# From Gene to Model – Linking Microorganisms to Microhabitat Functions

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## Abstract

A microcosm approach was designed to link the abundances of bacteria and fungi to microhabitat functions of soil microbiota. In microcosms, we used MCPA (2-methyl-4-chlorophenoxyacetic acid) as a model organic substance due to its well-known degradation pathways (*tfdA* genes) and due to the availability of primers targeting the *tfdA* gene encoding the enzyme responsible for the first step in degradation of this compound. We used maize litter to create a well-defined soil-litter interface (detritusphere). We studied microbial degradation, microbial abundance of degraders, adsorption, desorption and transport of MCPA along a gradient of decreasing availability of dissolved organic matter. Isotopic data ($^{14}CO_2$, $^{14}C_{mic}$, $^{14}$C-DOC, $^{14}C_{org}$, $^{14}$C-MCPA, $^{14}$C in the leachate) and molecular data (*tfdA*, 16S rDNA and 18S rDNA sequence copy numbers) were used as input variables for a mechanistic model. We showed that the quantification of the abundance of genes encoding specific functions of soil microbial communities helps to clarify the regulation of degradation of complex organic compounds at a biogeochemical interface.

### Key Words

MCPA, functional genes, *tfdA*, microhabitat, detritusphere, biogeochemical interface

## Introduction

The importance of close interdisciplinary approaches in soil science was recognized by the German Funding Agency (DFG). DFG is currently funding a special research programme SPP 1315 "Biogeochemical Interfaces in Soil" stimulating studies on biogeochemical interfaces in soils (*http://www.spp1315.uni-jena.de/General_Information.html*). In the frame of this programme, several organic compounds (e.g. MCPA, phenanthrene and hexadecan) are used as model substances to gain a mechanistic understanding of the interplay and interdependencies of the physical, chemical and biological processes operative at biogeochemical interfaces. K.U. Totsche, the coordinator of the programme, stated that "The grand challenges are to identify the factors controlling the architecture of biogeochemical interfaces, to link the processes operative at the molecular and organism scale to the phenomena active at the aggregate scale in a mechanistic way, and to explain the medium- to long-term behaviour of organic chemicals in soil within a general mechanistic framework."

We have chosen the widely-used phenoxy acid herbicide MCPA as well as the polycyclic aromatic hydrocarbon (PAH) phenanthrene for our project because these compounds show similar degradation rates, but different sorption properties. Our project aims to quantify the relative abundances of bacterial and fungal communities involved in the degradation of MCPA and phenanthrene in the detritusphere and to link these quantities to biogeophysical processes ruling the dynamics of degradation. During the first phase of our project we focused on degradation of MCPA. Therefore, metabolism and co-metabolism of MCPA by a diverse microbial community were studied in the detritusphere along decreasing availability of low molecular weight organic substances within a distance of 10 mm to the litter. We hypothesized that dissolved organic carbon (DOC) from the litter, which may be considered a natural analog of MCPA, might enhance MCPA biodegradation by serving either as substrate for catabolic enzymes and/or as cooxidized substrate during co-metabolism.

### Material and Methods

The experimental design includes the following treatments: (1) MCPA, no litter, (2) MCPA, litter, (3) control, no litter, (4) control, litter. Each treatment was replicated nine times (three cores were pooled to yield nine gram of sample per layer, an amount which is needed to cover all analyses). The soil was homogenized after thawing, amended with MCPA solution (50 mg $kg^{-1}$ soil) to a volumetric water content of 35.2% corresponding to a matric potential of -63 hPa (pF 1.8), filled into cylinders (PVC, diameter 5.6 cm,

height  3 cm) and compacted to a bulk density of 1.2 g $cm^{-3}$. The soil cores were subsequently placed on ceramic plates and a matric potential of –63 hPa was applied to ensure approximately homogeneous conditions (Figure 1). The day before the start of the incubation, 0.75 g of maize litter were weighed for each cyclinder and rewetted with 2 ml of 0.01 M $CaCl_2$ solution. The litter was placed on top of each soil core. The cyclinders were irrigated once with 4 ml and four times with 3ml of 0.01 M $CaCl_2$ solution. At the first irrigation event, 4 ml $CaCl_2$ solution was applied to account for differences in soil moisture originating from the preparation of soil cores. Leachate was collected and analysed for MCPA and dissolved organic carbon. $CO_2$ production was monitored on a daily basis. The microcosms were incubated for 20 days at 20°C.
The litter was removed at the end of the incubation. The cores were frozen and subsequently cut into slices using a cryostat microtome. These slices were taken at the following distances to the soil-litter interface: 0-1, 1-2, 2-3, 3-4, 4-5, 5-7 and 7-10 mm. Bacterial and fungal abundances were estimated by qPCR targeted on 16S rRNA and 18S rRNA sequences, respectively (Martin-Laurent *et al*., 2003). The MCPA degrading genetic potential of bacteria was estimated by qPCR targeted on *tfd*A and *tfdAα* sequences coding isoenzymes specifically involved in the first step of MCPA degradation (Ledger *et al*., 2006, Vieublè Gonod *et al*., 2006). Information on further chemical and physical analyses will be given by Poll *et al*. (2009, in prep.).

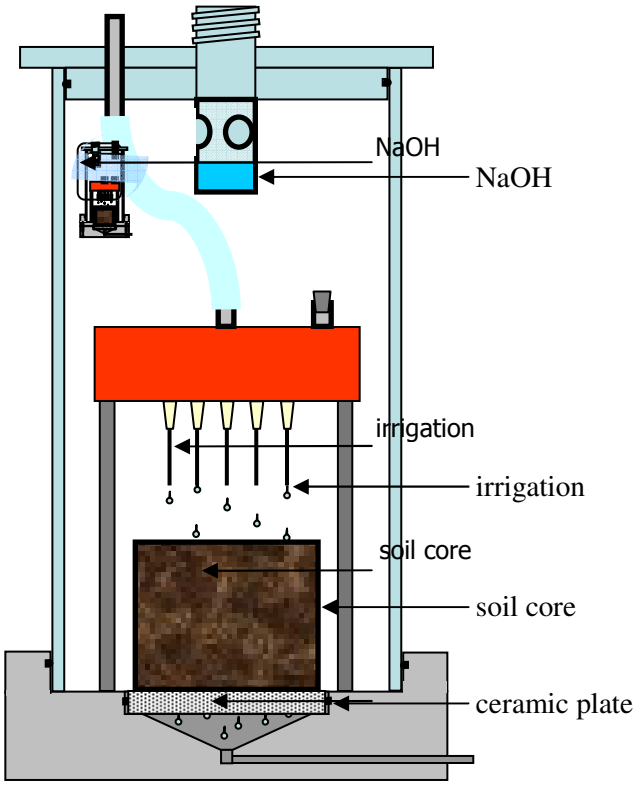


**Figure 1. Microcosm prepared to estimate $^{14}$C MCPA degradation within the detritusphere. $^{14}CO_2$ evolved from $^{14}$C-MCPA and column leachate were collected on a regular basis. A set up of 36 microcosms was settled with the following treatments: (1) MCPA, no litter, (2) MCPA, litter, (3) control, no litter, (4) control, litter.**

## Results and Discussion

Phenoxy acid degrading bacterial strains belonging to various species have been isolated from disparate regions of the world (Vallaeys and Soulas, 1992; Baelum *et al*., 2006), degradative pathways have been studied (Bell, 1957; Beadle and Smith, 1982; Evans *et al*., 1971), and genetic tools including probes and primers are available (Vallaeys *et al*., 1996; Leander *et al*., 1998). The *tfdA* gene encodes 2,4-dichlorophenoxyacetic (2,4)/α-ketoglutarate dioxygenase that catalyses the initial step in the degadation of phenoxy acid herbicides such as 2,4-D. The tfdA enzyme cleaves the ether bonds of MCPA to produce 4-chloro-2-methylphenol (MCP). This intermediate may then be transformed to chlorophenol by a chlorphenol hydroxylases encoded by the *tfdB* (Ledger *et al*., 2006).

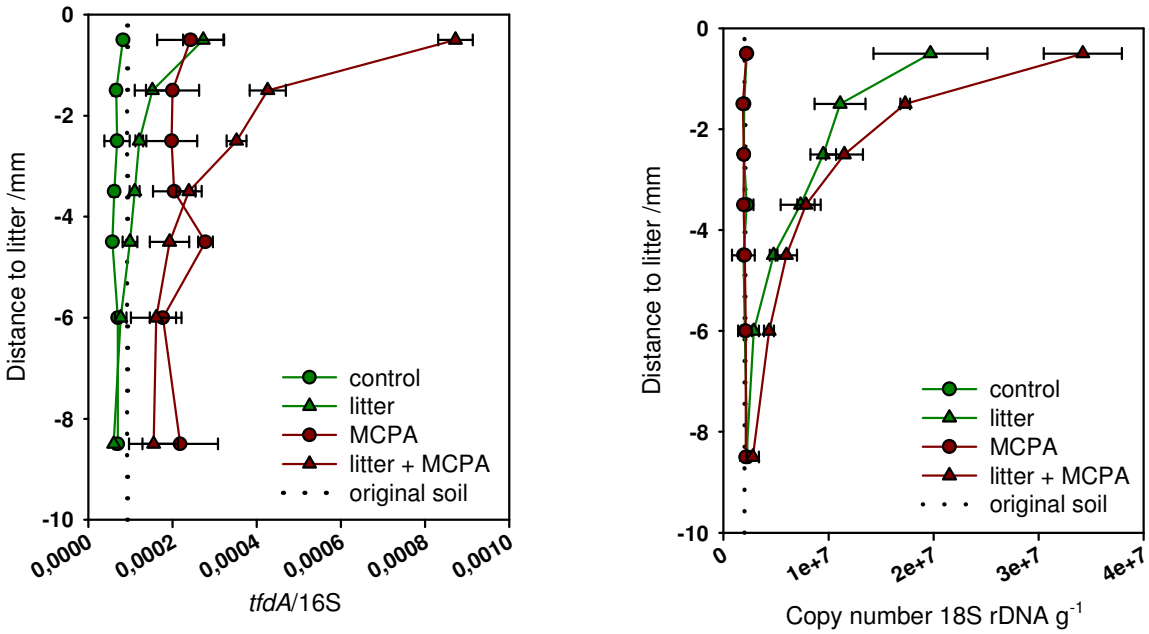


**Figure 2. Specific richness of *tfdA* functional community towards total bacterial community (ratio *tfdA*/16S rRNA) and abundance of fungal community (18S rRNA) (data modified from Poll *et al*., 2009, in prep.).**

Our microcosm study showed that the detritusphere provides a favourable environment for MCPA degradation: Isotopic measurements revealed that litter induced an additional release of $^{14}CO_2$ evolved from $^{14}$C-labeled MCPA, thereby enhancing the soil purifying capability (data not shown). Analyses of 16S rRNA and 18S rRNA sequence abundances in different layers of the detritusphere showed that the higher availability of DOC in the 0-4 mm zone stimulated both bacterial and fungal abundances (Figure 2, right side). In addition, *tfdA* sequence abundance in this zone significantly increased in specific richness of MCPA degraders (Figure 2, left side). Whereas the bacterial community increased in soil with MCPA even in the absence of litter, the fungal community increased only if MCPA as well as litter were added to soil cores (Figure 2, right side). Therefore, bacterial and fungal communities' regulation in response to MCPA exposure seems to be different in this environment. Further studies are required to show whether MCPA at the soil-litter interface is degraded by adapted bacterial and/or fungal populations that only partly use the carbon of the MCPA as a nutrient source for their growth.

Isotopic and molecular data are used to set up a computer model to simulate the dynamics of the total bacterial and fungal communities, and specific MCPA degrading microbial community as well as the fate of MCPA in the detritusphere. The model is formulated as a set of fully coupled (partial and ordinary) differential equations that are solved with a fully implicit finite difference scheme. The model describes sorption, transport and degradation of MCPA, transport and degradation of DOC through soil, and changes in bacterial and fungal communities and the MCPA degrading community, respectively. In accordance with the experimental set-up, modeling of the water regime is simplified by assuming steady state conditions. Transport of microorganisms is neglected. Model development is based on the detritusphere model by Ingwersen *et al*. (2008) which is an extended and modified version of the NICA model (Blagodatsky and Richter, 1998).

## Conclusion

Our study at a biogeochemical interface (in the detritusphere) offered the opportunity to better understand the biological mechanisms of degradation of xenobiotic compounds of varying complexity. Using MCPA as a model substance, we found that low molecular weight organic coumpounds in the first 4 mm of the detritusphere stimulated the development of bacterial and fungal communities as well as the development of the MCPA-degrading community. The efficient usage of carbon resources by a complex microbial community in the detritusphere could explain priming effects that are often described in soils. We will get an even closer mechanistic understanding by coupling taxonomical and physiological approaches. We will be able to find out, for example, whether fungal growth under MCPA and litter treatment is caused by changing competition within the soil microbiota, by cross-feeding or by direct fungal use of carbon from MCPA.

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