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Microbial catabolic diversity in and beyond the rhizosphere of plant species and plant genotypes

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ABSTRACT

Microorganisms in the rhizosphere drive important ecosystem processes such as nutrient cycling and organic matter decomposition. Microbial activity in the rhizosphere is a function of both rhizodeposition and the soil's inherent microbial community. In this study, we investigated plant species and genotype effects on microbial functioning in the rhizosphere and compared it to the corresponding bulk soil. We investigated the rhizospheres and bulk soils from eight natural grassland species (four grasses and four forbs) in a long-term biodiversity experiment and genotypes of two crop species (*Solanum tuberosum* and *Brassica juncea*) in a short term experiment. Soil microbial functioning was assessed by determining microbial catabolic diversity, which is the microbial response to addition of several carbon-rich substrates. Substrate-induced respiration was higher in the rhizosphere than in the bulk soil for all plant species and genotypes, except for the grasses *Agrostis capillaris*, *Anthoxanthum odoratum* and *Holcus lanatus*, which yielded similar microbial activities in the two soil zones. Microbial catabolic profiles in the rhizospheres of *Rumex acetosa*, *Leucanthemum vulgare* and *Plantago lanceolata* were most distinct from each other and from the other grassland species. The bulk soil's microbial community catabolic profile was also species dependent. For *S. tuberosum* we found a genotype effect on the microbial catabolic profile in the rhizsophere but not in the bulk soil. For *Brassica juncea* no such genotype effects were found in the rhizosphere or bulk soil. This is a first step to link microbial rhizosphere activities to soil functioning in natural and agricultural ecosystems.

1. Introduction

Soil microorganisms fulfill major roles in ecosystem processes such as nutrient cycling and organic matter decomposition. The activity of microorganisms in these processes is determined by the interactions among plants, soil characteristics and environmental factors (Degens et al., 2000; Fierer et al., 2009; Rout, 2014). Over the past decades, next generation sequencing techniques have significantly increased our knowledge with respect to soil microbial diversity and community composition (Rappé and Giovannoni, 2003; Mendes et al., 2011; Bulgarelli et al., 2013, 2015). However, despite the improved knowledge on 'who is there?', the question 'what are they doing?' is largely unanswered.

Plant roots exude a blend of carbohydrates. The chemical composition and the quantity of compounds exuded are – amongst others – dependent on plant species and plant genotype (Cheng et al., 2014; Gransee and Wittenmayer 2000; Lesuffleur et al., 2007). Plant rhizodeposits do not only fuel microbial activity and thereby accelerate organic matter decomposition ("priming"), but can also selectively affect microorganisms (Sugiyama et al., 2013), thereby potentially affecting essential ecosystem processes such as organic matter decomposition and nutrient cycling. For example, Weinert et al. (2009) found that genotypes of *Solanum tuberosum* exerted distinct effects on the microbial community composition in the rhizosphere. In addition, root exudates differentially affected

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growth and activity of beneficial bacteria (Carvalhais et al., 2013; Zhang et al., 2014).

Soil microbial activity has been investigated by determining the microbial catabolic diversity after the addition of a variety of C-rich substrates (e.g. Zak et al., 1994; Ellis et al., 2003; Blagodatskaya and Kuzyakov, 2013). Assessments of microbial activity with the MicroResp™ system typically need small volumes of soil (Campbell et al., 2003), which makes the method suitable for rhizosphere studies. The analyses need to be executed right after sampling, to mimick *in situ* observations. Knox et al. (2014) observed effects of cotton genotypes on the functioning of microbes in the rhizosphere using the MicroResp™ system. However, in their study only microbial functioning in the rhizosphere was reported, and bulk soil was not included. Including bulk soil measurements is necessary to understand plant-induced effects on the rhizosphere microbiome (e.g. Badri et al., 2013; Bulgarelli et al., 2015).

Most studies on the rhizosphere microbiome done so far have explored that associated with *Arabidopsis thaliana* or crop species (Bulgarelli et al., 2012; Mendes et al., 2013; Pfeiffer et al., 2013). Microbial activities in the rhizosphere of natural plant species remain thus unexplored. Therefore, we aim to assess effects of both natural and crop species on the microbial catabolic diversity in the rhizosphere and in bulk soil under field conditions, by testing eight grassland plant species, four *Brassica juncea* and five *Solanum tuberosum* genotypes. These species have been grown in monocultures for ten years, as part of a biodiversity experiment (Van Ruijven and Berendse, 2003). Microbial catabolic diversity of rhizosphere and bulk soil was measured upon the addition of a variety of C-rich substrates in the MicroResp™. We expected that: (i) microbial activities are higher in the rhizosphere as compared to bulk soil, and (ii) plant species and genotypes differentially affect the microbial catabolic diversity in the rhizosphere.

2. Material and methods

2.1. Experimental design and soil sampling

We performed three field experiments in which the catabolic diversity of the microbial communities in rhizosphere and bulk soil samples was assessed with the MicroResp™ system. Experiment 1 was a long-term field experiment with monocultures of natural grassland species. Experiments 2 and 3 were agricultural monocultures of *Brassica juncea* (mustard) and *Solanum tuberosum* (potato) genotypes, respectively.

Soil sampling was executed in the same way for all three experiments. Rhizosphere samples were taken by carefully lifting plants including root-adhering soil using a spade. Plants collected from subplots within the plots (Exp. 1: 0.04 m^2; Exp. 2 0.25 m^2; Exp. 3 0.50 m^2) were shaken twice to remove non-rhizosphere soil and the still adhering soil, i.e. the rhizosphere soil, was subsequently collected by gently brushing.

Bulk soil samples were collected using a soil corer (Ø2 cm, 0–15 cm depth) from which a few occurring roots and adhering soil were removed. Bulk soil samples consisted of a mixture of 15 cores per plot in Experiment 1, 30 cores per plot in Experiment 2, and 16 cores per plot in Experiment 3. In Experiment 2, a larger number of cores was taken because bulk soil samples were also used for nematode community profiling (Vervoort et al., 2014). All samples were refrigerated immediately (4 °C) and assessment of the microbial catabolic responses in the rhizosphere was done within 24 h after sampling. Before microbial catabolic assessment, the soil (rhizosphere and bulk soil) was mixed thoroughly and sieved over a 2 mm grid to remove organic debris and stones. For the sampling in Experiment 1 (grassland natural species) it is important to realize that these plants were performing poorly after 10 years of growth on sandy soil, where no fertilizer was added. So, there was a lot of bare soil in these plots to collect the bulk soil from. Samples were taken in March 2011. In Experiment 2 the bulk samples were taken between the rows, following a grid pattern of 3×10 m per plot (30 cores per plot) in September 2010. In Experiment 3 they were taken between plants within four ridges in September 2010.

2.2. Experiment 1

Experiment 1 was conducted within a plant biodiversity experiment at a former arable field in Wageningen, The Netherlands (Van Ruijven and Berendse, 2003). The topsoil of the arable field was removed up to 50 cm depth in the spring of 2000, after which square wooden frames were inserted in the sides of the holes. The plots, measuring $1 \times 1 \times 0.5$ m (l × w × d), were filled with a mixture of the sandy arable soil (2/3 v/v) and pure sand (1/3 v/v) to adjust the nutrient status of the soil to the grasslands in which the study species typically occur. In 2001, organic matter content of the soil mixture was 1.3% and pH ($CaCl_2$) 7.3 (averaged over all plots) (Cong et al., 2014). Plant species selected were four grasses (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., *Holcus lanatus* L.) and four forbs (*Centaurea jacea* L., *Leucanthemum vulgare* Lamarck, *Plantago lanceolata* L., and *Rumex acetosa* L.). Plant density was 144 plants per m^2. The plant species composition was maintained by removing seedlings of all other species at monthly intervals during the growing season.

2.3. Experiment 2

Experiment 2 comprised four *Brassica juncea* L. genotypes and was conducted in Münster, Germany. The soil was a loamy sand with 1.3% organic matter and pH ($CaCl_2$) 6.4. Four mustard genotypes were selected Terrafit, Terratop, Terraplus (all three genotypes were kindly provided by P.H. Petersen, Lundsgard) and ISCI 99 (Research Institute for Industrial Crops, Bologna) with distinct concentrations of the secondary metabolite 2-propenyl glucosinolate (sinigrin; Vervoort et al., 2014). Seeds of *B. juncea* were sown in rows (inter row distance 12.5 cm) July 2010 in plots of 4×15 m using a complete randomized block design with four replications. Seed densities were 12 kg ha^{-1} for genotype Terrafit, Terratop and Terraplus and 15 kg ha^{-1} for ISCI 99. Plants were fertilized at sowing with 70 kg nitrogen (N) ha^{-1} and 17.5 kg sulphur ha^{-1}.

2.4. Experiment 3

Experiment 3 comprised five potato (*Solanum tuberosum* L.) genotypes and was conducted in 2010 on an experimental field located in the province of Drenthe, The Netherlands. The field was on excavated peatlands, now sandy soils, with 25.5% organic matter (remains of peat) and pH (H_2O) 4.6. *S. tuberosum* genotypes Aveka, Aventra, Karnico (all three genotypes were kindly provided by Averis Seeds BV, Valthermond, Netherlands), Désirée (HZPC, Joure, Netherlands) and Modena (BASF GmbH, Limburgerhof, Germany) were planted in April 2010. Modena is a genetically modified *S. Tuberosum* variety (De Vetten et al., 2003), tubers do not contain amylose and as such it is an attractive genotype for a range of industrial applications. In this experiment, *S. tuberosum* was fertilised with 179 kg N ha^{-1}, 81 kg P ha^{-1} and 165 kg K ha^{-1}. A randomized complete block design with four replicates was used. Each plot contained 28 plants divided over four ridges.

2.5. Microbial catabolic profile

Assessment of the microbial community catabolic profiles was done using the MicroResp™ procedure. Samples were not pre-