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In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar

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ABSTRACT

Fungi are key to the functioning of soil ecosystems, and exhibit a range of interactions with plants. Given their close associations with plants, and importance in ecosystem functioning, soil-borne fungi have been proposed as potential biological indicators of disturbance and useful agents in monitoring strategies, including those following the introduction of genetically modified (GM) crops. Here we report on the impact of potato crop varieties, including a cultivar that was genetically modified for its starch quality, on the community composition of the main phyla of fungi in soils, i.e. *Ascomycota*, *Basidiomycota* and *Glomeromycota* in rhizosphere and bulk soil. Samples were collected at two field sites before sowing, at three growth stages during crop development and after the harvest of the plants, and the effects of field site, plant growth stage and plant cultivar (genotype) on fungal community composition assessed using three phylum-specific T-RFLP profiling strategies and multivariate statistical analysis (NMDS ordinations with ANOSIM test). In addition, fungal biomass, arbuscular mycorrhizal colonization of roots and activities of extracellular fungal enzymes (laccases, Mn-peroxidases and cellulases) involved in degradation of lignocelluloses-rich organic matter were determined. Fungal community compositions, densities and activities were observed to differ significantly between the rhizosphere and bulk soil. The most important factors determining fungal community composition and functioning were plant growth stage for the rhizosphere communities and location and soil properties for the bulk soil communities. The basidiomycetes were the most numerous fungal group in the bulk soils and in the rhizosphere of young plants, with a shift toward greater ascomycete numbers in the rhizosphere at later growth stages. There were no detectable differences between the GM cultivar and its parental cultivar in terms of influence on fungal community structure of function. Fungal community structure and functioning of both GM- and parental cultivars fell within the range of other cultivars at most sampling moments.

1. Introduction

One of the concerns surrounding the cultivation of GM crops is the possible impact on plant-soil ecosystems, including the soil-borne biota. Numerous studies have investigated the effects of GM-crops on soil bacterial community structure (Savka and Farrand, 1997; Dunfield and Germida, 2001; Rasche et al., 2006; LeBlanc et al., 2007; Weinert et al., 2009) and functioning (Heuer and Smalla, 1997; Di Giovanni et al., 1999; Hopkins et al., 2001; Griffiths et al., 2007). While some studies reported effects of modified crops on soil bacterial numbers (Siciliano and Germida, 1999; Dunfield and Germida, 2001), others have documented only minor or transient effects (reviewed by Kowalchuk et al., 2003). So far, the effect of the GM crops on soil fungi has received much less attention, despite the importance of fungi in terrestrial ecosystems (Carlile et al., 2001). A few studies have addressed the effects of GM-crops on general fungal community structures (Milling et al., 2004; Turrini et al., 2004; Götz et al., 2006; Hart et al., 2009; Wang et al., 2009). However, detailed studies on the effects of GM crops on the abundance, composition and functioning of fungi have not yet been reported. Moreover, most studies to date have focused on one time point and one field situation. Yet, effects of factors such as plant growth stage (Sessitsch et al., 2004; Hart et al., 2009), the plant community (Berg et al., 2002; Viebahn et al., 2005; Berg and Smalla, 2009) and tillage (Griffiths et al., 2007) are known to affect the microbial community considerably.

The 'true' fungi are ubiquitous in the environment and fulfil a range of important terrestrial ecological functions e.g. mineralization of soil organic matter and facilitation of plant nutrient acquisition (Christensen, 1989). Yet, the interactions between plants,

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plant residues and the soil fungal community and activities are not fully understood (Carlile et al., 2001). The most important fungal groups in most soils are the *Ascomycota*, *Basidiomycota* (Carlile et al., 2001) and *Glomeromycota*, comprising the arbuscular mycorrhizal fungi (AMF). Like all organotrophic soil microbes, fungi are influenced by plants. AMF as well as pathogens are in direct contact with plants, and saprotrophs are also influenced by the plant either directly via root exudates or indirectly via decomposition of litter and crop residues (Christensen, 1989; Buée et al., 2009). Members of the phylum *Basidiomycota* are perhaps the most important fungal decomposers, at least in forest soils, due to their ability to produce enzymes such as lignin peroxidases, manganese peroxidases and laccases that break down lignin-rich recalcitrant components of the litter (Lynch and Thorn, 2006). The relative importance of basidiomycetes in decomposition processes in agricultural soils is, however, not clear (Lauber et al., 2009), especially given the saprotrophic capabilities of many members of the phylum *Ascomycota*.

There are several mechanisms by which plants can influence soil-borne fungi. The chemical composition of root exudates, litter and other plant debris can vary strongly between plant species and even between cultivars of the same species (Lynch and Whipps, 1990; Kabouw et al., in this issue). Effects of plant species composition on bacterial community composition and functioning are well known, and some similar evidence has been reported for fungi (Lynch and Whipps, 1990; Kowalchuk et al., 2002; Bais et al., 2006; Broeckling et al., 2008; Badri and Vivanco, 2009).

In addition to the direct effects of plant residues on soil communities, there are many other factors that may affect soil-borne fungal communities, including soil type, past and present land use, management practices and crop species and -cultivars (Milling et al., 2004). Knowledge of these sources of natural variation in fungal communities is critical for the assessment of the relative effects of specific potential perturbations, such as transgenic crop cultivation. In this paper, we describe the development of fungal abundance, fungal community composition and fungal-related ligno-cellulolytic enzyme activities in two agricultural field sites planted with six different varieties of potato, including a GM-variety with modified starch quality. This approach facilitated an evaluation of the normal variation in fungal communities over time, between soils and under different cultivars, thereby providing the necessary baseline for assessing the potential impact of the GM variety. In order to provide a high resolution of site- and crop-related effects on fungal community composition, we adopted a terminal restriction fragment length (T-RFLP)-based approach to examine the composition of ascomycete, basidiomycete and glomeromycete communities separately. Resulting community profiles were related to environmental factors via multivariate statistics to determine the relative their importance in driving fungal community composition and function.

2. Materials and methods

2.1. Experimental design

The experiments were carried out during the 2008 growing season at two field sites in the north-eastern part of Netherlands, which is the main starch potato-producing region of the country. The sites VMD and BUI were located 10 km from each other and differed considerably in their soil characteristics: site VMD is characterized by a high organic matter content (average of 19%) and is categorized as sandy peat (silt fraction 2.8%, sand fraction 94.3%) whereas site BUI is a loamy sand (silt 5.7%, sand 90.5%) soil with an organic matter content of around 5%. pH of both soils was similar, around 5. Both fields had been under crop rotation and conventional agricultural practices for many decades. Six cultivars of potato (*Solanum tuberosum*) were grown in a randomized plot design consisting of four replicate plots per cultivar, each containing 28 plants. These cultivars comprised one modified potato line ('Modena') with altered starch quality used for industrial purposes, its parental cultivar ('Karnico') and four additional non-modified cultivars ('Aveka', 'Aventra', 'Désirée' and 'Premiere'). The altered starch composition was created by complete inhibition of the production of amylose via introduction of an RNAi construct of the granule-bound starch synthase gene inhibiting GBSS and amylose formation, which yields pure amylopectin. The modification was made without a marker gene as described by de Vetten et al. (2003). Cultivars 'Aventra', 'Aveka', 'Karnico' and 'Modena" produced tubers with relatively high starch content and had a low to medium growth rate, whereas cultivars 'Désirée' and 'Premiere' had lower starch content in the tubers and higher growth rates.

Soil samples were collected at five time points namely one day before planting, at three crop growth stages and after harvest. The growth stages sampled were: young plants (EC30), flowering plants (EC60) and senescent plants (EC90) (Hack et al., 2001). The bulk soil samples were collected from 0 to 15 cm depth, and 5 cores per plot were used to form a composite sample. Four plants per plot were used for a composite sample of the rhizosphere soil. In order to collect rhizosphere soil, the plants were shaken to remove the excess soil and the soil tightly adhering to the roots was collected by brushing. Bulk soils were homogenized and sieved (4 mm mesh) to remove possible root fragments and stones. Soil water content was determined from fresh material as weight loss after overnight drying at 105 °C.

2.2. Fungal biomass and enzyme activities

Quantification of ergosterol, via the alkaline extraction method, was used as an estimate of fungal biomass (de Ridder-Duine et al., 2006). Analyses of activities of enzymes involved in decomposition of lignocellulose-rich organic matter, *i.e.* laccase, cellulase and Mn-peroxidase were performed according to van der Wal et al. (2006).

2.3. Assessment of root colonization by AM fungi

Levels of mycorrhizal colonization and arbuscule and vesicle abundances were determined microscopically according to McGonigle et al. (1990). Briefly, randomly chosen 2 cm fine root pieces were cut, washed with water, cleared for 30 min at 90 °C in 10% KOH, incubated overnight in 1% HCl, subsequently stained with 0.05% tryptan and methyl blue in lactic acid: glycerol: water (1:1:1) and mounted onto slides. One hundred intersections per slide were counted.

2.4. Extraction of DNA from soil

DNA extractions were carried out using fresh soil material and the remaining soil was stored at −20 °C for enzymatic analyses. DNA was extracted from soil (0.5 g wet weight) with a Power Soil DNA isolation kit (MOBIO Laboratories, Inc.) using a bead beating system. Yields of genomic DNA were checked on 1% agarose gel and visualized under UV after ethidium bromide staining.

2.5. T-RFLP analyses

Terminal restriction fragment length polymorphisms (T-RFLP) combined with the construction of a small library of the most dominant operational taxonomical units (OTUs) was used to determine the fungal community compositions. Previously, T-RFLP has been used successfully to study total fungal communities or separate phyla like basidiomycetes or AMF in a variety of environments