The heterogeneous soil environment: Are there preferential pathways for fungal spread?

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Abstract: Most studies with soil-borne pathogenic fungi have been done with little explicit characterisation of soil structure within which fungi spread and biotic interactions occur. Soil, however, constitutes a framework of surfaces formed by old root channels, cracks or biopores in combination with aggregates. Using epidemiological and soil biological techniques in controlled environments we investigated the effect of soil heterogeneity on fungal growth dynamics. We show that cracks and larger pores can act either as preferential pathways or barriers for the spread of fungal plant pathogens through soil. Understanding the effect of soil structure on pathogen and antagonist dynamics is therefore critical for our understanding of epidemics and the development of control strategies in a heterogeneous environment.

Introduction

The structure of soil mediates all soil processes at a wide range of scales, and has significant effects on plant development, movement of water and particles, soil usage and on processes involving soil bacteria and fungi. Manipulation of soil structure is one of the principal means by which biological processes can be altered at scales ranging from the rhizosphere to the field scale (Young and Ritz, 2000). Many examples can be found where a change in tillage practice (e.g. reduced versus conventional tillage) had a significant impact on disease severity.

Field soil is not a uniform, homogeneous medium, but exhibits spatial heterogeneity on many scales. In tilled soil it can manifest itself in the form of beds of aggregates; in non-tilled soil, it may appear as a system of planar pores or cracks between soil peds. Biopores formed by roots of previous crops or macrofauna also are important aspects of soil structure. Foraging behaviour of soil micro-organisms therefore involves exploring a tortuous trajectory through a heterogeneous framework of cracks, biopores and aggregates. In spite of this the majority of studies involving soil organisms have been made in essentially homogeneous and structureless media. Such conditions favour repeatability but they conceal the heterogeneity in a natural soil.

In their response to soil heterogeneity, fungi may differ considerably from bacteria as the hyphal network enables translocation of water and nutrients and exploration of pore spaces in a highly efficient way. Recent work on the soil-borne fungus *Rhizoctonia solani* however has shown that the spatial and temporal dynamics of fungal colonies are limited by the network of suitable air-filled pores (Otten *et al*., 1999). A particularly striking feature of this work is that a small change in the air-filled pore space makes *R. solani* switch from a small dense colony to a larger faster expanding colony. The fungus also showed a preference to grow faster along surfaces than through soil (Otten and Gilligan, 1998). Questions need to be asked now as to

what extent structured soil provides a framework of surfaces formed by root channels, aggregate surfaces and cracks, and what the consequences are for fungal spread and biotic interactions. In this paper we use *Rhizoctonia solani* as a target organism and combine epidemiological and soil biological techniques that enable quantification of fungal growth dynamics in soil to test if belowground surfaces can act as highways for fungal pathogens.

Materials and Methods

Colonisation efficiency.

The complicated dynamics of mycelial growth through soil are summarized by the colonisation efficiency (Bailey *et al.*, 2000). For spread initiated from a localized substrate, the colonisation efficiency is given by the probability of successful colonisation of a particulate unit of substrate with distance from the source. Here we use the colonisation efficiency to assess the effect of cracks on fungal growth dynamics. In up to 60 replicated microcosms we introduced cracks in between the source of inoculum and the target particulate unit of substrate placed at 30 mm distance. We quantified the effect of the width, the orientation and the location of a crack for a fungus spreading over a surface and colonising a distant target.

In addition, we used the colonisation efficiency to summarize the fungal growth dynamics of *R. solani* spreading *through* replicated microcosms in the presence or absence of introduced larger pores. In short, 10 units of inoculum of *R. solani* were buried at predetermined distances below the surface in replicated microcosms. The surface was observed daily for outgrowth. The distances through which *R. solani* had to spread to reach the surface were 20, 30, 40, 45, 50 and 60 mm, with 30 replicated samples for each distance. In half of the replicates, 4 larger pores (1 mm wide) were vertically introduced from the surface down to the layer of inoculum. Fungal spread was quantified as the number of replicates in which the fungus had spread through the layer onto the surface over time.

Cracks in soil and hyphal density distribution.

A preliminary study was undertaken to test if a soil thin sectioning technique could be used to reveal hyphal distributions within bulk-soil and in proximity of a crack. Soil cores were prepared by packing sieved and wetted soil in polypropylene cylinders at a bulk-density of 1.2 Mg m^{-3}. Each ring contained a layer of inoculum comprising 15 g soil mixed with colonised poppyseeds (0.05 g g^{-1}) from which fungal growth was initiated. A vertical crack was introduced and the rings were subsequently incubated for 5 days at 23 ^{0}C. Thin sections were taken from horizontal layers through the soil cores (Harris *et al.*, 2002). Sections were taken at 20, 25 and 30 mm distance above the inoculum layer. In these sections, the mean hyphal lengths were estimated in relation to the distance to the crack.

Results

Fungal spread depends on width, location and orientation of a crack.

A narrow gap of approximately 0.5 mm wide was crossed by *R. solani* without any quantitative effect on fungal growth dynamics. Wider gaps up to 5.4 mm, were also easily crossed in all replicates, but did subsequently reduce the rate and extent of fungal spread. The reduction in spread after *R. solani* had crossed the crack is most likely caused by a smaller number of hyphae capable of crossing the crack with increasing crack width. The efficiency at

which fungi crossed cracks depended on the location of the crack relative to the source of inoculum. The closer the crack is located to the nutrient source from which fungal growth was initiated, the less is the effect on fungal growth. In contrast to the previous scenarios, fungal spread is significantly enhanced in the direction of the crack, with further and faster colony expansion. Considering two extreme situations with cracks either perpendicular or parallel to the direction of growth, the fungal expansion either decreased (crack acts as a barrier) or increased (crack acts as a preferential pathway).

Probability of spread through soil.

The probability of *R. solani* spreading through soil to colonise a discrete nutrient target decreased with distance (Fig.1). The gradual decline with distance reflects fungal morphology as the fungus becomes more sparse as it grows out from the locally provided nutrient source. Larger pores increased both the extent and rate of fungal spread. We conclude that when the fungus is spreading through the bulk-soil and encounters a crack, spread in the direction of the crack is enhanced as the fungus by-passes the more tortuous pathway through the bulk-soil.

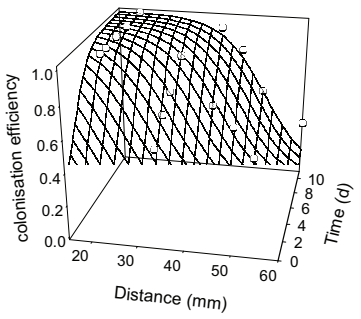

Figure 1. Colonisation efficiency of *R. solani* spreading through soil. Larger pores significantly enhance the colonisation efficiency (A) compared to samples without larger pores (B). With larger pores (C, closed circles) *R. solani* spreads faster and is more likely to colonise a target at 30 mm distance than in the absence of larger pores (C, open circles).

Hyphal densities in cracks and bulk-soil.

Rhizoctonia solani was clearly visible via microscopic observation of thin sections. This enabled quantification of lengths of fungal hyphae in the crack, and in the bulk soil in relation to distance from the crack. Close to the layer of inoculum, we could not detect an effect of the crack on the hyphal density: the density of hyphae was uniform and independent of the distance to the crack. In the middle layer, at 25 mm from the inoculum layer and in particular in the top layer, at 30 mm from the inoculum, there was a higher density of fungal hyphae in areas closer to the crack. In the top layer, fungal hyphae were almost completely restricted to an area within 2 mm surrounding the crack. We conclude from this that fungi move initially faster and further through the crack than through the bulk soil. The precise conditions that make the fungus preferentially follow these cracks are now topic of further investigation.

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