SOIL WATER REPELLENCY:

INVESTIGATING POTENTIAL BIOLOGICAL DRIVERS

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Introduction

Soil water repellency (SWR) is a phenomenon which leads to a reduction of wetting and infiltration of soils by water. SWR is a significant problem affecting large areas of land throughout the world, and is found in natural, intensively managed and man-made ecosystems (DeBano 2000). SWR can cause a decrease in plant-available water, reducing agricultural crop production. Coincident with the decreased water absorption is increased surface runoff, which causes nutrient losses for surface applied fertilisers, and soil erosion in extreme cases (Wallis, Scotter et al. 1991). Environmental and edaphic conditions can predispose soils to SWR; some, such as elevated temperature, cause changes in SWR over short time periods (Doerr and Thomas 2000).

It is generally accepted that SWR is caused by organic molecules coating the surface of soil aggregates, altering the crust to become hydrophobic (Mainwaring, Hallin et al. 2013). The exact nature and source of these is unknown, and possibly changes depending on soil location. Putative compounds include waxes, fatty acids, lignin (a polyphenol polymer from plant cell walls), plant root exudates, fungal hyphae exudates and condensates from forest or grassland fires (Atanassova and Doerr 2010). Artificial SWR was achieved by the application of organic compounds to soil aggregates, with the most hydrophobic effect from a mixture of alkanes and long chain fatty acids, yet in no case was the induced SWR near that observed in natural soils (Mainwaring, Hallin et al. 2013). An uncharacterised fungal protein, glomalin, has been implicated in SWR (Rillig 2005), and certainly fungi seem to be important both in the formation (Rillig, Mardatin et al. 2010) and amelioration of SWR (Liu, Zeng et al. 2013).

The limited understanding of the underlying causes of SWR necessarily restricts the development of management options to ameliorate the effects. The purpose of this research was to attempt to pin point a biological causal factor for SWR. To this end we looked at the interactions between depth in the soil profile, SWR, soil protein and the key fungal enzyme activities, peroxidase and tyrosinase, responsible for breakdown of lignin.

Methods

Soil was taken from a beef farm at Tihoi, west of Lake Taupo. This was a pumice soil, with properties as given in table 1. The samples were taken from two distinct physical locations: on the tops of small ridges running down a slope, and in the gullies running between the ridges. Vegetation colouration, brown on the ridges and green in the gullies, suggested that the ridge slope soil would be more hydrophobic than the gully soil. The samples were split into five depth layers (0-1, 1-2, 2-4, 4-6, 6-10 cm for gully and 0-2, 2-4, 4-6, 6-8 and 8-10 cm for ridges) and were passed through a 2 mm sieve.

Actual and potential water droplet penetration times (WDPT) were measured by water droplet penetration test (Dekker, Ritsema et al. 1998) and contact angles determined by the molarity of ethanol droplet (MED) method (Moody and Schlossberg 2010).

Peroxidase and tyrosinase activities were determined by a modification of the methods of Gallo, Amonette et al. (2004) and Bach, Warnock et al. (2013). Briefly, 0.5 g of fresh soil was homogenised in 50 ml of 50 mM acetate buffer, pH 5.0 for 1 minute in a Polytron blender. The suspension was mixed in a magnetic tumble stirrer (V&P Scientific, USA) and eight 200 µl samples were taken using a multipippete and added to a microtitre plate, with a further eight samples as control. For the peroxidase assay, 10 µl of 0.3% hydrogen peroxidase was added. The 50 µl of 10 mM L-DOPA was added to the samples, and the plates incubated at room temperature for three hours with constant tumbling. At the end of the period, substrate was added to the controls, and all plates were centrifuged at 1500 g for 3 minutes. Aliquots of 200 µl of the supernatant were transferred to a second microtitre plate, and the absorption read at 450 nm.

Bradford-reactive soil protein (BRSP) was extracted by the method of Rosier, Hoye et al. (2006). Air dry soil (1 g) was first extracted with 8 ml of 20 mM citrate buffer, pH 7.0 by autoclaving for 30 minutes at 121 C. This was centrifuged at 5000g for 15 minute, and the resulting supernatant contained the easily extractable (EE-) BRSP. The pellet was repeatedly extracted with 8 ml aliquots of 50 mM citrate buffer, pH 8.0 and autoclaving for 1 hour at 121 C followed by centrifugation, until the supernatant was clear or light yellow in colour. Total BRSP was found by adding the amount of protein in these combined extracts and the EE-BRSP. Protein estimation by the Bradford method did not work, as the soil extracts precipitated with Bradford reagent; instead soil protein concentrations were measured by the Lowry method, as described by Redmile-Gordon, Armenise et al. (2013).

Soil Property	**Average (±standard error)**
Bulk density (g/cm^3)	0.742 (±0.045)
Volumetric water content (m^3/m^3)	0.339 (±0.024)
pH (0-7.5 cm depth)	4.45 (±0.02)
pH (0-4 cm depth)	4.38 (±0.06)
Total carbon (%)	7.2 (±1.1)
Total nitrogen (%)	0.6 (±0.1)
C/N	12
HWC (µg C/g soil)	2000 (±303)

Table 1. Soil Properties.

Results and Discussion

Depth

SWR is thought to be a surface effect, yet most studies measure repellency in the top 5 to 10 cm of soil (Lozano, Garcia-Orenes et al. 2014). In this study, we measured the SWR in 1 or 2 cm bands for

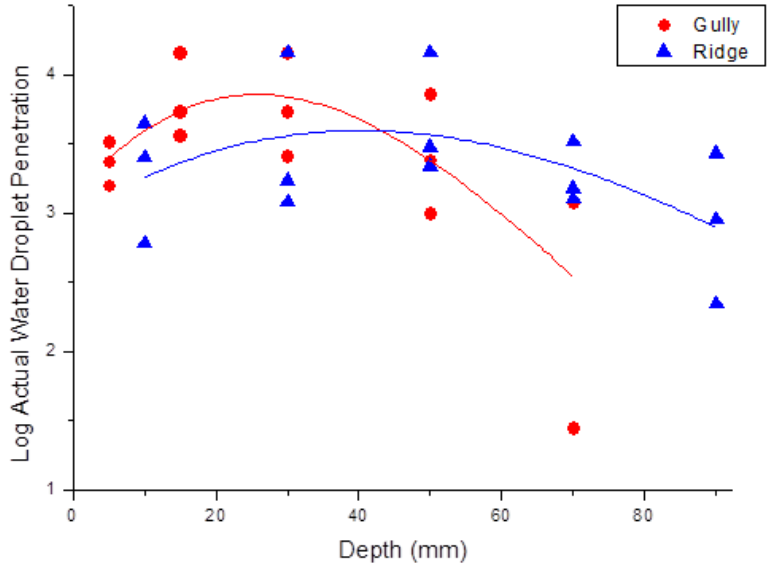

Figure 1. Relationship between actual WDPT and soil depth.

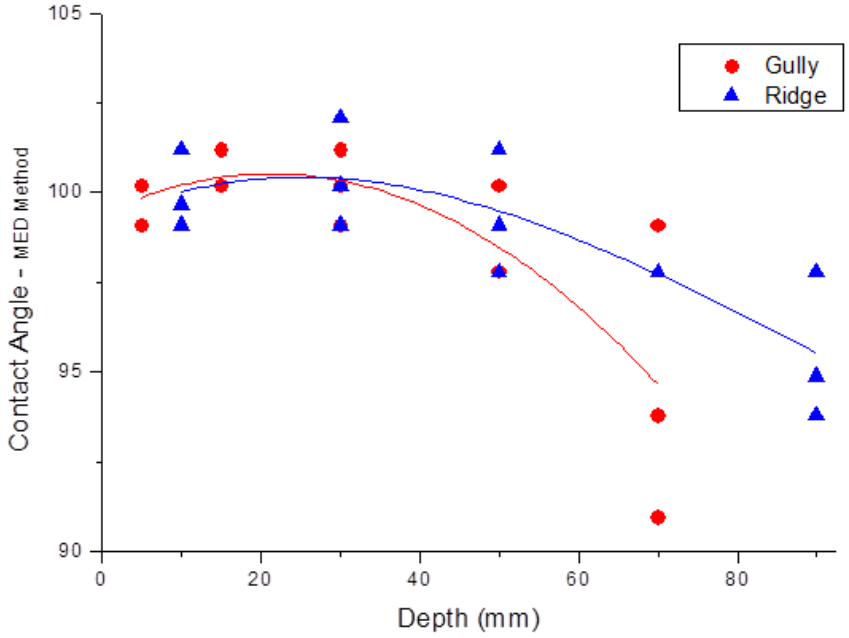

Figure 2. Relationship between contact angle and soil depth.

the top 10 cm of soil. Figures 1 and 2 show the relationship between depth and SWR as measured by actual WDPT and MED tests. Figure 1 shows that SWR increases from the top to a maximum around 3 to 4 cm below the soil surface before falling away; Figure 2 has a similar relationship, although the increase in SWR is much less marked. It is difficult to posit a mechanism that would lead to this distribution of SWR. Possibly leaching of hydrophobic compounds could lead to an increase in concentration at greater depths, but this does not explain the drop off at greater depths. If the disappearance at depth was caused by increased metabolism of the compounds by microbes, then it is difficult to explain why such break down in not occurring near the surface. At the time of sampling it was hoped that the two different locations, gully and ridge, would have differing SWR at the surface. However, this was not the case, instead it can be seen that the difference between these two localities is that the drop in SWR happens closer to the soil surface in gullies than in ridges. The greener vegetation in the gullies may then be the result of the roots being able to penetrate the water repellent layer to better access moisture for growth.

Lignin Digestive Enzyme Activity

The combination of the efficacy of a crude fungal enzyme extract in alleviating SWR (Liu, Zeng et al. 2013) and the implication that lignin is involved in the formation of SWR (Mainwaring, Hallin et al. 2013) led to the thought that SWR should be less in soil fractions where lignin degrading enzyme activity was high. Figure 3 shows the changes in the activities of tyrosinase and peroxidase with depth. The activity of tyrosinase remained low and fairly constant throughout the soil profiles, in contrast, peroxidase activity increased with depth. However, when enzyme activity was compared with contact angle as a measure of SWR, the relationship was less clear cut (Figure 4). While activity does seem to decrease with increasing contact angle, the correlation between the two was poor, and the 90% confidence limits could also include flat and even increasing relationships. The relationship between enzyme activity and actual WDPT (data not shown) was even more inconclusive. This would suggest that for this soil at least there is no relationship between lignin degrading enzymes and SWR.

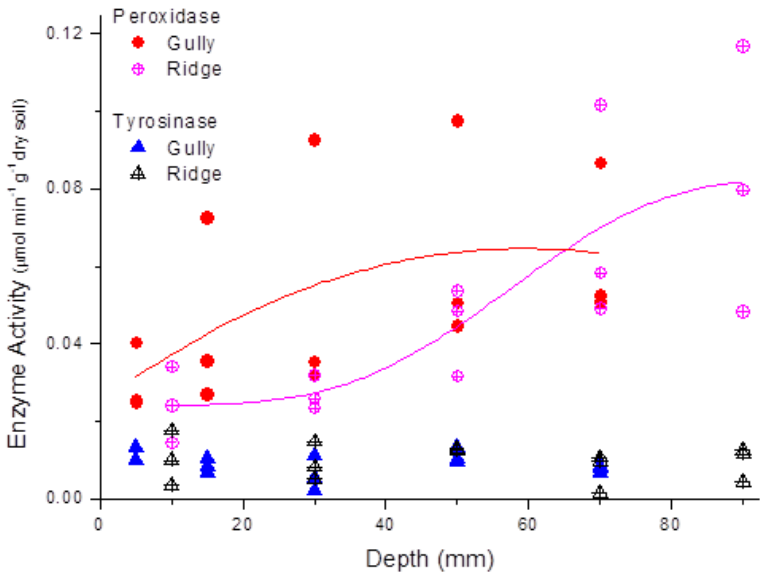

Figure 3. Relationship between enzyme activity and soil depth.

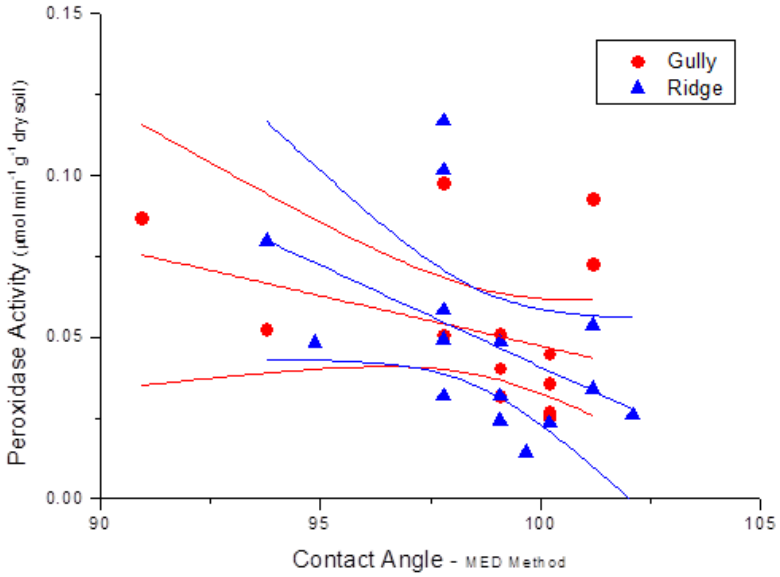

Figure 4. Relationship between peroxidase activity and contact angle. Striaght lines are linear regression fit, with curved lines marking the extremes of the 90% confidence level for the fits.

Bradford-Reactive Soil Protein

BRSP, as glomalin-related protein is currently known (Rosier, Hoye et al. 2006), is an unidentified protein or group of proteins from arbuscular mycrorrhizal fungi that have been implicated in the formation of SWR (Rillig, Mardatin et al. 2010, Wu, Srivastava et al. 2015). BRSP is extracted from soil by repeated autoclaving, and the total protein determined by the Bradford method (Janos, Garamszegi et al. 2008). However, we found that our extracts reacted with Bradford reagent, creating

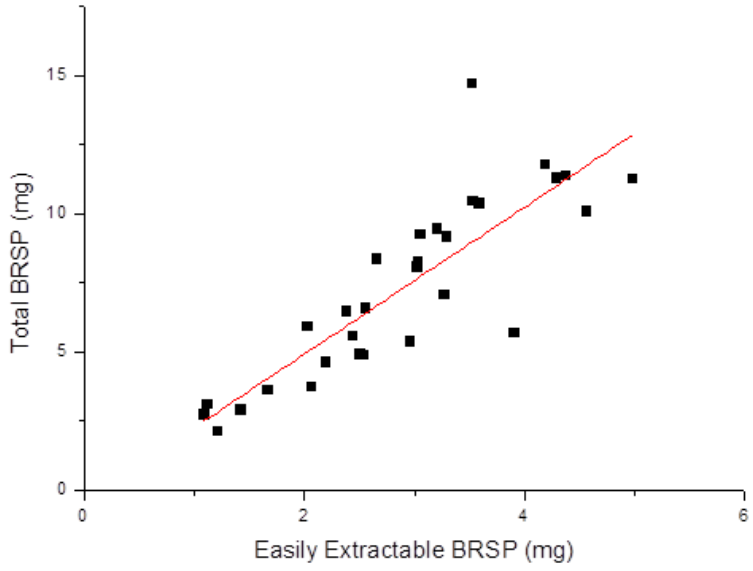

Figure 5. Relationship between easily extractable and total BRSP.

a precipitate. Other recent work has also shown that measurement of protein by the Bradford method can be interfered by humic substances (Jorge-Araujo, Quiquampoix et al. 2015), thus we used an alternative method to measure protein concentration which corrects for humic components, yet gives a similar result to the Bradford method in the absence of humic substances (Redmile-Gordon, Armenise et al. 2013). The two fractions of BRSP, easily extractable and total, showed a clear correlation between the two (Figure 5), as seen in most soils (Rosier, Hoye et al. 2006). When the relationship between EE-BRSP and depth was considered by the two different localities separately, there was less

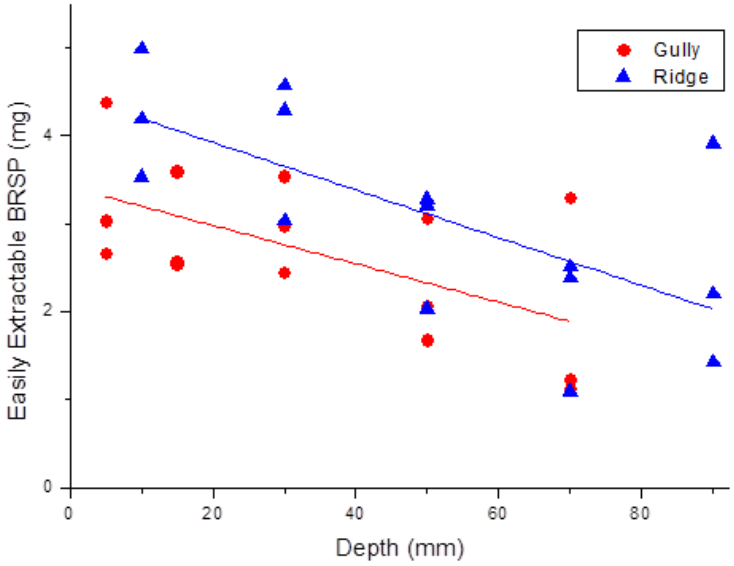

Figure 6. Relationship between easily extractable BRSP and depth.

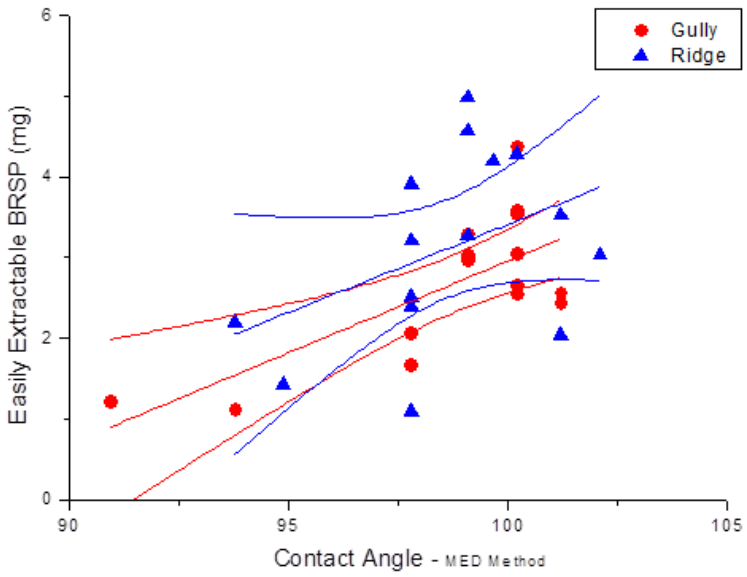

Figure 7. Relationship between easily extractable BRSP and contact angle.

BRSP as depth increased, and less overall in the gully compared to the ridge (Figure 6); however, this depth relationship was not seen if the two localities were pooled. In Figure 7, the relationship between BRSP and contact angle seems to be weakly positive. This is confounded by a few outlying results, and the relationship between actual WDPT (not shown) is almost completely random. This suggests that like Lignin digestive enzyme activity, there is simple relationship between BRSP and SWR.

Conclusions

This research has shown that SWR is not highest at the surface, but rather increases to a maximum at 3 to 4 cm below the soil surface before falling away. Further differences between gullies and ridges at this site are not due to differences in the amount of SWR at the two sub localities, but may be due to the difference in thickness of the SWR layer. No relationship was found between either lignin digestive enzyme activity or BRSP and SWR.

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