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VIEWPOINT/IDEA PAPER

# A connection between fungal hydrophobins and soil water repellency?

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## Summary

This Idea/Viewpoint paper aims to bring together two hitherto relatively disjointed areas of research: work on soil water repellency and our rapidly increasing knowledge about fungal hydrophobins. Water repellency is a significant problem worldwide, with important environmental consequences, for which proximate causes are poorly understood. Hydrophobins are a recently discovered, seemingly ubiquitous class of fungal proteins that have numerous roles in the life history of filamentous fungi through their ability to act at surfaces. Hydrophobins are potent surfactants; they also have the ability to self-assemble at hydrophobic–hydrophilic interfaces, with concurrent changes in surface properties. Potential, but as yet unexplored consequences of hydrophobin presence in soils are discussed, and it is concluded that the study of water repellency (and soil ecology in general) could profit by applying some of the knowledge obtained from molecular/biochemical studies on hydrophobins to the soil environment.


## Significance of water repellency

Water repellency is a significant problem affecting large areas of land and a wide variety of ecosystem types (Wallis and Horne, 1992; Bauters et al., 1998; DeBano, 2000). Hydrophobic soils occur in natural and agricultural situations in North America, Europe, Asia as well as New Zealand and Australia. Soils affected include also those found in intensively managed and man-made ecosystems, such as golf greens in many countries (e.g., Miller and Wilkinson, 1977; Kostka, 2000).

Consequences of soil water repellency include undesirable effects such as reduced water infiltration, increased surface run-off, leading in some instances to nutrient losses, leaching of agrochemicals, reductions in plant growth and increased soil erosion (e.g., Wallis and Horne, 1992). On the other hand, hydrophobicity can contribute to greater water stability of soil aggregates (e.g., Piccolo and

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Mbagwu, 1999), thus exerting a positive influence on soil structure and carbon storage (Spaccini et al., 2002).

While certain environmental and edaphic conditions predispose systems to displaying repellency, water repellency can significantly change over relatively short time periods in response to certain factors; for example, elevated atmospheric $CO_2$ concentrations changed water repellency within a few growing seasons in a New Zealand pasture (Newton et al., 2004). Incidentally, elevated $CO_2$ is a component of global change also known to elicit important responses in fungi (e.g., Rillig et al., 2002).

While factors contributing to soil water repellency have been extensively studied, the exact nature of the organic matter involved, the mechanism(s) behind the expression of hydrophobicity, and the precise involvement of soil biota are still little understood. This limited understanding obviously limits the development of management options (in addition to "symptom treatment", such as surfactant and clay addition). The purpose of this Viewpoint/Idea paper is to propose a mechanism involving soil fungal products, hydrophobins, in the development of non-fire related repellency.

## Microbes as contributors to soil water repellency

Numerous factors influence the development of repellency, including soil moisture, transformation of organic matter, fire, and soil texture (Wallis and Horne, 1992). In terms of hydrophobicity-causing source compounds, plant-produced compounds, such as waxes and others, have been implicated in the production of water repellency (Wallis and Horne, 1992), as have humic acids (Spaccini et al., 2002). However, soil microbes and their products or processing of other organic material can also contribute to soil hydrophobicity (Wallis and Horne, 1992; Hallett and Young, 1999).

Specifically, the involvement of fungi in water repellency has long been acknowledged. For example, the occurrence of basidiomycete-caused 'fairy-rings' in lawns frequently result in zones of localized, high hydrophobicity (e.g., Schantz and Piemeisel, 1917; York and Canaway, 2000).

## The hydrophobin link to soil water repellency

Hydrophobins are a relatively recently (Wessels et al., 1991) discovered class of small (about 100 amino acids), seemingly ubiquitous proteins found in filamentous fungi (Wessels, 1997; Wösten, 2001; Linder et al., 2005). There are currently 70 hydrophobin gene sequences known (Linder et al., 2005); sequence homology is relatively low, but hydrophobins share conserved cysteine residues. Their ubiquity and direct experimental evidence both suggest that these proteins are essential to the life style of filamentous fungi (Wösten and Willey, 2000). For example, hydrophobin knockout mutants cannot form aerial hyphae or fruiting bodies, and secretion of hydrophobins into the culture medium has been shown to lower the surface tension of water, such that hyphae can break through a water film (Wösten et al., 1999).

Research on hydrophobins to date has been carried out largely by molecular biologists and protein biochemists (Linder et al., 2005), focusing on occurrence of hydrophobins in different fungi, and description of hydrophobin structure–function relationships. It seems no research has been carried out on the behavior of hydrophobin proteins in the soil environment (Rillig, 2005).

What are the attributes that contribute to the potential importance of hydrophobins in soil repellency?

(1) **Ubiquity.** Hydrophobins appear to be so intricately involved with the life style of filamentous fungi that they have been found in these fungi universally (e.g., Wessels, 1997; Wösten, 2001). Since this group of proteins is seemingly produced by all filamentous fungi, they would most certainly be produced and present in soils.
(2) **(Potential) persistence**. We currently have no information about hydrophobin persistence in the soil environment, so it is unclear to what concentrations hydrophobins might accumulate in soil solutions and to what extent they are present on soil surfaces (e.g., aggregates, organic material). Nevertheless, if these molecules are deposited, at least in part, as a component of the fungal mycelium (Mankel et al., 2002), their decomposition may be delayed as it is part of this matrix. Additionally, the hydrophobic nature of the compound together with its propensity to form assembled nanostructures (see point (6) below) may in itself present an obstacle to microbial attack, contributing to its persistence in soil. For example, aggregates formed by the hydrophobin SC3 (from *Schizophyllum commune*) cannot be dissolved in detergents (like SDS) or most solvents, except strong acids, e.g. trifluoroacetic acid (Wösten et al., 1993).

(3) **Abundance of filamentous fungi in soils.** Fungi (including mycorrhizal and saprobic soil fungi) are frequently a large or dominant fraction of soil microbial biomass (Paul and Clark, 1996). For example, arbuscular mycorrhizal fungi alone can be present at lengths of tens of $m\,g^{-1}$ soil (Rillig, 2004a). Hence there would be a corresponding abundance of hydrophobin-producing biota in soils. Even if hydrophobins have an ephemeral existence in soils, the sheer abundance of producers in most ecosystems suggests that these molecules would be replenished at a significant rate.

(4) **Amphiphilic nature and surfactant activity of hydrophobins.** Hydrophobins are surface-active molecules. In fact, hydrophobins are among the most potent surface active proteins known (under in vitro conditions the hydrophobin SC3 could lower water surface tension from 72 to $24\,J\,m^{-2}$; Wösten et al., 1999). This occurs through the spontaneous formation of a hydrophobin film at the air-water interface. Amphiphilic molecules are such that have a hydrophilic and a hydrophobic portion. Hakanpää et al. (2004) have recently shown that an aliphatic side chain is presented at the protein surface, and hypothesized that this conserved feature is likely responsible for the amphiphilic nature of the protein (together with the observed rigidity of the molecule itself, i.e. this aliphatic region will always be exposed). This strong surfactant activity could obviously be quite important in determining water flow through soil pores, if sufficient quantities of secreted hydrophobin(s) were available in the soil solution.

(5) **Surface attachment of hydrophobins.** Hydrophobins do not only change surface tension of water, but they can also adhere to surfaces. Wösten et al. (1994) showed that hydrophobins adhere hyphae to hydrophobic surfaces. Hydrophobins are known to coat surfaces, such as fungal conidia and hyphae (Wösten and Willey, 2000). In fact, hydrophobins can assemble at any hydrophobic–hydrophilic interface into an amphipathic protein film (about 10 nm thick). This suggests that they may also coat surfaces in soils, such as aggregates, organic matter, and fungal mycelium itself. Their attachment at soil surfaces has two potentially important consequences: it may contribute to the relative stability of hydrophobins (see point 2), and through attaching to surfaces, water repellant properties of these surfaces may be altered (see point 6).

(6) **Self-assembly and aggregation/property changes.** Upon self-aggregation and assembly the hydrophobin-layer can have different properties. For example, Torkkeli et al. (2002) showed that non-dried and dried samples of the hydrophobin HFBII had different crystalline structure. Interestingly, in coating surfaces, hydrophobin films can reverse the polarity of a surface; i.e. make hydrophilic surfaces hydrophobic. Changes in soil hydrophobic properties are frequently attributed to conformational changes (of an unknown nature) in soil organic matter (Wallis and Horne, 1992). These changes can occur, for example, after drying of soils. It is hence interesting to hypothesize that a hydrophobin-layer would also form in water-filled soil pores in moist soils, but that upon soil drying this layer would contribute to a coating on soil aggregates, reversing surface polarity. This behavior could contribute to the explanation of drying-induced changes in water repellency (e.g., Dekker and Ritsema, 1994; de Jonge et al., 1999). In particular, it might help explain why drying in some cases increases repellency, while in other cases the opposite happens: hydrophobin coatings on soil surfaces may have caused changes in polarity from hydrophobic to more hydrophilic and vice versa.

## Glomalin-related soil protein as a potential parallel case?

Glomalin is a putative protein produced by arbuscular mycorrhizal fungi (Wright and Upadhyaya, 1996) with unknown biochemistry, currently operationally isolated and quantified from soil as glomalin-related soil protein (GRSP; Rillig, 2004b). A current working hypothesis is that glomalin is a hydrophobin, a claim that can only be substantiated once the molecular biology has been better explored. Nevertheless, some circumstantial evidence suggests a hydrophobin-like nature, including occurrence of GRSP-coating on soil aggregate and hyphal surfaces, formation of a GRSP layer at the water-air interface in pot cultures, presence of GRSP in river foam (Harner et al., 2004), localization of glomalin to the hyphal and spore wall of AMF (Driver et al., 2005), and very high concentrations of GRSP in some soils prone to extreme hydrophobicity (Rillig et al., 2000).

Irrespective of the actual biochemical identity of glomalin, research on this protein presents an interesting contrast to research on hydrophobins, because it has been almost exclusively soil-oriented, whereas the focus on hydrophobin research has been, as mentioned above, strongly on biochemistry/molecular biology. However, if GRSP has properties in common with hydrophobins, it may be possible to generally guide research on the soil environmental behavior of the latter by using some of the work done on the latter. For example, some of GRSP in soil seems to be in the slow SOM pool (turnover in the range of decades; Rillig et al., 2003), and concentrations of GRSP are typically highly correlated with soil aggregate water stability (Wright and Upadhyaya, 1998; Rillig, 2004b). Similar relationships may also hold for hydrophobins. Some of the recalcitrance of GRSP may be explained by the fact that it is tightly bound in fungal mycelium, in addition to being secreted (Driver et al., 2005); this could also apply to hydrophobins.

## Conclusions

The importance of soil repellency, the involvement of fungi in this process, and the potential mechanistic pathway for this involvement via hydrophobins strongly suggests that soil ecological implications of hydrophobin production should be examined. Tools available for experimental studies are isogenic strains of fungi with a hydrophobin gene deleted (e.g., ΔSC3; Wösten et al., 1994) and/or purified hydrophobins themselves. Much could also be gained by exploiting potential parallels of hydrophobins with GRSP, since these two areas of research have been initiated at diametrically opposed ends of the spectrum.

On the applied side, hydrophobins with suitable properties might become potential candidates for naturally based biosurfactants to treat repellency; and conversely, understanding their involvement in creating or diminishing hydrophobicity could open up management opportunities, for example using fungal field inoculants.

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