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Fungal plant pathogens on inoculated maize leaves in a simulated soil warming experiment

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ABSTRACT

Climate change will affect the survival of plant pathogens and the decomposition of crop residues on temperate arable soils. Information on the survival of fungal plant pathogens is important for assessing the subsequent infection risk of susceptible agricultural crops. Therefore, a soil warming experiment was performed to examine the effects of rising winter soil temperatures on the inoculum load of fungal plant pathogens (*Fusarium culmorum*, *F. graminearum*, *Rhizoctonia solani*) and the decomposition of infested maize leaves under field conditions. Using heating cables, an arable soil was subjected to temperature treatments simulating medium (+1.3 °C up to the year 2050) and long-term (+2.7 °C up to the year 2100) climate warming scenarios. Litterbags filled with pathogen-inoculated and non-inoculated maize leaves were placed on top of the soil. Soil microbial biomass below the litterbags, maize leaf decomposition as well as microbial colonisation of leaves were measured after 152 days. Pathogen load was estimated by DNA, total saprotrophic biomass by fungal glucosamine and bacterial muramic acid. Rising soil temperatures increased decomposition of pathogen-infested, but not that of non-inoculated maize leaves, without correlation to fungal and bacterial biomass. *F. culmorum* DNA produced the largest increase in DNA on maize leaves, but did not significantly respond to soil temperatures. In contrast, the increase in *F. graminearum* DNA was considerably lower, but revealed a significant positive response to rising soil temperatures. DNA from mycelia and sclerotia of *R. solani* strongly decreased. Rising winter soil temperatures will most likely cause shifts within the plant residue colonizing fungal community, especially between different *Fusarium* species.

1. Introduction

In wheat and other cereal crops, *Fusarium species* cause several diseases such as seedling blight, foot rot, *Fusarium* head blight, ear rot of maize and crown rot in wheat and barley (Doohan et al., 2003 Xu and Nicholson, 2009). *Fusarium culmorum* WG Smith and *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schw.) Petch) belong to the most important pathogens in crops worldwide, leading to high yield losses and to contamination of the grain with mycotoxins, such as deoxynivalenol and zearalenone (Basler, 2016; Pasquali et al., 2016; Popiel et al., 2008). The soil-borne fungus *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) with the anastomosis group (AG) *R. solani* AG 2-2 IIIB is responsible for diseases in sugar beet (*Beta vulgaris* L.), decreasing yields by up to 50% in parts of Europe, Japan and the United States (Kiewnick et al., 2001; Kühn et al., 2009).

Crop residues on the soil surface, in particular large amounts of maize debris, serve as substrate for these fungi; they represent a major source of inoculum for crop plant infection in the next season (Maiorano et al., 2008; Palumbo et al., 2008). However, fungal survival and inoculum production on crop residues is limited by residue decomposition. In contrast to plant residues buried in soil after ploughing, residues on the soil surface provide nutrients and serve as a substrate for a longer period, allowing fungal plant pathogens to survive for several years (Pereyra et al., 2004; Vogelgsang et al., 2011). Climate change may have a strong impact on pathogen population dynamics and decomposition processes of crop residues on temperate arable soils, as they remain close to freezing point throughout the winter (Henry, 2008). Here, temperature and water are key conditions influencing saprophytic growth and survival of fungal pathogens and the severity of diseases they cause (Doohan et al., 2003). In Europe and especially in Germany, climate change has led to increased mean air temperatures

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over the last 50 years (Haberlandt et al., 2010) and trend analysis has shown an increase in the mean annual temperature of about 0.8 to 1.1 °C from 1901 to 2000, accompanied by a marked increase in winter precipitation (Schönwiese and Janoschitz, 2008).

Knowledge concerning the effects of changing winter climate temperatures on infestation and colonisation of crop residues and the survival of fungal plant pathogens is important for assessing the subsequent infection risk of susceptible agricultural crops (Juroszek and von Tiedemann, 2011, 2013a,b; Launay et al., 2014). Currently, there is limited information available (Siebold and von Tiedemann, 2012a). Litterbag experiments are often used to investigate decomposition of plant residues (Knacker et al., 2003; Jacobs et al., 2011). They provide a useful tool for studying the effects of soil temperatures on the survival of fungal plant pathogens on crop debris under field conditions. Colonisation of decomposing plant material by fungi and bacteria can be specifically quantified by the analysis of the amino sugars glucosamine (GlcN) and muramic acid (MurN) (Joergensen and Wichern, 2008; Potthoff et al., 2008). In soils, the fungal cell membrane component ergosterol is a better indicator for fungal biomass, as it does not accumulate in soil organic matter (Joergensen and Wichern, 2008).

The current soil temperature manipulation experiment with litterbags containing maize leaves addresses the following questions: (1) How strong is the infestation and survival of fungal plant pathogens on inoculated maize leaves under increased winter soil temperatures? (2) Is the fungal plant pathogen load related to the fungal and bacterial biomass colonizing maize leaves during decomposition? (3) Do increasing winter soil temperatures and inoculum loads of fungal plant pathogens affect soil microorganisms below the litterbags?

2. Material and methods

2.1. Soil warming facility

The experimental site is located in the northern part of Göttingen, Lower Saxony, Germany (51°33′29.28″N, 9°55′59.46″E) at 180 m ASL. The mean annual temperature of Göttingen is 8.7 °C and the mean annual precipitation is 644 mm. Exact information on precipitation during the experimental period was given by Siebold and von Tiedemann (2013) as Supporting information. Briefly, the soil warming facility with a total size of 60 m^2 was designed in 2009 (Siebold and von Tiedemann, 2012b), where the original stony and heterogeneous soil was replaced to a depth of 1 m with an arable top soil, i.e. a silty clay loam (Tu4, pH-H_2O 8.3), classified as Calcaric Cambisol (FAO-WRB, 2014).

The experiment had a completely randomized block design with the following three temperature treatments, each replicated four times: (ST1) ambient soil temperature, (ST2) ambient +1.6 °C, and (ST3) ambient +3.2 °C. The experimental site consisted of 12 plots (2 m × 2.5 m each) arranged in two rows. Heating cables were buried at a depth of 10 cm in each plot, also in the control plots, to ensure equivalent physical conditions. The soil warming treatments were applied to simulate warming scenarios for Lower Saxony up to the year 2050 (medium-term) and the year 2100 (long-term), respectively (Werner and Gerstengarbe, 2007).

2.2. Experimental procedure

The soil used for the experiment was taken from the upper 10 cm of an arable field near Witzenhausen (51°23′N, 9°55′E, Northern Hessia, Germany) in September 2011, sieved and stored at 4 °C for about two weeks. The soil was classified as a Haplic Luvisol (FAO-WRB, 2014) with the following characteristics: 3.3% sand, 83.4% silt, 13.3% clay, a pH ($CaCl_2$) of 6.3, 1.4% total C, 0.14% N and a C/N ratio of 10. Polypropylene cylinders (10 × 10 cm), closed at the bottom with polyethylene mesh (1 mm mesh size), were filled with moist soil equivalent to 350 g dry soil and transferred to the experimental site at the beginning of October 2011. Per plot, two cylinders were inserted to a depth of 5 cm (24 cylinders in total).

Green maize leaves (*Zea mays* L.) were dried (60 °C), chopped at < 2 cm and stored in a paper bag at 40 °C until the beginning of the experiment. The maize leaves contained 41.2% C and 2.4% N. Macroconidia of *Fusarium culmorum* DSM 62184 (isolated from mouldy maize grain in Germany by E. Seemüller) and *Fusarium graminearum* 210 (isolated from a wheat ear in Göttingen, Germany) and a mixture of mycelium and sclerotia of *Rhizoctonia solani* AG2-2 were used for inoculation. Macroconidia were obtained as described by Becher et al. (2010). Briefly, 50 mL of mung bean (*Vigna radiata* (L.) R. Wilczek) broth was inoculated with potato dextrose agar (PDA) plugs, colonised with *F. graminearum* and *F. culmorum*. These plugs were filled into 300-mL flasks and incubated for 7 days at ambient light and temperature while shaking at 50 rev min^{-1}. PDA medium in a single Petri dish was inoculated with an *R. solani* AG2-2 strain and incubated for 4 weeks at room temperature without light. Agar with *R. solani* mycelium was homogenised in a blender and the slurry was transferred into nutrient solution. The inoculum solutions were centrifuged at 4500*g* for 10 min in 120 mL polypropylene containers to rule out possible effects of the mung bean broth and the sclerotia nutrient solution during the decomposition of maize leaves. The supernatant was discarded carefully and the conidia/sclerotia pellets were dissolved in autoclaved water. This step was repeated until colourlessness of the suspension indicated complete removal of the nutrient solution. The stock solutions were then kept at 4 °C.

Polyethylene litterbags (LB) (8 × 5 cm; 1 mm mesh) were filled with 3 g of the oven-dried maize leaves, closed with staples and placed in a desiccator with moist paper towels for 24 h to reduce water repellence of maize leaves. After remoistening, the maize leaves of 12 litterbags were inoculated at the same time by pipetting 2 mL of each inoculum solution containing 30,000 macroconidia of *F. culmorum* and *F. graminearum*, respectively, and 133.7 mg mycelium/sclerotia of *R. solani*. After inoculation at the end of October, the litterbags were immediately brought to the experimental site together with another 12 litterbags with non-inoculated maize leaves, which received 6 mL autoclaved water and served as controls. Each of the two cylinders per plot received one pathogen-inoculated or one non-inoculated litterbag, placed on top of the soil. Then, the cylinders were covered with a 2 mm polyethylene mesh to protect the litterbags, but allowing precipitation to pass.

At the end of the experiment, litterbags and soil-filled cylinders were recovered. Soil loosely adhering to the litterbags was removed carefully with a brush and a knife prior to opening the bag itself. The maize residues of each litterbag were dried at 40 °C for 48 h, weighed and milled for further analysis.

2.3. Microbial biomass indices

Soil microbial biomass C and N were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987). A sub-sample of 20 g moist soil was separated into two portions of 10 g. One portion was fumigated at 25 °C with ethanol-free $CHCl_3$, which was removed after 24 h. Fumigated and non-fumigated samples were extracted for 30 min with 40 mL of 0.5 M K_2SO_4 by horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C and total N in the extracts were measured using a multi N/C 2100S automatic analyser (Analytik Jena, Germany). Microbial biomass C was calculated as E_C/k_{EC}, where E_C = (organic C extracted from fumigated soil) − (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as E_N/k_{EN}, where E_N = (total N extracted from fumigated soil) − (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985).

Ergosterol was extracted from 2 g moist soil with 100 mL ethanol (96%) according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC analysis (Gynkotek 480, Germering,