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Cropping history shapes fungal, oomycete and nematode communities in arable soils and affects cavity spot in carrot

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ABSTRACT

Fungal, nematode and oomycete communities in arable soil serve diverse ecological functions. However, little is known about the dynamics among these three taxonomic groups. In order to identify the driving factors and networks shaping these communities, and the influence of communities on the development of carrot cavity spot, we collected soil samples from more than sixty fields in which carrot was planned as the next crop, and analyzed the microbial communities using metabarcoding. Crop grown before sampling was best in explaining variation in community composition, but also soil type was shaping fungal and nematode communities, but not oomycete communities. Network analysis revealed that correlations between taxa within taxonomic groups were mostly positive whereas negative correlations dominated between taxa across the different taxonomic groups. *Pythium intermedium* and *P. irregulare* from soil samples were found to be associated with cavity spot disease in carrot, and were also found in the lesions of cavity spot. Our study demonstrate that previous crop and soil type significantly affect microbial communities and the development of symptoms of cavity spot in carrot. We also found that differences in co-occurrences within and between taxonomic groups exist.

1. Introduction

Even though soil is an important part of the crop environment, the understanding of microbial communities in arable soils, and the drivers that shape these communities has only recently begun to accumulate (Bardgett and van der Putten, 2014; Peay et al., 2016). Fungi, oomycetes and nematodes are the major part of soil eukaryotic diversity and are of high economic significance to crop productivity: fungi provide plants with nutrients by forming symbiotic associations with plants (van der Heijden et al., 2015) or by serving as decomposers of organic material (van der Wal et al., 2013), and fungi and oomycetes make dense mycelial networks facilitating soil structure thus allowing movement of water, nutrients and microbes (Bronick and Lal, 2005); nematodes are the most abundant metazoans in soil and include bacterial and fungal feeders together with plant and animal parasites (Morise et al., 2012; Porazinska et al., 2012) and they play important roles in soil nutrient cycling and act as indicators of soil health (Porazinska et al., 1999). In a few but important instances, however, fungi and oomycetes may cause plant diseases, and nematodes cause significant crop losses as plant feeders. Therefore these groups of organisms are of great economic concern to farmers and a significant amount of effort is put into their management.

Several biotic and abiotic factors are important in shaping soil microbial communities. Cultivation practice such as organic farming may result in distinct microbial communities and in addition, different types of organic substrates also influence community structure and diversity (Hartmann et al., 2015). A higher abundance of bacteriovore nematodes and a higher diversity of fungi were observed in organic soils in comparison to conventionally managed soils (Briar et al., 2007; Sugiyama et al., 2010), and soil organic matter was found to affect fungal communities by increasing decomposers and reducing soilborne pathogens (Yu et al., 2013). Similarly, suppression of plant parasite nematodes was found after organic matter application (Briar et al., 2007) and nematode composition was found to be highly dependent on tillage and crop residues (Zhong et al., 2016). Soil pH was found to be the most important driver of bacterial diversity and richness in soil (Fierer and Jackson, 2006), whereas the influence of pH on fungal communities was found to be relatively weak in arable soils (Rousk et al., 2010). Plants are shown to select microbes from soil (Garbeva et al., 2004), suggesting that the crop affects the soil microbial population. In yet another study of cultivated soil, soil type was the major factor influencing the structure of bacterial and fungal communities in newly cultivated crops, whereas crop was the major driver in long-term cultivated fields (Jesus et al., 2015; Li et al., 2014). Also oomycete community structures were shown to be affected by soil properties and environmental factors in soybean fields (Rojas et al., 2017).

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Unravelling fungal, nematodes and oomycete communites can have potential for understanding crop health. In this repect, a number of metabarcoding studies of fungal communities in relation to crop health have been published (e.g. Xu et al., 2012a; Kuramae et al., 2013; Nallanchakravarthula et al., 2014; Bazghaleh et al., 2015). In contrast, metabarcoding studies focusing on oomycete and nematode communities are limited, although recent studies highlight the potential for providing insights into their diversity and effect on plant disease (Rojas et al., 2017; Sapkota and Nicolaisen, 2015a,b).

In the present study, cavity spot of carrot was used as a model to study interactions of fungi, oomycetes and nematodes and their effects on the development of plant disease. Cavity spot is one of the most important soilborne diseases in carrot grown in temperate regions and is often referred to as a disease complex including several species of *Pythium* in single lesions (Hiltunen and White, 2002). Previous studies isolated *P. violae, P. sulcatum, P. intermedium* and *P. sylvaticum* amongst others from cavity spot lesions (Allain-Boulé et al., 2004; Suffert and Guibert, 2007; Hermansen et al., 2007), yet the understanding of the dynamics of this complex in relation to the disease, and how it is affected by crop management strategies, is limited. Therefore, a next step would be to study the factors that influence *Pythium* communities in soil and to determine whether these soil communities would reflect the microbial communities in symptomatic tissues of cavity spot in carrot.

Metabarcoding studies and especially studies taking all three groups of eukaryotic organisms into account, are limited. To better understand the major drivers of soil microbial community structures and their effect on plant health, many components associated to crop production system such as previous crop, soil texture and cultivation practice must be evaluated simultaneouly. We hypothesize that soil microbial community structures in arable soil are determined by the soil properties and also by the crop, and further that the community plays a vital role in disease development of the following crop. Therefore, the aim of this study was to evaluate crop grown just before sampling and soil properties for their effects on fungal, nematode and oomycete communities in arable soils, and to evaluate the development of cavity spot in the following carrot crop. Further, we studied co-existence patterns within and between different taxonomic groups. For this, we sampled soils from fields with no crop in the autumn from more than sixty fields in which carrot was planned as crop in the following year, and assessed the development of cavity spot in the carrot crop. Oomycete, fungal and nematode communities from these arable soils were analyzed using metabarcoding in relation to soil properties and disease severity index of cavity spot in the cultivated carrots.

2. Materials and methods

2.1. Soil sampling and preparation

Sixty three fields were sampled during autumn in the years 2013 and 2014. The fields were distributed throughout the northern region of Jutland, Denmark (Fig. 1). All fields were planned for carrot cultivation in the following season, but did not have any crop at the time of sampling. Fields represented both organic (30) and conventional (33) management practices. The organically managed fields had been organic for at least two years. The crops grown before harvest included barley (20), wheat (14), oat (9) and rye (10) along with other crops such as potato (3), triticale (3), clover (6), and corn (1) (Table S1). Carrot cultivation 6 years back was recorded for each field. In each field, 20 cores of soil were collected in a 'W' shape using a soil sampling probe (diameter 5.7 cm) taking the upper 15 cm soil layer. These 20 samples were subsequently pooled and carefully mixed before subdivision into two subsamples, one sample for analysis of soil properties and the other sample for DNA extraction. Soil samples for DNA extraction were frozen (−20 °C) within 24 h of sampling. Before DNA extraction, 100 g of soil was sub-sampled and subsequently freeze-dried for 48 h and then ground in a bead mill (Retsch MM301 mixer mill, Haan, Germany) for 10 min. Soil characteristics such as pH, phosphorus, potassium, boron, copper and magnesium content were measured at OK Laboratorium for Jordbrug, Viborg, Denmark. Soil type (JB no.) was recorded based on the Danish soil classification's textural classes ranging from 1 to 7 on a scale (Breuning-Madsen et al., 1999), which corresponds to sandy (JB1), loamy sand soil (JB 2), sandy loam (JB 3 and 4), sandy clay loam (JB 5 and 6) and loamy soil (JB7) in the soil texture triangle classification (Brady and Weil, 2002).

2.2. Disease scoring after crop harvesting

Carrots were scored for symptoms of cavity spot after harvest from 41 fields out of 63 (some fields were not planted with carrot). The severity of disease was assessed on 100 randomly selected carrots from each field starting from '1' indicating the lowest disease score (up to 1% coverage of symptoms) and up to '4' indicating the highest disease score (more than 10% coverage).

2.3. Sampling of cavity spot lesions

Carrots with symptoms of cavity spot from 15 fields were selected randomly for metabarcoding in order to identify oomycete communities in the symptomatic lessions. The lesions were gently washed with tap water to remove soil and were cut out in a conical shape and frozen. Healthy carrots were included as negative controls. Frozen samples were freeze dried for 48 h and ground by shaking with 5 sterilized steel beads in a Geno/Grinder 2000 (SPEX CertiPrep, Metuchen, NJ, USA) at 1500 rpm for 3 × 30 s. The ground powder was used for DNA extraction.

2.4. DNA extraction, DNA amplification, and metabarcoding

From the lyophilized and homogenized 100 g soil sample, 250 mg was used for soil DNA extraction using the PowerLyzer™ PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions, except that samples were homogenized in a Geno/Grinder 2000 at 1500 rpm for 3 × 30 s. DNA was extracted from ground plant tissue (20 mg lyophilized) using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were stored at −20 °C until used for PCR.

Three different amplification procedures were employed for DNA amplification. The fungal ITS1 DNA region was amplified using primers ITS1-F (Gardes and Bruns, 1993) and 58A2R (Martin and Rygiewicz, 2005); the oomycete ITS1 region was amplified in a semi-nested approach using the primers ITS6 and ITS4 in the first amplification, and ITS6 and ITS7 in the second amplification as described in an earlier report (Sapkota and Nicolaisen, 2015a); a fragment of the nematode ribosomal DNA 18S was amplified in a semi-nested PCR using the primers NemF and 18Sr2b in the first amplification and NF1 and 18Sr2b in the second amplification as described earlier (Sapkota and Nicolaisen, 2015b). PCR was performed in a reaction mixture of 25 µl consisting of 1 × PCR reaction buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 µM of each primer, 1 U of GoTaq Flexi polymerase (Promega Corporation, Madison, USA) and 1 µl of DNA template. Ten base pair multiplex identifiers (MIDs) were attached to the forward primers. The amplicon size of PCR products was confirmed by visualization in a 1.5% agarose gel using ethidium bromide staining. PCR products were pooled, ethanol precipitated and redissolved in 50 µl of TE buffer. The pooled DNA was separated on a 1.5% agarose gel and the band of the expected size (300–450 bp) was extracted using a QIAquick Gel Extraction Kit (Qiagen), and the concentration was evaluated using a spectrophotometer. All samples were shipped to Eurofins MWG (Ebersberg, Germany) for sequencing on a GS Junior 454 Sequencer (Roche Diagnostics). The raw data were deposited in the sequence read archive, number SRP115464 and bioproject number PRJNA398354.