to a matric potential of −63 hPa. Finally, soil equivalent to 90 g dry soil was filled into cylinders (diameter = 5.6 cm, height = 4 cm) and covered with 0.5 g litter for the litter treatments. The litter was rewetted with 2 ml 0.01 M $CaCl_2$ before addition to the microcosms. The soil cores had a height of 3 cm and were compacted to a bulk density of 1.2 g cm^{-3}. For each treatment, 9 soil cores were prepared. Subsequently, each soil core was placed into an air-tight microcosm (Fig. 1) with a saturated ceramic plate beneath each cylinder. Ceramic plates were kept at a defined water suction of −63 hPa to maintain a defined lower boundary condition regarding water transport in the soil columns. The microcosms were incubated at 20 °C. After 20 days, $^{14}CO_2$ production indicated a strong decrease in MCPA degradation activity and the experiment was ended. During the incubation, we irrigated the microcosms one time with 4 ml and four times with 3 ml 0.01 M $CaCl_2$ solution. We used $CaCl_2$ to avoid dispersion of clay. At the first irrigation event, we applied 4 ml $CaCl_2$ to account for differences in soil moisture originating from the preparation of soil cores. Leachates were sampled one day after each irrigation event.

2.3. Sample preparation

The litter was removed after the incubation and the soil cores were frozen and subsequently cut into thin slices using a cryostat

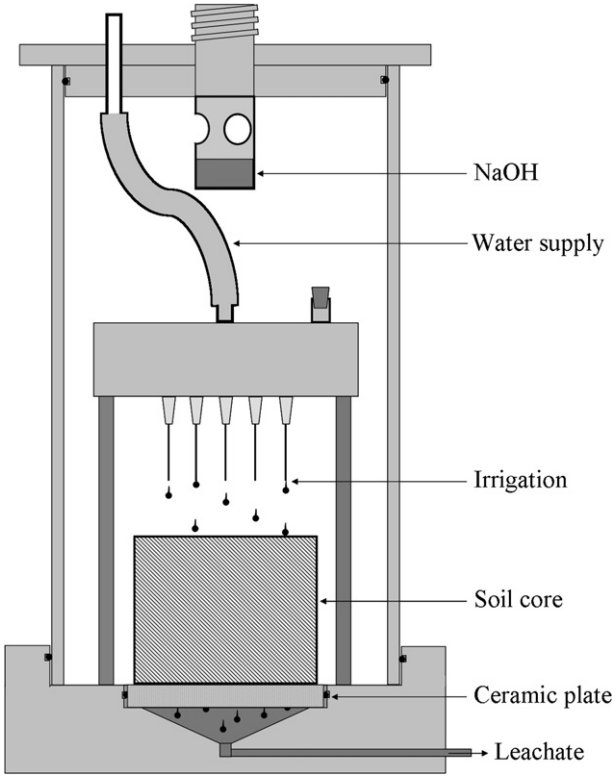

Fig. 1. Illustration of the microcosms used for the incubation experiment.