THE EFFECT OF SOIL WATER REPELLENCY AND FUNGAL HYDROPHOBICITY ON SOIL WATER DYNAMICS IN THE ATHABASCA OIL SANDS

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By

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ABSTRACT

Surface mining of the Athabasca Oil Sands of Canada is occurring at an unparalleled rate resulting in large scale disturbances over vast areas. Soil water availability for plants is one of the key issues faced when reclaiming the landscape. A factor which limits the soil water availability is soil water repellency (SWR). Soil water repellency is found on both natural and disturbed sites in this region and can cause reduced infiltration, reduced soil water storage, enhanced runoff, increased preferential flow, and reduced ecosystem productivity. Effective characterization of SWR, determination of the causes of SWR and understanding how it affects soil pores and water flow are important for environmental management.

The main objective of this study is to examine the effect of SWR and fungal hydrophobicity on soil water dynamics in Athabasca Oil Sands. This was accomplished by determining the relationship between the measurement of severity and persistence of SWR and the critical water content (CWC) where SWR is greatest between different soils in the region. Examining how the water conducting porosity and soil pores are affected by SWR. Developing methods to quantify fungal strains that cause SWR and testing of these fungal strains for their ability to alter the SWR and infiltration into soil.

Results show that a high severity (Contact angle) of repellency does not necessarily denote long persistence (Water Drop Penetration Time) or high CWC in soils from the region. A high severity of SWR in larger diameter pores decreased the water conducting porosity due to the larger pore contribution to the total liquid flux. The modified microscopy approach and the alcohol percentage test (APT) resulted in improved characterization of fungal hydrophobicity. Fungal strains were classified as hydrophilic, hydrophobic and chrono-amphililic based on their surface properties from these measurements. The surface property of selected fungi strains can alter the SWR in both a repellent and wettable soil and can also change the water infiltration rate.

This research highlights the importance of characterization of SWR, the effects on water flow, and how fungal hydrophobicity can alter the SWR and infiltration. This will aid in improving our understanding of SWR and improve remediation efforts on water repellent soils in the Athabasca Oil Sands region.

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LIST OF ABBREVIATIONS

AE1	A ecosite 1
AE2	A ecosite 2
ALFH	Aurora LFH capping study
APT	Alcohol Percentage Test
ATS	Albian Tailing Sands
CPA	Center pit at Aurora
CA	Contact Angle
CONRAD	Canadian Oil Sands Network for Research and Development
CV	Coefficient of variation
CWC	Critical water content
SCB	Syncrude Coke Bulk
LFH	Leaf, litter and humus layer on soil surface
MED	Molarity of an ethanol droplet
RI	Water repellency index
SW	Water sorptivity
SE	Ethanol Sorptivity
SS Trial	Albian Shallow Stripping Trial
SV10	Soil vegetation plot 10
SV26	Soil vegetation plot 26
SV27	Soil vegetation plot 27
SWR	Soil water repellency

SW30	South west 30
WDPT	Water droplet penetration time

1. GENERAL INTRODUCTION

1.1 Introduction

Soil water repellency (SWR) has received increased attention by the scientific community in recent years since it has been found to be a much more widespread property in soils than previously thought (Hallett, 2008; Müller and Deurer, 2011; Jordán et al., 2013). Soil water repellency is a surface property which causes soil to reduce its affinity to water. This has a direct effect on the infiltration of water in and flow through soils. The presence of SWR in soil often affects whether water moves to surface water as runoff, infiltrates into soil water storage, contributes to drainage into groundwater, or is lost by evaporation (Doerr et al., 2000). Likewise, SWR will also affect the soil's filtering and buffering capability of nutrients and contaminates. The efficient use of water for agricultural crops, forest development, reclamation practices and effective protection of fresh water from contamination could be greatly enhanced by a better understanding of relationship between SWR and soil water.

The Athabasca Oil Sands of Canada is where surface mining of the oil sands is occurring at an unparalleled rate, resulting in large scale disturbances over vast areas. In order for the oil companies in the region to operate within a regulatory framework set within the Land Surface Conservation and Reclamation Act 1973 and the Environmental Protection and Enhancement Act 1992 (Government of Alberta, 1999), they are obligated to conserve and reclaim their disturbed lands. The aim of reclamation entails the complete re-creation of landforms and ecosystems at the landscape scale, with the goal of producing suitable and sustainable habitats for plants and animals (Government of Alberta, 1999).

The soils and materials in the area used for the recreation of landforms originated from coarse textured glacial fluvial and eolian deposits (Turchenek and Lindsay, 1983). Additionally, the oil sands are also situated under the peat lands (bogs and fens) of the boreal forests. As such, reclamation entails using salvaged soil materials, tailings sand, peat, and surface organic matter for reclamation practices. Soil water availability for plants is one of the key issues facing reclaiming disturbed landscapes in the Athabasca Oil Sands Region of Canada due to the dominance of these materials. A limiting factor to soil water availability is soil water repellency. Soil water repellency is found on both natural and disturbed sites in this region (Hunter et al., 2011). Observation of low infiltration rates on reclaimed / distributed landscapes compared to natural landscapes, suggest differences in the severity and persistence of SWR (Hunter et al., 2011). As such, effective characterization of the severity and persistence of water repellency must be done to accurately determine the influence on hydrological processes. To better understand water flow in repellent soils, understanding what pores sizes are influenced by SWR and how SWR influences the conducting porosity is also important. Soil water repellency is dependent on many inter-related and dynamic factors including soil organic matter content, hydrocarbon concentration, fungi and plant exudates, fire, and water content (Doerr et al., 2000). Examination of the causes of SWR is important to provide us with more information on factors to consider when determining the implications of SWR. As well, research into the causes of SWR will also aid in management and remediation practices of severely repellent sites. One of the main causes of SWR is fungi; however correlations between SWR and specific fungal causes have been weak (Savage et al., 1969; Smits et al., 2003). This is mainly due to difficulties with the classification of fungi based on their surface hydrophobicity which are known to cause SWR. The roughness of fungi and fungi mycelia does not allow for effective characterization of contact angle on fungal surfaces (Smits et al., 2003). More efficient methods are needed to quantify fungal hydrophobicity and to classify fungi by their ability to cause SWR. The main objective of this research is to examine the effect of SWR and fungal hydrophobicity on soil water dynamics in the Athabasca Oil Sands, starting from the fundamental principles of SWR characterization (1) by examining the relationship between degree, persistence and the critical water content in water repellent soils, (2) examining SWR effects on the conducting porosity and water flow, (3) development and, (4) modifications of methods to quantify fungal strains based on their surface property that can cause SWR and (5) by examining the effect of fungal strains in soils on SWR and infiltration.

1.2 Organization of the Dissertation

The research presented in this dissertation is organized in a manuscript format. Following this introduction and the literature review presented in Chapter 2, five studies are presented in Chapters 3 through 7. Chapter 3 focuses on the measurement of SWR. The issue with SWR measurement is there is little literature on the effect of water content on SWR measurement (DeJonge et al., 1999; Beatty and Smith, 2013). In addition, measurement techniques only focus on the severity or persistence as an indication of the presence of repellency. Since SWR is a dynamic property affected by soil water content, the objective of this chapter was to examine the severity as a function of persistence and water content in water repellent soils. This will aid our understanding of SWR under different water content conditions. In the fourth chapter the objective is to examine the effect of SWR on the conducting porosity. As the soil water content increases, the severity and persistence of SWR decreases. This phenomenon will influence the water flow in certain pores. Understanding how repellency affects water flow in certain pores is important in terms of water management.

Chapter 5 focuses on how to measure the hydrophobicity of fungal strains that cause water repellency. The objective of this chapter was to develop a method to measure the hydrophobicity of different fungal strains. This is useful for determining fungal strains that have the ability to change SWR. A challenge for both classification of water repellency in soil and hydrophobicity of fungal strain based on contact angle is surface roughness (Unestam, 1991; Smits et al., 2003). This surface roughness is due to rough particle surfaces of soil and the mycelia of fungi. In chapter 6, a previously developed method using different concentrations of ethanol solutions with different surface tension to measure the spread of solution on the soil surface or infiltration as indication of the severity (Chau et al., 2010) was tested on fungi. The objective of this chapter was to examine if the contact angles measured on the fungal surface are related to the percentages of alcohol droplets. This method would be suitable for measurement of fungal culture with large aerial mycelia which would obscure the view of CA on fungi.

Application of surfactants, amendments are not a economically and environmentally viable option to reclaim repellent soils due to cost of transport and potential negative impact to the environment (Doerr et al., 2000). In Chapter 7, the objective was to examine the use of fungal strains or the stimulation of fungal strains to change the SWR in soil. In this dissertation, I have provided better measurement techniques for determination of SWR, determined which fungal strains could cause SWR, and improved our understanding of how the wetting behaviour of repellency is affected in different pore sizes and the influence on water flow. Chapter 8 is the conclusion of this dissertation, where I summarize major findings, limitations and future directions of the research.

2. LITERATURE REVIEW

2.1 Soil Water Repellency

Soil water repellency or hydrophobicity is defined as the state whereby the soil does not wet spontaneously when water is applied, and is increasingly recognized as a problem (Wallis, 1992). Water repellent soils are found throughout the world on grasslands (Dekker and Ritsema, 1994), forests (Buczko et al., 2002), agricultural lands (Hallett and Young, 1999), and also on disturbed/reclaimed areas (Roy and McGill, 1998; Wallach et al., 2005). The main effects of SWR are reduced infiltration (DeBano, 1971; Wallis et al., 1993) and water storage (DeBano, 1981; Hendrickx et al., 1993), increased overland flow and soil erosion (King, 1981; Dekker and Ritsema, 1994; Shakesby et al., 2000; Ellies et al., 2005; Cerdà and Doerr, 2007), development of fingered flow or preferential flow paths, creation of unstable, irregular wetting fronts (Hendrickx et al., 1993; Dekker and Ritsema, 1994; Bauters et al., 2000; DeBano, 2000; Buczko and Bens, 2006; Carrick et al., 2011) and delayed seed germination (Osborn et al., 1967). However, low levels or sub critical SWR has been shown to be important for stabilizing soil structure (Tillman et al., 1989) and soil aggregates (Hallett and Young, 1999), improving soil water storage (Kobayashi and Shimizu, 2007), and preventing dispersion and erosion of soil (Ellies et al., 2005).

During the past years, SWR has been studied intensively. Studies examining the impacts of SWR have focused on water repellency in natural soils (Crockford et al., 1991; Woche et al., 2005), finger flow and finger formation (Ritsema et al., 1997; Bauters et al., 1998), water flow in field studies (Wang et al., 2000b; Wallach et al., 2005), infiltration of water into water repellent soils (Wang et al., 2000b; Wallach et al., 2005; Lamparter et al., 2006; Carrick et al., 2011; Ganz et al., 2013), and finally the effect of water content on water repellency (DeJonge et al., 1999; Doerr and Thomas, 2000; Liu et al., 2012). Management strategies such as reducing evaporation, increasing infiltration, optimizing water retention, controlling water movement in soils and amelioration of repellency have been implemented to improve soils affected by this condition (Ritsema and Dekker, 2003; Hallett, 2008; Müller and Deurer, 2011). However, these strategies are neither cost effective nor entirely practicable due to our gaps in our understanding of how SWR functions in soil.

2.2 Causes of Soil Water Repellency

Soil water repellency occurs in many soils around the world (Leelamanie et al., 2010). Coarse textured soils are more susceptible to SWR (Tschapek, 1984; Harper and Gilkes, 1994; DeJonge et al., 1999; Woche et al., 2005; Karunarathna et al., 2010) due to lower surface area per unit volume than fine textured soils, meaning less hydrophobic material is required to coat soil to have the same severity of SWR (Doerr et al., 2000). Soil water repellency is primarily caused by hydrophobic compounds coating the mineral surfaces of soil particles (Tschapek, 1984; Ma'shum et al., 1988; Doerr et al., 2000; Ellies et al., 2005; Diehl and Schaumann, 2007; Karunarathna et al., 2010). Soil mineral particles have a high affinity for water (Tschapek, 1984), but when coated with hydrophobic or amphiphilic compounds they become repellent in nature (Hudson et al., 1994; Ellies et al., 2005; Buczko and Bens, 2006). These organic compounds are derived from sources from vegetation (Bond, 1964; Franco et al., 2000), fungi (Bond and Harris, 1964; Savage et al., 1969; Dekker and Ritsema, 1996), microorganisms (Schaumann et al., 2007; Fisher et al., 2010; Bond and Harris, 1964; York and Canaway, 2000), humic acids (Roberts and Carbon, 1972; Chen and Schnitzer, 1978), decomposed plant material (McGhie and Posner, 1987; Doerr, 1998; Ellies et al., 2005), fires (Shakesby et al., 1993), and hydrocarbons (Roy and

McGill, 1998). Soil water repellency is a dynamic phenomenon that changes with water content (Dekker and Ritsema, 1994). Change in water content causes hydrophilic portions of amphiphilic organic compounds to reorient themselves, leaving mainly hydrophobic areas exposed (Savage et al., 1969; DeBano, 1981; Doerr, 1998; Lichner et al., 2007). Just as drying induces SWR, prolonged exposure to water weakens repellency by re-exposing hydrophilic portions of organics (Doerr et al., 2000).

The origin of SWR remains elusive, making it difficult to assess management strategies that control its occurrence in soil. Despite years of research (Wallis and Horne, 1992; DeBano, 2000; Doerr et al., 2000; Hallett, 2008; Jordán et al., 2013), a comprehensive understanding of the repellency phenomenon from a biological perspective is still lacking. Although studies are mixed on the causes, fungi are generally thought to be a prime cause of water repellency in soils (Hallett, 2008). Hallett and Young (1999) found that stimulating the microbial biomass with nutrients can greatly enhance repellency in soils. As well, Feeney et al. (2006) reported a strong relationship between fungal biomass and SWR. Furthermore, Hallett et al. (2001) selectively inhibited either fungi or bacteria on a sandy soil with biocides to separate the influence of each group on SWR. Inhibition of fungal growth decreased the development of SWR after 10 days of incubation in a nutrient amended soil. By inhibiting bacterial proliferation, SWR was greatly enhanced, possibly because bacteria can degrade hydrophobic compounds and/or the native fungi experienced less competition (Hallett et al., 2001b). Research to date into soils has identified fungi as the dominant microbial group that causes water repellency, while bacteria may decrease repellency (Roper, 2004). However, certain fungal strains do not express hydrophobic surface properties; instead express hydrophilic surface properties (Unestam and Sun, 1995). Little is known about how these surface properties from fungi can alter SWR.

Hydrophobins, a class of small amino acids which are found in filamentous fungi (Wessels, 1996), has created interest in its connection to SWR (Rillig, 2005). Linder (2009) found that the increase in fungal surface hydrophobicity is related to the amount of hydrophobins produced on fungal surfaces. Rillig et al. (2010) was the first to report a causal relationship between the growth of AM fungal mycelia and SWR. This relationship is due to the presence of a hydrophobin-related protein: glomalin. They both speculated the hydrophobins and glomalin-related surface proteins (GRSP) on fungal surfaces might be the cause of the increased SWR (Rillig, 2005). However, conflicting evidences suggest that hydrophobins and GRSP in some fungal strains does not necessarily confer water repellent surface properties (Hallett et al., 2009; Mosbach et al., 2011). As such, the cause of SWR due to fungi remains inconclusive. However, characterization of water repellency from fungal surfaces properties as a whole and its effect on SWR may give a better indication on the role that fungi play in SWR (Spohn and Rillig, 2012). Additional, the presence of fungal hyphae in soil will cause clogging of soil pores and effectively decrease infiltration (Fig. 1). Coupling hyphae clogging of soil pores with fungal hydrophobicity would result in further decrease in infiltration (Fig.1.) (Seki et al., 1998; Fisher et al., 2010). Since fungi are one of the primary causes of SWR in soils, examining its effect on SWR will also improve our understanding of how SWR can alter infiltration in soils.

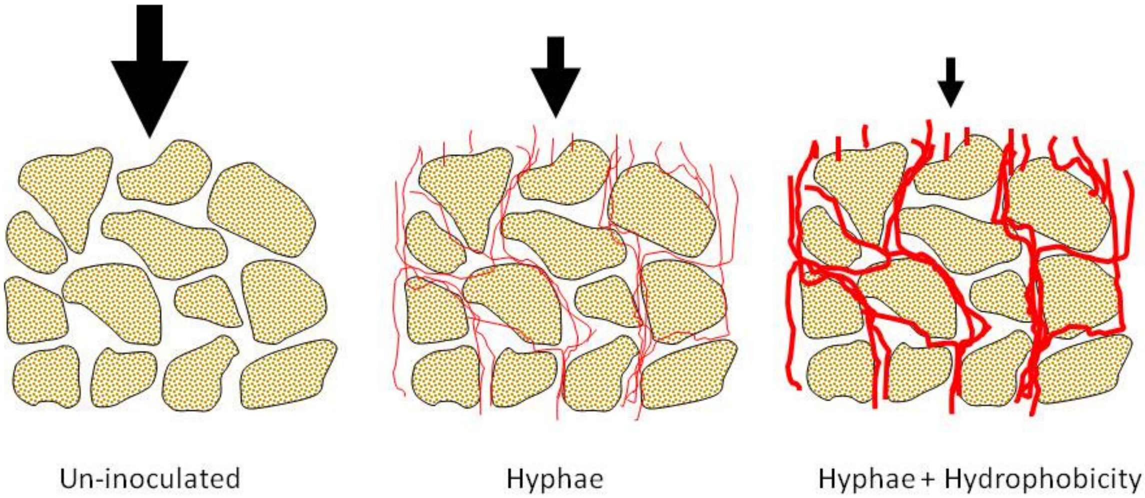

Fig. 1. Diagram of decreasing infiltration due to presence of hyphae and presence of hyphae and fungal hydrophobicity

2.3 Measurement and Characteristics of Soil Water Repellency

Several approaches exist for measuring and quantifying SWR. These include: water drop penetration time (WDPT) test, molarity of ethanol droplet (MED), repellency index (RI), and contact angles (CA) (Wallis and Horne, 1992; Letey et al., 2000). Although these are simple tests which are easy to reproduce, the correlation between their results is often rather weak (Czachor, 2006). The most direct way to determine SWR is to measure the contact angle (CA) of water on the soil surface. Quantitative measurements of soil contact angles are influenced by composition of the soil, porosity, surface roughness and chemical heterogeneity of natural soil grains (Busscher et al., 1984; Woche et al., 2005; Shang et al., 2008). Several methods have been used to calculate or directly measure CA, including capillary rise (Emerson and Bond, 1963) and modified capillary rise (Bachmann et al., 2003), Wilhelmy plate (Bachmann et al., 2003) and sessile drop (Bachmann et al., 2000) methods. The sessile drop method (Bachmann et al., 2000) is the preferred method to determine the CA directly from a droplet on the soil surface.

This method allows for measurement of CA in the range of 0-180º with an accuracy of < 6º (Bachmann et al., 2003). This direct measurement of CA is simple, accurate and requires the least amount of extrapolation. Shang et al. (2008) found that the sessile drop method yielded the most consistent results as compared to other contact angle measurements.

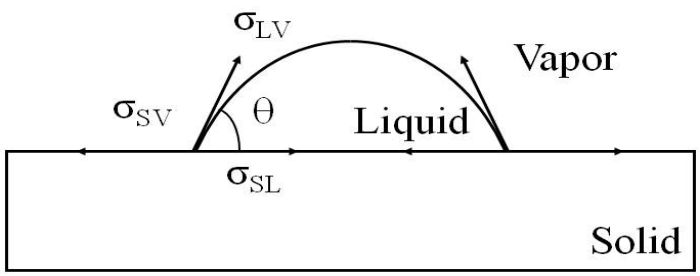

Fig. 2. Diagram of the contact angle formed by a water droplet placed on a repellent surface.

The severity can be described as how strongly the soil repels water, which is determined by measuring the CA (Fig. 2, 3a). When a water drop is placed on a repellent surface, the droplet is not absorbed, forming a bead on the surface which depends on the relation between the three interfacial energies; liquid-vapour (σ_{LV}), solid vapour (σ_{SV}) and solid liquid (σ_{SL}) formulated in Young's equation (Eq. 2.1);

$$\sigma_{LV} \cos(\theta) = \sigma_{SV} - \sigma_{SL} \qquad \text{(Eq. 2.1)}$$

where θ is the contact angle formed between the soil and the water droplet. Classification scheme for SWR using contact angles is described by King (1981). The severity (degree) of SWR can range from sub-critical (slightly) to extremely repellent. Subcritical SWR, defined as the contact angle of a water droplet on a soil surface larger than 0° but less than 90°, is often ignored when examining the effects of SWR on water flow (Tillman et al., 1989). This is due to less severe changes to hydrologic processes in the soil as observed in an extremely water repellent soil.

Severity provides information about the risk of runoff and erosion during a rain fall event, since water will not enter the soil when the severity is high (Cerdà and Doerr, 2007; Miyata et al., 2007).

A water droplet may remain as a drop in a finite area (static), or it may spread or be absorbed over the surface (dynamic), which is indicative of a decrease in the persistence (Letey, 1969; Letey et al., 2000). Persistence can be defined as how long the soil remains water repellent in the presence of water (Fig. 3a). Because SWR is temporally variable, its persistence is also of interest. An understanding of the persistence of water repellency provides information about wettability of the soil in the long term. In general, quantitative classification of persistence is measured by the time it takes for water droplets to be absorbed/infiltrate, defined as the water drop penetration time (WDPT) (Dekker and Jungerius, 1990). A decrease in the persistence is associated with the surface energy required to shift the soil from a repellent state to a more wettable state. Surface energy gained in forming the solid liquid interface should exceed the liquid air surface for spreading to occur (Eq. 2.2):

$$\sigma_{SV} - \sigma_{SL} > \sigma_{LV} \qquad \text{(Eq. 2.2)}$$

The surface energy is determined by the composition of the hydrophobic compounds, functional groups, orientation, and the nature of the intermolecular forces between them (Roy and McGill, 1998). Non-hydrophobic compounds are associated with non-polar molecules and dispersion forces, while hydrophobic compounds are associated with polar molecules, which have hydrogen bonding and dipole/dipole interactions (Roy and McGill, 1998). There are both hydrophobic and non hydrophobic compounds that coat soil particle surfaces (Doerr et al., 2000). The arrangement of the molecules oriented on the surface of soil particles determines how the molecules reorientate themselves during the rewetting processes. This causes a shift the soil from a repellent state to more a wettable state, relating to the persistence of the SWR. The time of infiltration of a water droplet is directly relevant to the erosion potential and potential water runoff since water will not infiltrate until the persistence of SWR is gone (Wessel, 1988).

Another method for characterization of the severity of SWR is molarity of an ethanol droplet (MED) test (Watson and Letey, 1970; King, 1981; Doerr, 1998), dilutions or concentrations of ethanol and water, with known surface tensions (or energies), that are applied to the soil for determination of the surface energy of the soil. The MED test is recorded as the lowest ethanol:water concentration to penetrate the soil (Crockford et al., 1991; Dekker and Ritsema, 1994; Doerr, 1998; Doerr and Thomas, 2000; Cofield et al., 2007). Roy and McGill (2002) found that the MED test is not reliable at field moist conditions as it is not sensitive to subcritical water repellency.

The repellency index (RI) compares sorptivity of water and ethanol from the infiltration of these two liquids into soil (Fig. 3b) (Wallis et al., 1991). This uses the sorptivity, calculated from the unsaturated flow rate of two liquids in soil, determined using the tension infiltrometer (Tillman et al., 1989; Wallis et al., 1991). For a liquid to enter into soil, its surface energy must be less than that of the soil (Doerr, 1998). Thus, the infiltration of water will be impeded if the soil has a high surface energy, as is the case for water repellent soils. The test liquid will infiltrate if the surface energy (tension) is sufficiently lowered (Van’t Woudt, 1959). (95% v/v) Ethanol, with a much lower surface energy than water behaves in water repellent soil in a similar way that water would in the same soil if the soil were wettable (Letey et al., 1962). Because SWR affects infiltration of water but not ethanol, comparisons of their sorptivies in soil are used to characterize SWR (Roy and McGill, 2002; Lamparter et al., 2010). The tension infiltrometer infiltrates a test liquid (water and ethanol for these studies) into soil under a negative tension to exclude macropore flow. Sorptivity can be determined when cumulative infiltration is plotted against the square root of time. Initial or early time sorptivity, calculated from infiltration and time measurements is described by Philip, (1954) (Eq. 2.3) as

$$i = St^{1/2} \qquad \text{(Eq. 2.3)}$$

where, *i* is the cumulative infiltration (L T^{-1}), for each measured pressures, *S* is sorptivity (L T^{-1}) of infiltrating liquid and *t* is the time (T). Sorptivity (*S*) can be determined at steady state by (Leeds-Harrison et al., 1994) (Eq. 2.4)

$$S = \sqrt{\frac{Qf}{4br}} \qquad \text{(Eq. 2.4)}$$

where *Q* is the steady state infiltration rate, *b* is the parameter dependent on the soil water diffusivity function, taken as 0.55 for soil with unknown *b* parameter (White and Sully, 1987), *r* is the radius of disc, and *f* is the fillable (air filled) porosity.

Soil-water sorptivity is affected by SWR, whereas soil-ethanol sorptivity is not (Letey et al., 1962). As such, the corrected soil-ethanol sorptivity is used as the measure of the intrinsic property of soil against which the impeded soil-water sorptivity is compared to. The repellency index (RI) can be calculated with (Tillman et al., 1989) (Eq. 2.5).

$$RI = 1.95 \frac{S_E}{S_W} \qquad \text{(Eq. 2.5)}$$

where S_E is the sorptivity of 95% ethanol (L $T^{-1/2}$), S_W is the sorptivity of water (L $T^{-1/2}$), and 1.95 is a constant to correct for viscosity and density differences between water and 95% ethanol (Tillman et al., 1989; Wallis et al., 1991). Multiple methods of measuring SWR are required to get a clear picture of the state of SWR in soils. Furthermore, the conditions under which SWR are tested should be carefully controlled and clearly reported (Shirtcliffe et al., 2006; Douglas et al., 2007).

The knowledge of the severity and persistence of SWR in soil is crucial for understanding and predicting how SWR affects hydrological processes; for optimizing plant growth, and for reducing groundwater contamination risk and improving infiltration. However, there is a poor understanding of the persistence of SWR and its effect on soil water infiltration and flow (Doerr et al., 2000). Although it is well known that soils can lose their water-repellent characteristics during a long period of wetting (Crockford et al., 1991), little is known about the exact wetting mechanisms involved, and the threshold conditions needed for SWR to disappear (Doerr et al., 2000). Leelamanie and Karube (2009) performed a study on the severity of SWR and its relation to SWR persistence in hydrophobized sand. They concluded that WDPT, or the persistence, is positively correlated to the initial contact angle. Further, the persistence measured at any point during the wetting processes is the same as that of a soil at the same initial contact angle. Therefore, the relationship between persistence and contact angle is unique, regardless of the initial conditions. If this was truly the case, a single measurement for either the severity or persistence is needed to assess SWR. However, not all water repellent soils will follow this scenario as some soils may have a lower initial severity, but a longer persistence (Dekker and Ritsema, 1994). If the severity and persistence operate independently, management scenarios become more complex. To assess the relationship between severity and persistence, multiple soils must be tested to prove if a high severity equates to high persistence and vice versa (Ju et al., 2008; Lamparter et al., 2010).

Another important factor controlling SWR is the critical water content (CWC). The CWC is an important transition zone where soil turns from a repellent state to a wettable state (Fig. 3c) (Ritsema and Dekker, 2003; Shirtcliffe et al., 2006; Liu et al., 2012). It is the water content at which the effects of SWR are no longer present. Since the measurement of the severity and temporal persistence of repellent soils are usually performed at a single water content, measurements of CWC should be performed because it explains how SWR behaves under different water contents. The soil moisture-related aspect of SWR has important repercussions for land use management due to effects of SWR in soils which have not reach the threshold of the CWC. Determining the relationship between the contact angle, WDPT, and the CWC would be helpful in understanding SWR because the measured WDPT data could be correlated to a range of CA or CWC. Additionally the applicability of this relationship in different types of soils needs further attention.

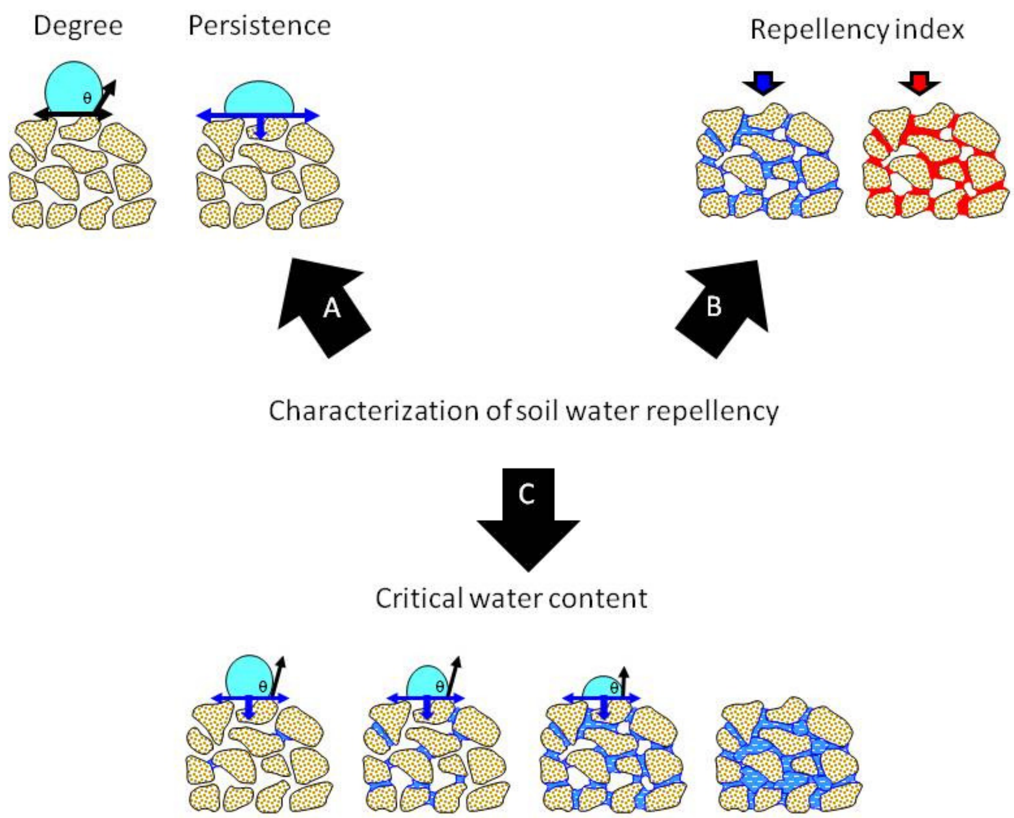

Fig. 3. Diagram illustrating the characterization of soil water repellency. a) Degree and persistence measured as contact angles formed on the soil surface and water drop penetration or spreading time respectively. b) Water repellency index determined by sorptivity ratio of 95 % ethanol and water through infiltration. c) Critical water content is the water content at which soil water repellency is no longer present (no contact angle on soil surface or instantaneous spreading or penetration of water droplet).

2.4 Conducting Porosity in Water Repellent Soils

The soil water conducting porosity is crucial for understanding water, solute, and pollutant infiltration and movement through soils (Beven and Germann, 1982; Ankeny et al., 1990; Luxmoore, 1990). Macroporosity (diameter >1.0 x 10^{-3}m) and mesoporosity (diameter from 1.0x 10^{-5} – 1.0 x 10^{-3} m) are the major fractions of the total soil porosity that contributes to the water conducting porosity in saturated soil (Luxmoore, 1981). Not all these pores contribute to the flow as they can include pores that are non-continuous, dead ended and have irregular pore geometry, which restricts the transport of water (Bodhinayake et al., 2004). Pore toruosity and surface roughness are also known to decrease the conducting porosity.

Since the entry and transport of water from the soil surface is controlled by the conducting porosity (Watson and Luxmoore, 1986), examination into the relationship between SWR and the conducting porosity must be performed. Soil water repellency is highly spatially variable (Hallett et al., 2004) and it operates at the millimeter-scale, making measurements extremely difficult. However, with recent advances using the miniaturized infiltrometer for assessing SWR (Tillman et al., 1989), more accurate characterization at smaller scales has been done (Hallett et al., 2004). Tension infiltrometers measure the infiltration rate at negative water pressures with respect to the atmosphere (Clothier and White, 1981). Since capillary pressure is related to the equivalent pore diameter, the change in the tension or pressure head can estimate the range of pore size contribution to infiltration (Jarvis et al., 1987). Alternatively, tension infiltrometers are being used to observe hydraulic properties or time dependent changes in water repellent soils (Doerr and Thomas, 2000; Hallett et al., 2004; Lichner et al., 2007). Pore water pressures need to be or become negative before infiltration occurs as compared to ponded infiltration techniques. Given that SWR is water content dependent and infiltration under tension is responsive to initial positive pore water pressures and air-entry pressures, the changes in the rate of infiltration into soil or discharge from the tension infiltration instruments can provide useful indications of changes in surface tension of soil (or contact angle change) and associated changes in wettability as time of exposure to water increases (Beatty and Smith, 2013).

Ethanol is considered to be a completely wetting liquid due to its low surface tension as discussed above. As such flow of ethanol into and through water repellent soil is independent of the severity of water repellency of the soil (Watson and Letey, 1970). Tillman et al. (1989) found that infiltration using ethanol under tension, the measurement of the intrinsic sorptivity can be determined. The intrinsic sorptivity is defined as soil sorptivity independent of surface properties and only depends on the pore geometry of the porous system. Surface properties of the soil are an issue when sorptivity is measured with an incomplete wetting liquid, such as the case of water in a water repellent soil. Lamparter et al. (2006) and Jarvis et al. (2008) both used ethanol and water as the infiltrating liquids under tension to evaluate the effect of SWR on hydraulic processes. They found reductions in water infiltration rates caused by SWR as compared to ethanol (Lamparter et al., 2010). The ratio of the two sorptivities from the early time or steady state infiltration curve can also give an indication of how repellent the soil is under different water content situations. In this way, the overall contribution of SWR to the total conducting porosity excluding the macropores is assessed. However, this does not distinguish the contribution of specific water repellent pore sizes to the total water flux. There is little research on how the RI changes with exclusion of different pore sizes (decreasing pressure head).

Many methods have been developed to calculate the water conducting porosity. Watson and Luxmoore (1986), Dunn (1991) and Bodhinayake et al. (2004) utilized minimum equivalent pore radius, mean pore radius, and a range of pore radii for determination of the conducting porosity, respectively. Due to the overestimation of the conducting porosities from Watson and Luxmoore (1986) and Dunn (1991), Bodhinayake et al. (2004) is the preferred method for characterization of the conducting porosity. Nevertheless these conducting porosity estimations do not take into account the presence of SWR. Using 95% ethanol infiltration, the intrinsic conducting porosity can be determined which is the conducting porosity independent of soil surface properties (Lamparter et al., 2010).

3. RELATIONSHIP BETWEEN THE SEVERITY, PERSISTENCE OF SOIL WATER REPELLENCY AND THE CRITICAL SOIL WATER CONTENT IN WATER REPELLENT SOILS[1]

3.1 Preface

Characterization of SWR is difficult due to fact that SWR is dependent on water content and exposure time to water (Shirtcliffe et al., 2006). Previous studies focus only on the presence or absence of repellency in site as indicator of SWR (Letey, 1969; Müller and Deurer, 2011). Soil water repellency is often measured for severity, persistence or the soil critical water content independently. Measurements of severity and persistence are related to the differences and changes in surface energy between water and the soil surface respectively and may operate independently of each other. However, these measurements will not give consistent results (Shang et al., 2008). Characterizing and understanding the severity, persistence and CWC together are valuable when determining how SWR will affect soil water flow. The objective of this study was to determine the relationship between the severity of SWR and its persistence and to determine the critical water content (CWC). The severity of SWR as a function of persistence was assessed by measuring the change of water drop contact angles (modified sessile drop method) with time (WDPT, water drop penetration time) and by water content on soils.

3.2 Abstract

Soil water repellency (SWR) causes reduced soil water storage and enhanced runoff and reduced ecosystem productivity**.** As such, characterization of SWR is a prerequisite for effective

[1] This work has been previously published in Chau, H.W., Biswas A, Vujanovic, V. and Si, B.C. (2013), Relationship between the severity, temporal persistence and the critical soil water content in water repellent soils. Accepted in Geoderma (GEODER-S-13-00167). Minor modifications have been made for consistency.

environmental management. The objective of this study was to determine the relationship between the severity of SWR and its persistence and to determine the soil critical water content (CWC). Soils were collected from thirteen soil sites; five from natural jack pine (*Pinus banksiana*) ecosites (AE1, AE2, SV 10, 26 and 27), six from reclaimed/disturbed sites (ALFH, CPA, SS trial, ATS, SCB, and SW30) located in the Athabasca Oil Sands region and two from agricultural sites (Goodale and Melfort) in Central Saskatchewan, Canada. The severity of SWR as a function of persistence was assessed by measuring the change of water drop contact angles (modified sessile drop method) with time (WDPT, water drop penetration time). The CWC was determined for all the soils by measuring water drop contact angles on soils with predetermined water contents from oven dried to 0.20 kg kg^{-1}. In natural, reclaimed and agricultural soils, a high severity (contact angle) of repellency does not necessarily denote long persistence (WDPT) or high CWC. Measurement of severity and persistence are related to the differences and changes in surface energy between water and the soil surface respectively. Although the CWC gives us the water content at which above it SWR is negligible, the trend between contact angle and increasing water content proved to be more informative. Characterizing and understanding the severity, persistence and CWC together are valuable when determining the effects of SWR on hydrological processes as the mechanisms involved may differ from one another.

3.3 Introduction

The Athabasca Oil Sands of Canada are estimated to contain over 170 billion barrels of oil, making it the second largest viable oil deposit in the world (Kraemer et al., 2009). Surface mining of the oil sands is occurring at an unparalleled rate, resulting in large scale disturbances over vast areas. The oil companies in the region are obligate to operate within a regulatory framework to conserve and reclaim their disturbed land as set out within the Land Surface

Conservation and Reclamation Act 1973 and the Environmental Protection and Enhancement Act 1992 (Government of Alberta, 1999). The aim of reclamation entails the complete re-creation of landforms and ecosystems at the landscape scale, with the goal of producing suitable and sustainable habitats for plants and animals (Government of Alberta, 1999).

The soils in the area originated from coarse textured glacial fluvial and eolian deposits (Turchenek and Lindsay, 1983). Additionally, the oil sands are also situated under the peat lands (bogs and fens) of the boreal forests. As such, reclamation entails using salvaged soil materials, tailings sand, peat, and surface organic matter for reclamation practices. Given the presence of hydrocarbon, and that coarse textured and organic soils dominate the Athabasca Oil Sands region (Government of Alberta, 1999), the issue of water repellency in soil has yet to be addressed (Hunter et al., 2011). The low surface area per unit volume of coarse textured soils (Doerr et al., 2000; Lehrsch and Sojka, 2011) and excessive drying of both soil and organic materials stockpiles (Moskal et al., 2001) for future reclamation projects may contribute or enhance expression of water repellency in soil.

Soil water repellency (SWR) has an effect on hydrological processes due to reduced infiltration, increased overland flow, increased preferential flow, decreased soil water storage, and increased soil erosion (Doerr et al., 2000). It has been reported in many types of soils at variable severities (DeBano, 1981) and is considered as the “norm rather than the exception” (Wallis et al., 1991). Water repellent soils are found throughout the world on grasslands (Dekker and Ritsema, 1994), forests (Buczko et al., 2002), agricultural land (Hallett and Young, 1999), and also on disturbed mining sites in Athabasca Oil Sands (Roy and McGill, 1998; Wallach et al., 2005). Additionally SWR has been found to affect the spatial distribution of vegetation in landscapes leading to patchiness of growth (DeBano, 1981; Lozano et al., 2013). As such, the role SWR plays in water flow processes is important for effective drought mitigation and water management to improve vegetation growth.

The effect of SWR on soil is related to its severity and persistence. The severity can be described as how strongly the soil repels water, which is measured by water drop contact angles. When a water drop is placed on a repellent surface, the droplet does not absorb, forming a bead on the surface which depends on the relation between the three interfacial energies; liquid-vapour, solid-vapour, and solid-liquid. The severity (degree) of SWR can range from sub-critical (slightly) to extremely repellent. Subcritical SWR, defined as the contact angle of a water droplet on a soil surface larger than 0° but less than 90°, is often ignored when examining the effects of SWR (Hallett et al., 2001a). This is due to less severe changes to hydrologic processes in the soil as observed in an extremely water repellent soil. Additionally, methods such as MED (Molarity of ethanol droplet method), are unsuitable for determination of the severity of SWR in sub-critically repellent soils since different subcritical severities of water repellency are difficult to distinguish (King, 1981).

A water droplet may remain as a drop in a finite area (static), or it may spread or be absorbed over the surface (dynamic), which is indicative of a decrease in the difference between the soil surface tension and liquid surface tension. Persistence can be defined as how long the soil remains water repellent in the presence of water. In general, quantitative classification of persistence is measured by the time it takes for water droplets to be absorbed/infiltrate, defined as the water drop penetration time (WDPT) (Doerr, 1998). Persistence of SWR can be classified by WDPT as described by Dekker and Jungerius (1990). Persistence of SWR is associated with the surface energy required to shift the soil from a repellent state to a more wettable state. Surface energy gained in forming the solid liquid interface should exceed the liquid air surface for spreading to occur. The surface energy is determined by the composition of the hydrophobic compounds, functional groups, orientation, and the nature of the intermolecular forces between them (Roy and McGill, 1998; Cheng et al., 2009, 2010). Non-hydrophobic compounds are associated with non-polar molecules and dispersion forces, while hydrophobic compounds are associated with polar molecules, which have hydrogen bonding and dipole/dipole interactions (Roy and McGill, 1998). There are both hydrophobic and non hydrophobic compounds causing coatings on the soil particle surface (Doerr et al., 2000; Horne and McIntosh, 2000). The arrangement of the molecules orientated on the surface of soil particles determines how the molecules reorientate themselves during the rewetting processes (Roy and McGill, 2000). This is necessary to shift the soil from a repellent state to more a wettable state.

The knowledge of the severity and persistence of water repellency in soil is crucial for understanding and predicting how it affects hydrological processes; for optimizing plant growth, and for reducing groundwater contamination risk on reclaimed land. However, there is a poor understanding of the persistence of SWR and its effect on soil water flow (Doerr et al., 2000; Ganz et al., 2013). Although it is well known that SWR may decrease or disappear during long wetting periods (Crockford et al., 1991), little is known about the exact wetting and rewetting mechanisms involved, and the threshold conditions needed for SWR to disappear (Doerr et al., 2000; Jordán et al., 2013). Leelamanie and Karube (2009) performed a study on the severity of SWR and its relation to persistence in hydrophobized sand. They concluded that persistence of SWR (measured as WDPT) is positively correlated to the initial contact angle. Further, the persistence measured at any point during the wetting processes is the same as that of a soil at the same initial contact angle. Therefore, the relationship between persistence and contact angle is unique, regardless of the initial conditions (Leelamanie and Karube, 2009). If this was truly the case, a single measurement for either the severity or persistence is needed to assess SWR. However, not all water repellent soils will follow this scenario as some soils may have a lower initial severity, but a longer persistence (Dekker and Ritsema, 1994; Ganz et al., 2013). If the severity and persistence operate independently, management scenarios become more complex. To assess the relationship between severity and persistence, multiple soils must be tested to prove if a high severity of SWR equates to high persistence of SWR and vice versa (Ju et al., 2008; Lamparter et al., 2010). Additionally, naturally water repellent soil material should be tested to assess moisture dependent wettability.

Another important factor controlling SWR is the critical water content (CWC). The CWC is an important transition zone where soil turns from a repellent state to a wettable state (Ritsema and Dekker, 2003). It is the water content at which above it the expression of SWR is no longer present. Since the measurement of the severity and persistence of repellent soils are usually performed at single water content, measurements of CWC should be performed because it explains how SWR behaves under different water contents. The soil moisture-related aspect of SWR has important repercussions for land use management due to the effects of SWR in soils which have not reached the threshold of the CWC. In an effort to better quantify the effect that different SWR has on hydrological processes such as infiltration. It is important to determine relationship between severity and persistence in water repellent soils. The purpose of this study was to determine the relationship between the severity, persistence and the CWC in soils with varying SWR. I hypothesized that there is a difference in the relationship between the severity, persistence of SWR and the critical water content.

3.4 Materials and Methods

3.4.1 Study Sites and Soil Description

The study areas are located in the Canadian Boreal Forest Region within the Central Mixed Wood Region in Alberta, Canada and in Central Saskatchewan, Canada within the Aspen Parkland Region (Natural Regions Committee, 2006). Sites used in this study from the Canadian Boreal Forest Region were located in Athabasca Oil Sands, characterized by a continental boreal climate with long, very cold winters and short cool summers. The climate of the study area is humid continental with long term mean annual precipitation of 455 mm and mean daily temperature of -18.8°C in January and 16.8°C in July (Environment Canada, 2003). The average elevation of the study site is 369 m (Huang et al., 2012). The area is comprised of valleys incised into broad muskeg covered plains (Carrigy and Kramers, 1973). Approximately, 20% of the area is comprised of coarse textured glacial fluvial and eolian deposits on which Brunisolic (Inceptisol) soils have developed (Turchenek and Lindsay, 1983). Sites were selected from natural jack pine (*Pinus banksiana*) ecosites (long term soil and vegetation plots) and disturbed/reclaimed sites located on Suncor Energy Inc., Shell Albian Energy Inc. and Syncrude Canada Inc. leases. The long term soil and vegetation plots: SV 10 (N 57°07’44”, W 111°59’44’’), SV 26 (N 57°51’92’’, W 111°43’04’’) and SV 27 (N 57°50’51’’, W 111°43’70’’) as well as AE1 (N 57°26’68’’, W 111°55’48’’) and AE2 (N 57°20’55’’, W 111°51’95’’) sites were on undisturbed jack pine stands with a lichen covered forest floor on coarse-textured, nutrient poor, eolian and glaciofluvial parent material, classified as an A ecosite (Beckingham and Archibald, 1996). Ecosites are ecological units developed under similar environmental conditions (climate, moisture, nutrient regime) (Beckingham and Archibald, 1996). A ecosites are developed under dry conditions with fast drainage and pore nutrient status in the soil.

Additional sites from reclaimed and disturbed open pit oil sands mining operations include the Albian Shallow-Stripping trial (SS trial) (N 57°25'55''. W 111°52'017''), Syncrude South West (SW 30) (N 56°99'13'', W 111°61'85''), Aurora LFH Capping Study (ALFH) (N 57°07'18'', W 111°50'66''), Center-pit at Aurora (CPA) (N 57°32'46'', W 111°54'05''), Albian Tailings Sands (ATS) (N 57°25'55'', W 111°52'01''), and Suncor Coke Bulk (SCB) (N 57°00'56'' W 111°50'16''). The SS trial had a reclamation prescription with 0-10 cm of LFH/Ae horizon mix overlaid on to 10-50 cm of peat/mineral sand mix and 50-100 cm of tailing sands. The Aurora LFH Capping Study site was constructed of 0-10 cm of LFH (litter, fermentation and humus) overlaid onto 10-100 cm peat mineral mix soil located on a southeast facing complex slope of a saline sodic overburden. The SW 30 site is a large overburden shale site constructed with 15 cm layers of peat/mineral mix, overlaying a layer of 20 cm of glacial till or glaciolacustrine clay. The CPA site was a disturbed open pit mining site, which comprised of Brunisolic soils typical of the region. The SCB site is mixture of sand and coke, a waste product formed during the heavy oil upgrading processes of bitumen. Coke is proposed to be added between tailings sand and a peat layer for reclamation covers. The ATS site is composed of by-products of the oil sand extraction process; settled sand, silts, clays and hydrocarbon residues from tailing ponds that will be used for future reclamation prescriptions. Additionally, two agricultural sites, Melfort (N 52°81'21'', W 104°51'18'') and Goodale (N 52°03'66'', W 106°35'38'') from central Saskatchewan were selected for comparison of SWR with soils with finer particle sizes (Fig. 4). The two agricultural sites in Central Saskatchewan were previously cropped with canola (*Brassica napus*). These areas are characterized with warm summers and very cold winters. The climate of the study area is humid continental with long term mean annual precipitation of 350 mm and mean daily temperature of -17°C in January and 18.2°C in July

(Environment Canada, 2003). The average elevation of the study site is 480 m. The soils in these sites are Dark Brown Chernozemic soils under the Canadian system (Typic Borolls, USDA taxonomy system).

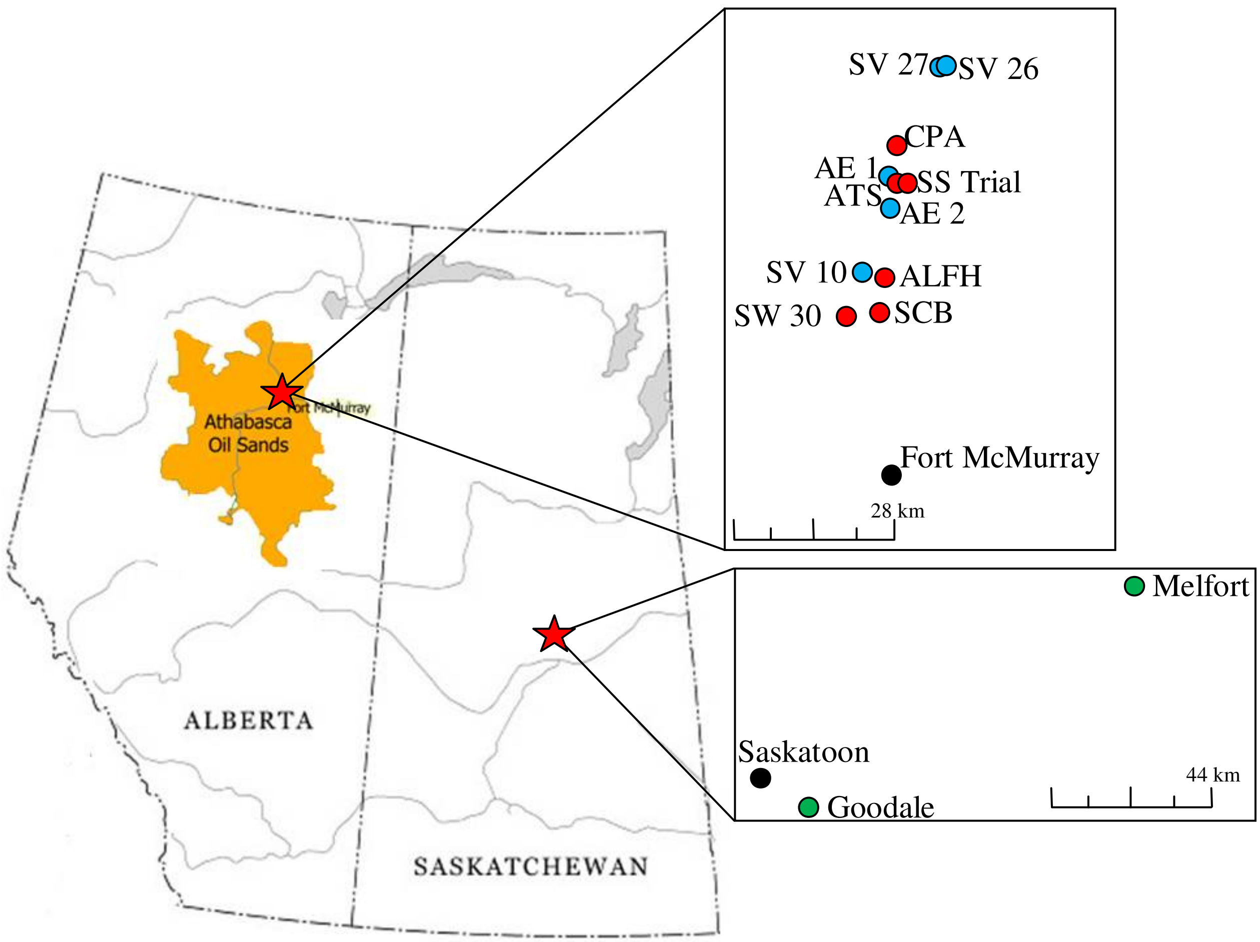

Fig. 4. Map of 13 sites in Northern Alberta and Central Saskatchewan (● natural jack pine sites, ● reclaimed/disturbed sites, and ● agricultural sites).

3.4.2 Laboratory Analysis

Total Carbon and Nitrogen contents were determined using a LECO CNS-2000 analyzer (LECO Corp., St. Joseph, MI). Particle size distribution was also analyzed for determination of soil texture classification (USDA) using a Laser Scattering Particle Size Distribution Analyzer

(Horiba LA - 950, Horiba Instruments Inc., Irvine, CA) after air-drying, sieving to 2 mm and removal of organic matter using hydrogen peroxide (35% v/v). Bulk composite soil samples were taken from 0-5 cm soil surface (excluding the LFH whenever possible) where repellency is the greatest on the surface (Miyata et al., 2007). Soils were passed through a 2 mm sieve to remove plant roots and debris, as well to ensure a uniform and smooth surface for contact angle determination. Samples were then oven dried to 40 °C for 24 hrs and kept air dry at 20°C before testing to obtain the potential SWR and minimize extreme alteration to the soil surface (Dekker et al., 1998). Modified sessile drop contact angle was measured on a thin layer of sieved soil fractions affixed to a glass slide using double sided adhesive tape (Bachmann et al., 2000).

3.4.3 Measurement of Severity and Persistence

The severity and persistence were measured on eight of the sites (natural sites: AE1, SV 10, SV 26, and SV 27; reclaimed/disturbed Sites; CPA, SW 30, ALFH, and SS Trial) by measuring the mobile sessile drop contact angle and water drop penetration time. Measurements were performed in an enclosed chamber with relative humidity (RH) at 80-85 % and temperature at 22.5°C, using saturated salt (KCL) solution to mitigate the evaporation influence of the soil surfaces. Control samples were taken to measure the amount of water content change within the samples during the experiment in the chamber due to evaporation and condensation. The water loss and gain from the soil using the modified sessile drop method was negligible. A PG-X goniometer (FIBRO System AB) was placed on top of prepared soil surfaces. Drops of 4 μL of distilled water were deposited on the soil surface and pictures of the static contact angle were taken from an integrated camera - captured 6 images min^{-1} (640x480 pixels) until the droplet spread across the soil surfaces. Contact angles were measured on images from 5 replicate droplets from a composite sample of the soil. The measurement of severity and persistence was categorized by scheme developed by King, (1981) and Dekker and Jungerius, (1990), respectively (Table 1). At a significance level of $P = 0.05$, Paired t tests were used to compare the severity and persistence of SWR among sites. Pearson Correlation Coefficient was used to assess the linear relationship between severity, persistence of SWR, total C, and total N at significance level of $P = 0.05$.

Table 1. Soil water repellency (SWR) classification of soils based on the severity (Contact angles: King, 1981) and the persistence (WDPT: Dekker and Jungerius, 1990).

Severity		**Persistence**	
Contact angles (°)	**SWR Classification**	**WDPT (s)**	**SWR Classification**
<75*	Not significantly water repellent	<5	Wettable
75-80*	Very low water repellent	5-60	Slightly repellent
81-86*	Low water repellent	60-600	Strongly water repellent
87-93*	Moderately repellent	600-3600	Severely water repellent
94-97	Severely repellent	>3600	Extremely water repellent
>97	Very severely repellent		

*Subcritical soil water repellency (0° > contact angle < 90°)

3.4.4 Measurement of Critical Water Content (CWC)

Soil CWC was determined on all 13 soils by measuring sessile drop contact angle with increasing water contents on the soil surface until the soil changed from a repellent to a wettable state. The range of water contents were obtained by separating oven dried soil samples into 100 gram fractions into sealed containers. Distilled water was applied manually by spraying soil samples with predetermined amounts of water calculated by mass. Soil water contents ranged from 2.5, 5.0, 7.5, 8.5, 10.0, 12.5, 15, 17.5 and 20.0 % gravimetric water content. Oven dried and ambient air dried soil samples were also included in the measurement. Samples were subsequently shaken, mixed thoroughly and left for a week to equilibrate. As shown in King (1981), and Badía et al. (2013) the action of sieving and mixing of soil sample has the potential to decrease the severity of repellency as hydrophobic coatings may be removed from the soil surface. As such all samples at specific water contents including oven and ambient air dried were subject to the same manipulations to minimize the influence of degradation of SWR due to the mixing. The actual water content was determined by taking a subsample and measuring gravimetrically the water content before contact angle measurements were taken. Five droplets of 4ul were placed on each of the five subsamples (25 measurements per water content). The experiment was replicated twice. By comparing contact angle (severity) versus water content, the CWC was determined to be the water content at which the contact angle reaches 0 degrees (Point at which the soil changes from repellent to wettable state and vice versa).

3.4.5 Contact Angle Measurements

Measurement of contact angles from the images obtained by the PGX goniometer were measured by using open source multi-platform java image processing program Image J, available at http://rsb.info.nih.gov/ij/ as well as using a Low Bond Axisymmetric Drop Shape Analysis

Model of Drop Shape Analysis (LB_ADSA) approach (Stalder et al., 2010). This method allowed for fitting of the whole drop profile based on the Young-Laplace equation. It allows for contact angle determination on obscured view due to roughness of soil particles, overcoming the baseline issues of the goniometer approach as the whole drop profile is taken into account when determining the contact angle.

3.5 Results and Discussion

3.5.1 Severity and Persistence

The eight soil tested exhibited some severity of SWR, of which six sites showed a high severity with contact angles above 90° (Table 2). Ranking the sites with initial contact angles in ascending order were SV 10, SW 30, SV 26, ALFH, SS trial and AE1 with contact angles of 95±3°, 121±1°, 129±2°, 132±4°, 145±3° and 145±1°, respectively. This resulted in SWR classification of severely water repellent (SV 10) to very severely water repellent (SW 30, SV 26, ALFH, SS trial and AE1) based on the contact angle and classification scheme in Table 1 (Fig. 5a). The WPDT values as a measure of persistence of SWR in SW 30, SV 10, SS trial, AE1, ALFH, and SV 26 were 5±0.2, 86±2, 94±4, 135±2, 158±6 and 167±17 minutes, respectively (Table 2). This resulted in an extremely water repellent SWR classification for five of the six soils based on WDPT and classification scheme (Table 1). Though these soils have the same classification based on both the contact angle and the WDPT measurement, the severity as a function of persistence for these soils is different as the curves cross (Fig. 5a). This suggests that a high contact angle (severity) does not necessarily denote long WDPT (persistence). The SW 30 site, however, had a severely water repellent classification based on its persistence (WDPT) (Table 2). This difference in persistence compared to severity could be due to its peat/mineral mix composition (Table 2). The peat composition of the soil would be water repellent when dry; however when wet it can absorb a substantial amount of water (Lachacz et al., 2009). This resulted in a high severity (contact angle) initially, but with the breakdown of repellency in this soil, it absorbs water rapidly as compared to the other repellent soils. The short persistence is likely due to the reversible nature of repellency under the presence of water and the composition of the peat material in the site (Lachacz et al., 2009).

Table 2. Soil texture and soil water repellency (SWR) classification of the soils based on the severity (contact angles) and persistence (WDPT) independently and the soil critical water content

Soils	Soil Texture	Severity		Persistence		Soil Critical Water Content
	USDA	Contact angles (°)†	SWR Classification	WDPT (Min)†	SWR Classification	θ (kg kg^{-1})
SV 27	Sand	65±5	Not significantly water repellent	95±12	Extremely water repellent	4%
CPA	Sand	75±2	Very low water repellent	60±7	Extremely water repellent	0%
SV 10	Sand	95±3	Severely repellent	86±2	Extremely water repellent	3%
SW 30	Clay	121±1	Very severely repellent	5±0.2	Severely water repellent	>17%
SV 26	Sand	129±2	Very severely repellent	167±17	Extremely water repellent	8%
ALFH	Sand	132±4	Very severely repellent	158±6	Extremely water repellent	6%
SS Trial	Sandy Loam	145±3	Very severely repellent	94±4	Extremely water repellent	16%
AE1	Sand	145±1	Very severely repellent	135±2	Extremely water repellent	5%
AE2	Sand	89±3	Moderately repellent	n/a	n/a	8%
SCB	Loam Sand	114±2	Very severely repellent	n/a	n/a	>19%
ATS	Sand	111±3	Very severely repellent	n/a	n/a	2%
Melfort	Clay Loam	81±1	Low water repellent	n/a	n/a	>12%
Goodale	Sandy Loam	79±1	Very low water repellent	n/a	n/a	12%

†Contact angles and WDPT were measured on 5 replicates with standard error in severity and minutes respectively.

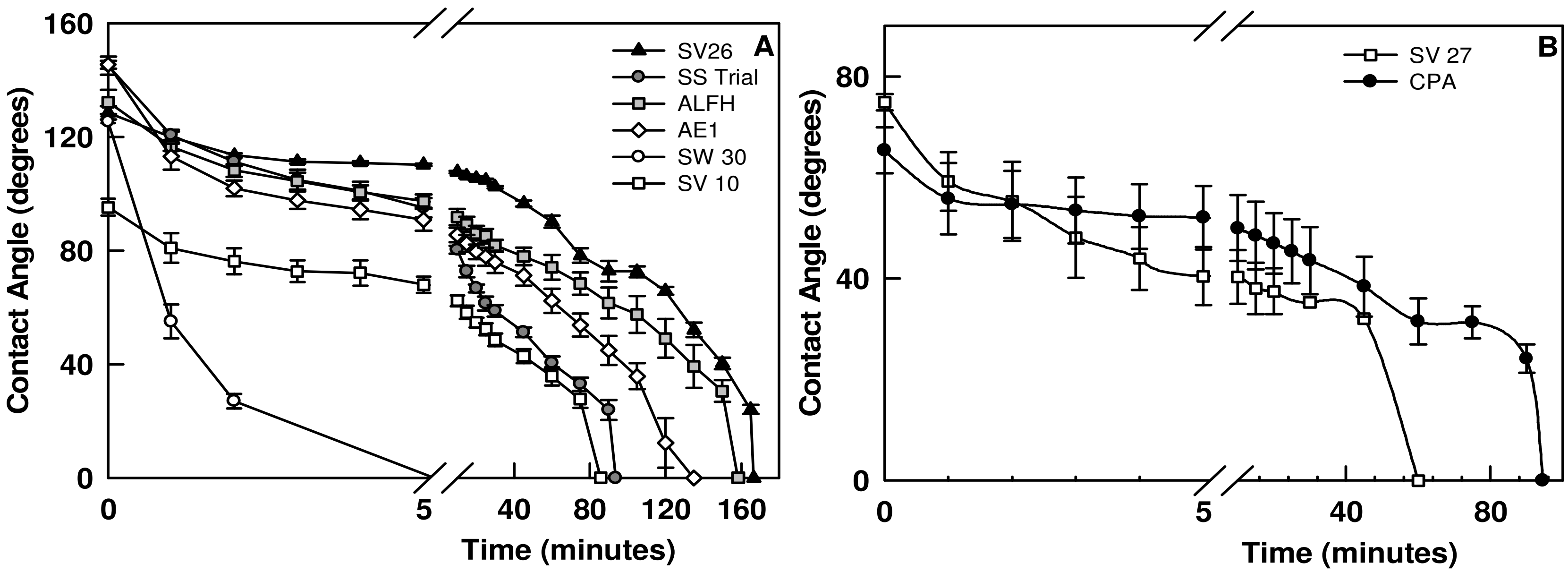

Fig. 5. a) The severity of SWR (contact angles) as a function of persistence (time) on extremely water repellent sites classified by contact angles measured; AE1, SS trial, SV 26, ALFH, SW30 and SV 10. b) The severity of SWR (contact angles) as a function of persistence (time) on subcritical repellent soils classified by contact angles measured; SV 27 and CPA.

There was no obvious trend between CA and the carbon and nitrogen content with r of 0.21 (p>0.05) and 0.20 (p> 0.05) respectively. Additionally WDPT was negatively correlated with carbon and nitrogen content with r of -0.70 (p>0.05) and -0.71 (p>0.05) and was not statistically significant. Since all the soils originated from different locations, it is expected that the relationship normally found between carbon and repellency is not present in this study. The arrangement and source of hydrophobic and non hydrophobic molecules on the different soil surfaces could be the cause for the differences observed between SWR severity and persistence (Douglas et al., 2007).

Site SV 26 had significantly lower severity (smaller contact angle) of SWR than other extremely repellent soils: AE1 (P<0.01), SS Trial (P<0.01), and SW 30 (P<0.01). According to the assumptions of Leelamanie and Karube (2009), contact angle should decrease exponentially with soil water contact time and WPDT should respond to initial contact angle, but not to reductions in contact angle with soil water contact time. This suggests that high degree should be associated with high persistence. Therefore, SV 26 theoretically should have a shorter persistence than these repellent soils. However, this soil showed significantly larger WDPT at 167±17 minutes (Fig. 5a) as compared to SS Trail (P<0.01) and SW 30 (P<0.01). This implies that the initial severity of SWR is not necessarily related to persistence (Dekker and Ritsema, 1994; Shirtcliffe et al., 2006). The SWR in site SV 27 and the CPA was in the sub-critical range (CA < 90°) and classified as not significantly water repellent and very low water repellent, respectively (Table 2). The CPA had a higher severity with a contact angle 75±2° of SWR compared to SV 27 with a contact angle of 65±5°, however it was not statistically significant (P>0.05). SV 27 did however have statistically significant longer persistence (95±12 minutes) (P<0.05) than the CPA (60±7 minutes) (Fig. 5a, 5b). Therefore, in this subcritical range, the same severity of water repellency does not always equate to long persistence as indicated by the crossing of the soil curves (Fig. 5b). Subcritical SWR has the ability to buffer the water storage property of sandy soils to facilitate plant and microbial growth (Doerr et al., 2000). It may be advantageous in decreasing the hydraulic conductivity of the soil to slow down deep drainage and allow for longer water residence time in soil for plant water uptake (Lichner et al., 2007). As discussed by Doerr et al. (2000) and DeBano (1981), the presence or absence of a soil water repellent layer can result in drastic change in hydrological processes. These results demonstrate that severity is not always related to persistence in both an extremely repellent and subcritical repellent soil. Contact angles were not strongly correlated with WDPT and were not statistically significant ($r = 0.37$, $p > 0.05$). This suggests that the role SWR plays in hydrological processes is more complex. For example, a soil with a high severity of SWR but low persistence would result in more initial runoff and less infiltration, but will subsequently become wettable, negating the influence of the water repellency. Assessing differences between severity and persistence is important when determining runoff scenarios considering the magnitude and frequency of the rainfall events (Beatty and Smith, 2013). The difference between contact angle and WDPT is due to wetting mechanisms occurring at the surface of soil particles. Contact angles formed on the soil surface are caused by the difference in surface energy between water, soil surface and air. The larger the difference in surface energy between the soil and liquid, the larger the contact angles will be. Assuming that the surface energy of water and air remains constant, the change in soil surface energy causes persistent repellency measured by WDPT. Although a soil surface may have a large surface energy compared to water, the rate at which surface energy changes is dependent on the composition of hydrophobic coating on the soil surface (Chen and Schnitzer, 1978). As such, comparison between the severity and persistence measured by contact angles and WDPT between soils are much more complex considering they measure different properties for determination of SWR.

3.5.2 Critical Water Content

Critical water contents ranged from 0% in the CPA site to > 19% in the SCB site (Table 2, Fig. 6). This is similar to the finding by Doerr and Thomas (2000) who indicated that repellency can occur in soils with water content up to 28%. A soil with a high CWC would be more difficult to remediate as more surfactant or water will be needed to overcome the repellent nature of the soil. Four trends were observed in the determination of the CWC from the change in contact angle as a function of water content (Fig. 6, 7). As water content increased in the SV 10, SV 27, CPA, and ATS sites from 0%, the severity of repellency or the water drop contact dropped drastically to zero (Fig. 6). This suggests that repellency is easily reversible in these sites and when water is present repellency disappears rapidly. In sites SV 26, SS Trial, AE 1, AE 2, the severity of repellency or the water drop contact angle decreases slowly until it reaches its CWC (Fig. 6). This indicates that repellency is not as easily reversible in these sites and is more persistent as soil is wetted from oven dried moisture conditions. As shown in sites SCB and Goodale, with increasing water content, the contact angle does not decrease until water content reaches > 8% (Fig. 6). This suggests that the SWR in these soils are very persistent at water contents < 8%. After reaching 8%, SWR drops slowly until the CWC is reached. With increasing water content in sites SW 30 and Melfort, the decrease in contact angle was not reached in this study. The exact critical water content was not determined for these soils, however the CWC would be expected to be >17% and > 12% for SW 30 and Melfort respectively. Additionally, SW 30 and Melfort sites had persistent repellency at lower water contents. The critical water content was not reached in the Goodale and Melfort sites. In sites

SCB, SW 30, Goodale, and Melfort the contact angle also increased initially from 0% water content and then decreased slowly until the CWC was reached (Fig. 6). It has been suggested that as water content goes from oven dried conditions (0%) to the soil's permanent wilting point, the soil increases to its maximum SWR, followed by a decrease in SWR as it approaches the water content at field capacity (King, 1981). Through the examination of CWC between different soils, we observe that the value CWC is not only important but the trends observed are also important. The trends show how repellency changes as a function of water content (Fig. 7). A more rapid decrease in repellency (measured by contact angle) with increases in water content would indicate less severe repellency even though the initial severity may be high (Fig. 7a, 7b). A slow decrease or persistent repellency at low water contents would indicate more severe repellency in a site (Fig. 7c, 7d). As water content increases such as after a rainfall event, the soil will approach its critical water content. The expression of repellency is important to determine as the soil increases in water content. If the soil does not reach the critical water content, soil infiltrability in the soil matrix will be reduced, with a increased chance of preferential flow and runoff causing a decrease in soil water storage in the soil profile.

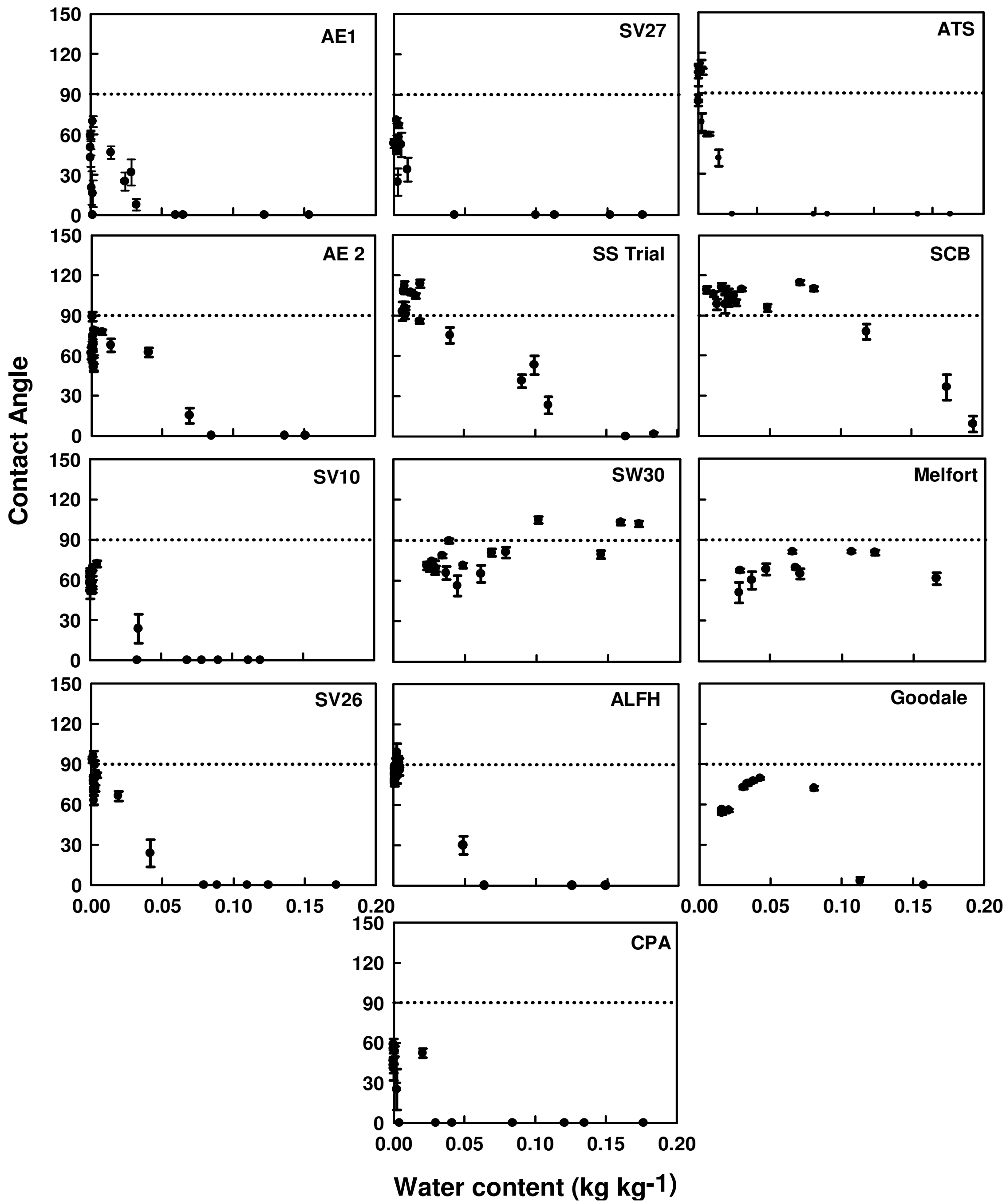

Fig. 6. Contact angle as a function of water content for all sites for determination of the critical water content. Error bars represents the standard error of mean for 5 droplets on 5 subsamples.

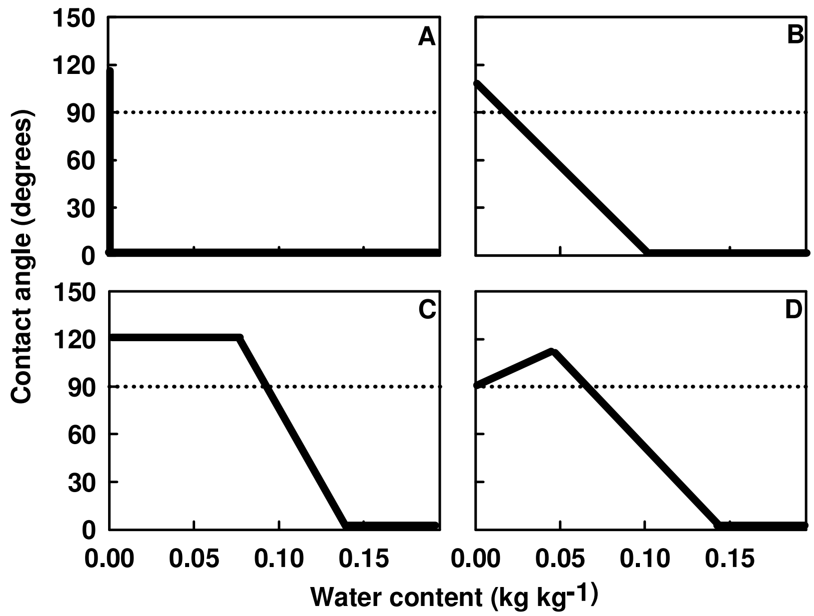

Fig. 7. Trends observed from contact angle (severity) as function of water content (kg kg-1): a) Contact angles decreases rapidly with increase in water content, b) Contact angles drops slowly with increase in water content, c) Contact angles are persistent until a water content is reached, thereafter contact angles drops slowly with increase water content. d) As water content increases past 0% there is increase in contact angle initially followed by decrease in contact angle.

In terms of reclamation, natural sites did show lower CWC compared to reclaimed/disturbed sites (Fig. 6). This is more likely due to the composition or nature of repellent materials in each of the soils (Horne and McIntosh, 2000). In the agricultural sites, the effects of SWR can also be seen in finer textured soils as illustrated by high CWC (Fig. 6). Additionally similar cropped sites with finer soil textures showed differences in CWC, suggesting that the repellency is site specific soil. CWC is important to assess the effect of water repellency in soils as it determines at which water contents the effect of SWR are negligible. A soil with a high CWC would be more susceptible to preferential flow and runoff due to the soil remaining repellent at higher water contents. Additionally it was found that severity of repellency is not necessarily related to critical water content as some of the soils with high severity of repellency had low CWC (Table 2). Furthermore soils with low severity of repellency were also found to have a high critical water content. This is most likely due to the composition of hydrophobic compounds causing the repellency. Hydrophobic compounds in soils with high CWC indicate that the difference between the surface tension from water and the soil surface at low water contents is persistent. However when water is present and the soil reaches the CWC, the particles reorientate rapidly, making the effect negligible and making SWR manageable (Doerr et al., 2000; Lehrsch and Sojka, 2011). While hydrophobic compounds in soils with lower initial severity and higher critical water contents have more complex organic compounds which take substantially longer time to reorientate when subject to wetting. The complex nature of compounds that cause repellency further complicates measurement as differences in amount and type will determine the severity, persistence and CWC of repellency in the soil (Horne and McIntosh, 2000).

Classification of SWR could be inconsistent between soils; depending on if the classification is based on the contact angles, the WDPT, or the critical moisture content due to fact that they measure a different property of repellency under different conditions (Table 2). The ability to measure contact angles with time during rewetting and CWC gives us a more detailed understanding of how the severity of SWR is changing with persistence and at which water content repellency is still present. On reclaimed land, soils with a high severity of SWR and CWC may increase the chance of runoff and evaporation, particularly for materials placed on a slope (Arbel et al., 2005). However, the shorter persistence and CWC means that a storm may remove the SWR more rapidly. This will mitigate the effect of SWR on soil due to its disappearance after a rainfall event. Much of the soils in the Athabasca Oil Sands are comprised of coarse textured sandy soils. These sandy textured soils have a lower water retention and higher hydraulic conductivity as compared to finer textured soils. This makes re-vegetation of plant communities difficult due to less available water in the soil profile. However, the expression of subcritical SWR in sandy soils does slow the rapid infiltration of water through the root zone (Hallett et al., 2004). Therefore, sandy soils displaying sub-critical repellency will have a smaller hydraulic conductivity compared to non-water repellent soils, resulting in an increased soil water residence time in the soil profile (Andry et al., 2009). This would potential increase the water storage capability of sandy soils or prevent the loss of water to deep percolation. The severity and persistence and CWC are major indicators of SWR and must be examined accurately to determine the role water repellency plays in the environment.

3.5.3 Conclusions

In this study, the relationship between severity, persistence of SWR, and critical water content was determined using soils from the Athabasca oil sands region and agricultural sites in central Saskatchewan. Regardless of the land use, a high severity (Contact angle) of repellency does not necessarily denote long persistence (WDPT) or high CWC. Measurement of severity and persistence are related to the surface tension differences and changes in surface energy between water and the soil surface respectively. Critical water content allowed us to determine how persistent SWR was under different water contents. This is important for predicting runoff scenarios due different magnitude and frequency of rainfall events in water repellent sites. Characterizing and understanding the severity, persistence and CWC together are valuable tools when determining the effects of SWR on hydrological processes as they operate under different mechanisms.

4. DETERMINATION OF THE CONDUCTING POROSITY AND WATER REPELLENT AFFECTED PORES SIZES IN SOILS UNDER DIFFERENT TENSIONS[2]

4.1 Preface

Water in soil primarily flows through long continuous connected pores. The conducting porosity is the percentage of long continuous connected pores in soil compared to the total volume. Pore properties including tortuosity, surface roughness, discontinuity and dead end pores can impede water flow through soil (Bodhinayake et al., 2004). Although the presence of soil water repellency (SWR) has been documented in many soils (Doerr et al., 2000), the issue of water repellent pores has yet to be examined. Infiltration using tension infiltrometer at different pressure heads and using water and equivalent pressures using 95% ethanol, the contribution of specific pores sizes (derived by the capillary rise relationship) to water flow can be determined. Also the repellency index (RI) is dependent on including all pores size excluding macropores to determine the SWR in soil. Does the exclusion of certain pore size influence the measurement of the RI? The objective of this study was to determine if SWR affects the conducting porosity in soil and if the measurement (repellency index (RI)) of SWR is influenced by pores sizes. This study will aid in our understanding of how water moves through repellent soil and how to best measure SWR using RI.

[2] This work has been previously submitted in Chau, H.W., Li, M., Biswas, A, Vujanovic, V. and Si, B.C. (2013). Soil water repellency and the conducting porosity in water repellent soils under different tensions. Under review European Journal Soil Science (EJU 165-13). Minor modifications have been made for consistency.

4.2 Abstract

The conducting porosity in soil is of critical importance to the movement of water, and transport of solute and pollutants through soil. Pore properties including tortuosity, surface roughness, discontinuity and dead end pores influence the conducting porosity in soil. As soil water repellency (SWR) is present in all types of soils to a certain degree, the issue of water repellent pores has yet to be addressed. The objective of this study was to determine if SWR affects the conducting porosity in soil and if the repellency index (RI) is dependent on different tensions. Tension infiltrometer measurements were taken at 5 pressure heads using water (-0.3, -3.0, -7.0, -10.0 and -13.0 cm) and equivalent pressures using 95% ethanol (-0.11, -1.31, -2.64, -3.77 and -4.9 cm) at five randomly selected locations on a Jack Pine (*Pinus banksiana*) stand (SV 26) in Northeastern Alberta and two agricultural fields (Goodale and Preston) in Central Saskatchewan. The total conducting porosity in soil was higher under ethanol infiltration compared to water at all the locations in all the three sites. The effect of SWR on the conducting porosity is related to the severity of repellency and at which pore diameter range water flow is impeded. The RI under -3 cm was more representative of the total repellency in the soil as more contributing diameter pores to the water flux were included in the measurement. A high RI affecting larger diameter pores has more influence on the conducting porosity due to the contribution to the total liquid flux. To accurately determine the actual conducting porosity in soil, SWR must be taken into account.

4.3 Introduction

Soil water repellency (SWR) has been recognized as a worldwide phenomena affecting many types of soils (Wallis and Horne, 1992). It has considerable influence on soil water dynamics through decreasing infiltration and increasing preferential flow, runoff and erosion.

Since the transport of water from the soil surface is controlled by the conducting porosity (Watson and Luxmoore, 1986), examination into the relationship between SWR and the conducting porosity must be performed. The soil water conducting porosity is crucial for understanding water, solute, and pollutant movement through soils (Beven and Germann, 1982; Ankeny et al., 1990; Luxmoore, 1990). Characterization of this property is paramount for research and practical application in agriculture, forestry, pedology and hydrology. With the development of the tension infiltrometer (Perroux and White, 1988), determination of the water conducting porosity has improved. This also allowed for the determination of which pore diameters contribute more to infiltration. Macroporosity (diameter >1.0 x 10^{-3}m) and mesoporosity (diameter from 1.0 x 10^{-5} – 1.0 x 10^{-3} m) are the major fractions of the total soil porosity that contributes to the water conducting porosity in soil (Luxmoore, 1981). Not all these pores contribute to the flow as they can include pores that are non-continuous, dead ended and have irregular pore geometry, which restricts the transport of water (Bodhinayake et al., 2004). Pore toruosity and surface roughness are also known to decrease the water flow in soils.

As we know SWR is highly variable in site (Hallett et al., 2004); it can also operate at the millimeter-scale, making measurements extremely difficult. However, with recent advances using the miniaturized infiltrometer for assessing SWR (Tillman et al., 1989), more accurate characterization at smaller scales has been done (Hallett et al., 2004). Tension infiltrometers measure the infiltration rate at negative water pressures with respect to the atmosphere (Clothier and White, 1981). Since capillary pressure is related to the equivalent pore diameter, the change in the tension or pressure head can estimate the range of pore size contribution to infiltration (Jarvis et al., 1987).

Calculation of the repellency index (RI) is a common method for determination of the severity of SWR. This method involves the infiltration of two liquids: commonly water and 95% ethanol (not influenced by repellency) under -3 cm pressure head to minimize macropore flow. The ratio of the two sorptivities from the early time or steady state infiltration curve gives an indication of how repellent the soil is. In this way, the overall contribution of SWR to the total conducting porosity excluding the macropores is assessed. However, this does not distinguish the contribution of specific water repellent pore sizes to the total water flux. There is little research on how the RI changes with exclusion of different pore sizes (decreasing pressure head).

Many methods have been developed to calculate the water conducting porosity. Watson and Luxmoore (1986, 1988), Dunn (1991), and Bodhinayake et al. (2004) utilized minimum equivalent pore radius, mean pore radius, and a range of pore radii for determination of the conducting porosity, respectively. Due to the overestimation of the conducting porosities using minimum equivalent pore radius and mean pore radius (Watson and Luxmoore, 1986; Dunn, 1991), using a range of pore radii (Bodhinayake et al., 2004) is the preferred method for characterization of the conducting porosity. Nevertheless these conducting porosity estimations do not take into account the presence of SWR. Through using 95% ethanol infiltration we can determine an intrinsic conducting porosity; that is the conducting porosity independent of soil surface properties (Lamparter et al., 2010). The objective of this study was to examine how SWR affects water conducting porosity at different pore sizes by examining the change in RI under different pressure heads. I hypothesized that SWR will decrease the conducting porosity in soil and the extent will be determined by which pores sizes SWR is affecting.

4.4 Materials and Methods

4.4.1 Site Information

The study areas are located in the Canadian Boreal Forest Region within the Central Mixed Wood Region in Alberta and in Central Saskatchewan within the Aspen Parkland Region (Natural Regions Committee, 2006). Three field experiments using a tension infiltrometer to infiltrate water and 95 % (vol vol^{-1}) ethanol were performed on one field site in Northeastern Alberta, Canada (N 57º 30’ 39”, W 111º 25’ 48”) and two sites in Central Saskatchewan, Canada (N 52 º 03’ 66’’ W 106 º 30’ 38’’, N 52º 07’ 98” W 106º 37’ 53’’). The site in Northeastern Alberta is characterized by a continental boreal climate with long and cold winters and short cool summers. The average annual precipitation is 455 mm, with mean daily temperature of -18.8°C in January and 16.8°C in July (Environment Canada, 2003). This site is an undisturbed jack pine (*Pinus banksiana*) stand with a lichen covered forest floor on coarse-textured, nutrient poor soils, designated as SV 26 (Long Term Soil Vegetation Plot 26), an A ecosite, respectively (Beckingham and Archibald, 1996). This long term soil vegetation plot is a typical site that the Athabasca Oil Sands industries need to recreate in their reclamation/restoration projects (Johnson and Miyanishi, 2008). The soil in the area is described as Dystric Brunisol (Dystrochrept inceptisol, USDA taxonomy system) developed on glacial fluvial and eolian parent material. The two agricultural sites in Central Saskatchewan are the Goodale (Goodale Crop Research Farm) and Preston (Animal Science Field) sites, which were previously cropped with canola (*Brassica napus*). This area is characterized with warm summers and very cold winters. The average annual precipitation is 350 mm, with mean daily temperature of -17°C in January and 18.2°C in July for these sites. (Environment Canada, 2003). The soils in these sites are Dark Brown Chernozemic soils under the Canadian system (Typic Borolls, USDA taxonomy system).

Site SV 26, Goodale, and Preston sites were classified with a Canadian/USDA texture of sand, sandy loam and loam designation respectively. Initial assessment showed the presence of natural SWR in the sites (Hunter et al., 2011). Soil texture was determined by measuring particle size distribution using a Laser Scattering Particle Size Distribution Analyzer (Horiba LA - 950, Horiba Instruments Inc., Irvine, CA) after air-drying and sieving to 2 mm. Gravimetric water content and bulk density was determined at locations adjacent to where the infiltration occurred before the experiment (Table 3). To test for SWR in the soil initially, contact angles were determined using the sessile drop method and Low Bond Axisymmetric Drop Shape Analysis (Bachmann et al., 2000; Stalder et al., 2010).

Table 3. Location specific information: bulk density and initial volumetric water content from each location in each of three sites; SV 26, Goodale, and Preston before infiltration.

	SV 26		Goodale		Preston	
	Bulk density (g cm^{-3})	Initial field water content (θ_v)	Bulk density (g cm^{-3})	Initial field water content (θ_v)	Bulk density (g cm^{-3})	Initial field water content (θ_v)
Location 1	1.39	0.17	1.08	0.09	0.85	0.06
Location 2	1.29	0.13	1.25	0.27	0.87	0.05
Location 3	1.34	0.17	1.24	0.26	0.76	0.15
Location 4	1.42	0.12	0.98	0.10	0.86	0.17
Location 5	1.32	0.16	0.91	0.08	1.00	0.05

4.4.2 Tension Infiltrometer

Before infiltration measurements were performed, an area of 0.6 cm diameter was selected and surface debris was carefully removed from the surface to ensure good contact. Tension infiltrometers with a 0.2 m diameter disc (Soil Moisture Measurement Systems, Tucson, AZ) were used to measure infiltration rate as a function of time at different pressure heads for two liquids on 5 locations randomly selected in each of the three fields. SWR can vary at the millimeter scale due to the presence of unevenly distributed hydrophobic material (Orfánus et al., 2008). The five locations were not taken as replicates as averaging the locations would not allow us to see the true effect of repellent pores on the RI and the conducting porosity. The nylon mesh attached to the tension infiltrometer disc had an air entry value of about -3.0 to 3.2 kPa pressure head. Tension infiltrometer measurements were taken at five pressure heads using water (-0.3, -3.0, -7.0, -10.0 and -13.0 cm water pressure head corresponding to 1×10^{-2}, 1×10^{-3}, 4.2×10^{-4}, 3.0×10^{-4}, and 2.3×10^{-4} m equivalent pore diameters). Pressure heads were adjusted for infiltration of ethanol to account for differences in surface tension and density as compared to water. Using the capillary rise equation (Eq. 4.1) with γ of 2.19×10^{-2} N m^{-1} and ρ of 8.07×10^{2} kg m^{-3} for 95% ethanol the pressures head used were -0.11, -1.31, -2.64, -3.77 and -4.9 cm. The water and 95% ethanol pressure heads corresponded to 1×10^{-2}, 1×10^{-3}, 4.2×10^{-4}, 3.0×10^{-4}, and 2.3×10^{-4} m equivalent pore diameters. Since the pressure head were equivalent between the water and 95% ethanol, the liquid content in soil at each pressure head is equal. Water and ethanol (95%) were infiltrated side by side, on the same location from low to high pressure heads, beginning with the -13.0 cm and -4.9 cm for water and ethanol respectively to reduce the effect of spatial variability (Logsdon and Jaynes, 1993). This reduces the hysteresis where drainage occurs close to the tension infiltrometer disk while wetting continues near the wetting front (Reynolds and Elrick., 1991). The water infiltrating into the soil was measured by recording the drop in water level as a function of time. When the rate of water infiltrating in soil did not change with time for three consecutive measurements taken at 30 second intervals, steady state was assumed. Steady state occurred around 18-20 minutes.

4.4.3 Estimation of the Conducting Porosity

The capillary rise equation, (Eq. 4.1) (Bear, 1972) gives the maximum filled pore size, *r*, (L) at a specific pressure head, *h* (L).

$$r = \frac{2\,\gamma \cos(\alpha)}{\rho\, g\, h} \qquad \text{(Eq. 4.1)}$$

which γ is the surface tension of the liquid (M T^{-2}), α is the contact angle between the liquid and the pore wall (assumed to be zero), ρ is the density of the liquid (M T^{-2}), and g is the gravity acceleration constant (L T^{-2}). Modification of the capillary rise equations for different liquids for determination of the maximum filled pore size must be corrected for differences in the surface tension and the density of the liquids.

The steady state infiltration rate obtained from measurement under the five pressure heads was used to determine unsaturated hydraulic properties using Gardner's (1958) exponential hydraulic conductivity function, (Eq. 4.2);

$$K(h) = K_{fs} \exp(\alpha h) \qquad \text{(Eq. 4.2)}$$

where $K(h)$ is the unsaturated hydraulic conductivity (L T^{-1}), as a function of pressure head, *h* (L), α is the inverse macroscopic capillary length parameter (L^{-1}) and K_{fs} is the saturated hydraulic conductivity (L T^{-1}). Under a circular disc and steady state condition, Eq. 4.3 can be used for determination of the unknown parameter K_{fs} and α (Wooding, 1968).

$$q\infty(h) = (1 + \frac{4}{\alpha\pi r_d})K_{fs}\exp(\alpha h) \quad \text{(Eq. 4.3)}$$

$q\infty$ is infiltration rate at steady state (L T^{-1}) as a function of pressure head, h (L), r_d is the radius, (L), of the circular disc, and K_{fs} is the saturated hydraulic conductivity (L T^{-1}).

Given K_{fs} and α, the soil water conducting porosity, ε, for a given range of pore radii or pressure heads was estimated by (Bodhinayake et al., 2004) (Eq. 4.4)

$$\varepsilon(a,b) = \frac{2\mu\rho g K_{fs}}{\gamma^2}\left\{\exp\left[\frac{-2\gamma\alpha}{\rho g b}\right]\left[\frac{4\gamma^2}{(\rho g b)^2} + \frac{4\gamma}{\rho g b\alpha} + \frac{2}{\alpha^2}\right] - \exp\left[\frac{-2\gamma\alpha}{\rho g a}\right]\left[\frac{4\gamma^2}{(\rho g a)^2} + \frac{4\gamma}{\rho g a\alpha} + \frac{2}{\alpha^2}\right]\right\} \quad \text{(Eq. 4.4)}$$

where a is the lower limit for the pore diameter (L), b is the upper limit of the pore diameter (L), α is the hydraulic property (L^{-1}), ρ is the density of liquid (M L^{-3}), g is the gravity acceleration, (L T^{-2}), K_{fs} is the saturated hydraulic conductivity (L T^{-1}), γ is the surface tension (M T^{-2}), and μ is the viscosity (M L^{-1} T^{-1}). Estimation of the conducting porosity was done on the following pore diameter ranges from 1.0 x 10^{-3} to 1.0 x 10^{-2}, 4.2 x 10^{-4} to 1.0 x 10^{-3}, 3.0 x 10^{-4} to 4.2 x 10^{-4}, 2.31 x 10^{-4} to 3.0 x 10^{-4} and 2.3 x 10^{-4} to 1.0 x 10^{-2} m. We assumed that the maximum pore diameter at the site is 1.0 cm.

4.4.4 Water Repellency Index

Early time sorptivity was determined by (Philip, 1954) (Eq. 4.5)

$$i = St^{1/2} \quad \text{(Eq. 4.5)}$$

where, i is the cumulative infiltration (L T^{-1}), for each measured pressures, S is sorptivity (L T^{-1}) of infiltrating liquid and t is the time (T). Sorptivity (S) was determined at steady state by (Leeds-Harrison et al., 1994) (Eq. 4.6)

$$S = \sqrt{\frac{Qf}{4br}} \qquad \text{(Eq. 4.6)}$$

where Q is the steady state infiltration rate, b is the parameter dependent on the soil water diffusivity function, taken as 0.55 for soil with unknown b parameter (White and Sully, 1987), r is the radius of disc, and f is the fillable (air filled) porosity. The repellency index (RI) can be calculated with (Tillman et al., 1989) (Eq. 4.7).

$$RI = 1.95 \frac{S_E}{S_W} \qquad \text{(Eq. 4.7)}$$

where S_E is the sorptivity of 95% ethanol (L T^{-1}), S_W is the sorptivity of water (L T^{-1}), and 1.95 is a constant to correct for viscosity and density differences between water and 95% ethanol. However, utilizing different pressure heads to assess the conducting porosity of soil, the viscosity and density were factored in to the pressure head differences at different pore sizes for infiltration, so the 1.95 correction factor is not needed, and RI can be calculated with (Eq. 4.8);

$$RI = \frac{S_E}{S_W} \qquad \text{(Eq. 4.8)}$$

An RI greater than 1 indicates the presence of SWR in the site. The RI was determined for each of the 5 pressure heads and compared to the equivalent pore diameter ranges.

4.5 Results and Discussion

The RI in each of the five locations in SV 26, Goodale and the Preston site using water pressure heads (-0.3,-3.0,-7.0, -10.0, and -13.0) and corresponding 95% ethanol pressure heads (-

0.1, -1.3, -2.6, -3.7 and -4.9) are displayed in Fig. 8 a) and 8 b) for early time and steady state conditions, respectively. The pressure heads were set sequentially from -13.0, -10.0, -7.0, -3.0, and -0.3 cm; corresponding to increasing equivalent pore diameter sizes. This will allow us to determine the differences in RI excluding larger pore sizes or increasing pressure head to -0.3 cm. Steady state RI was lower than early time RI (Fig. 8). This is related to initial water content in soil before the measurement (Table 3). Parameter θ, contact angle is an indication of the wettability of soil; however it is often neglected as it is assumed to be constant at 0° in fully wettable soil. Assuming the wetting fluid does not change in surface tension due to increase in hydrophobic compounds leaching into the fluid during transport through soil (Arye et al., 2007). Additionally, discussion of fractionally wettability, in which some pores expressing contact angle, may not be filled at a given pressure (Beatty and Smith, 2013). This is further complicated when we have subcritical contact angle (CA<90°), suggesting partially filled pore, or completely empty pore not contributing to water flow. The pores contributing to flow must be wettable or else it would not contribute to water flow due to the discontinuity in pores not being filled. Under steady state conditions, if the persistence of repellency is low, we can expect that all the pores filled and conducting water will contribute to the conducting porosity. If the persistence of repellency is long, then we can assume that the some of the pores that are repellent will not fill and conduct water, which will not contribute to the conducting porosity.

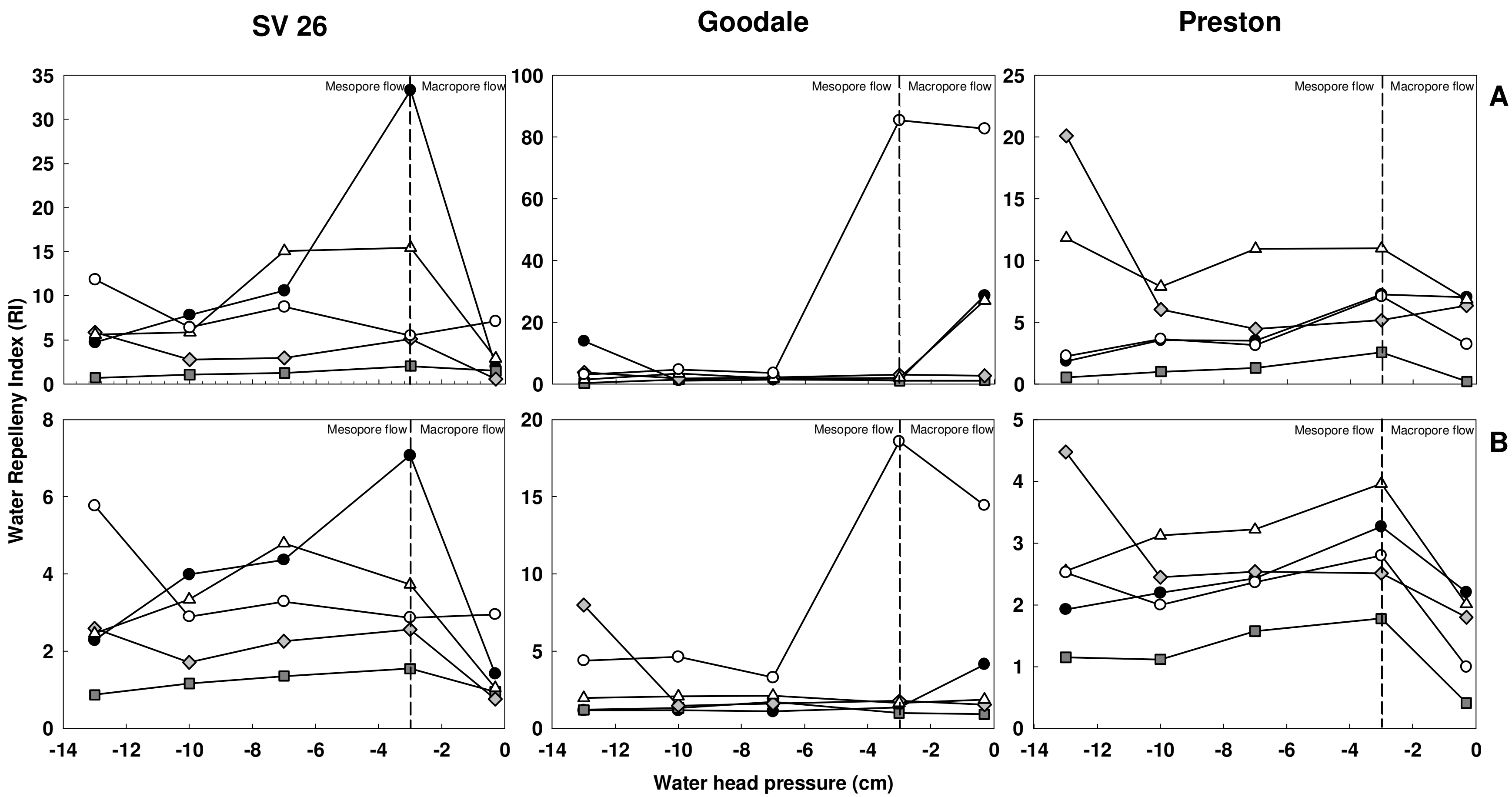

Fig. 8. Water repellency index (RI) as a function of water pressure head (cm) for the 5 locations calculated from sorptivity obtained at a) early time and b) steady state on SV 26, Goodale and Preston. Macropore and mesopore contributions to flow separated at -3 cm pressure head. Location 1 (●), 2 (■), 3 (◆), 4 (△), and 5 (○). Dashed vertical line indicates RI values at different locations under -3 cm tensions.

Water repellency indexes calculated from either the early time or the steady state varied with pressure head (Fig. 8). For example, in location 1 for SV 26, RI varies from approximately 5 at the pressure head of -13.0 cm, to 7 at the pressure head of -10.0 cm, to 9 at pressure head of -7.0 cm, to 33 at the pressure head of -3.0 cm and to 2 at the pressure head of -0.3 cm (Fig. 8a). However, the patterns of the RI variation with pressure head were different from location to location and from site to site (Fig. 8). This gives a indication of how variable the severity and persistence of SWR is between sites and within site. In most cases, RI increased with the increase in pressure head from -13.0 cm to -3.0 cm, and then decreased from pressure head = -3.0 to -0.3 cm (Fig. 8). This suggests that the role of repellency is less of a factor since the smaller pores contribute less to the total flux and repellency affects capillary flow more than gravitational flow. However, there were locations where the patterns were different. For example, at location 5 of SV 26, RI decreased from pressure head = -13.0 cm to -10.0 cm, increased from pressure head =-10.0 to – 7.0 cm and then decreased from pressure head =-7.0 to -0.3 cm (Fig. 8a). This is likely due to the highly variable nature of repellency in soils and in soil pores and that repellency affects water transport at smaller scales and larger scales like in macropores (Hallett et al., 2004).

Though the general patterns in the RI vs. pressure head relationship were similar between the early time and the steady-state at a location (Fig. 8), the repellency indices calculated from the early time were much larger than that calculated from the steady-state as discussed previously. For example, the largest RI value from location 1 of SV 26 was 33 at pressure head of -3.0 cm from the early time and was 7 from the same water pressure head from the steady-state. According to Hunter et al. (2011), who examined only early time infiltration, severely water repellent soil has RI > 19.5, and sub critically water repellent soil has RI between 1.95 and

19.5. According to this classification, location 1 of SV 26 could be classified as severely water repellent from the early time analysis, and sub critically water repellent from the steady-state analysis. This is due to fractionally wettability of the pores, with more exposure to water the soil will reach the critical water content and the equivalent pore under a certain pressure head will be filled and conduct water.

The early time analysis and steady-state analysis lead to different RI values. Determination of the RI from sorptivities at early time infiltration, would give us an indication of the repellency in the soil at the water content initially before infiltration. As well, the RI at steady state would be more related to repellency after all the equivalent pore diameters (corresponding to the tension applied from the disc) are filled with water. Therefore, the RI at steady state as compared to early time is lower due to the change in water contents (Fig. 8) (Logsdon and Jaynes, 1993). For examining the influence of SWR on the RI excluding the certain pore diameters, the RI at steady state would be more useful as all the conducting pores should be filled regardless of the persistence of SWR. As such our discussion will focus on repellency at steady state conditions.

At the highest pressure head, -0.3 cm, we see that all the locations in site SV26, Preston and Goodale (except for location 5 in Goodale) have a RI below 5 and have a lower RI than at pressure head -3.0 cm (Fig. 8). This suggests that when all the pores with diameters $\leq 1.0 \times 10^{-2}$ m are included, the contribution of non-water-repellent pores may reduce the overall water repellency. This is also likely due to the temporal removal of water repellency under this pressure head as we see a drop in RI from early time to steady state (Fig. 8). When the water content increases as more conducting pores are involved and filled, repellency can be temporally removed as the soil has passed its critical water content (Wang et al., 2000a). At -3.0 cm pressure head, we see that RI increases for most of the locations for the 3 sites, because SWR measured in soil theoretically excluded in all the macropores (diameters ≥ 1 x 10^{-3} m) (Fig. 8). At this pressure head, the macropores were empty and did not contribute to the flow measurement. Only the mesopores contributed to the flow much like at field capacity where all gravity water has drained out of the macropores (Luxmoore, 1981). This indicates that SWR still affected water flow at these conditions. We know that SWR will affect the infiltration rate (Wang et al., 2000b) during a wetting event when soil water content increases past a certain water content. However, the effect of SWR during gradual drying is still unknown.

As the pressure head decreases, we notice that repellency is still being detected by the repellency index at lower pressures heads (smaller pore diameters) (Fig. 8b). This suggests that SWR affects both flow through macropores and mesopores (Nyman et al., 2010). Repellency at smaller pore sizes may be less influential as liquid flux is smaller in pores with smaller diameters. As such repellency index measured under the -13.0 cm pressure head would be less significant as the liquid through pores are less influential to the total flux. The result that repellency was present under different pressure heads validates that the measurement under different pressure head is important to determine the role SWR plays in certain pores (Hunter et al., 2011). If the repellency index was determined under only one pressure head neglecting a specific pore size range, the overall influence of SWR to soil water transport would be incorrect. This is the first demonstration of the RI as a function of pressure head or exclusion of certain filled pore diameter.

Fig. 9 depicts the steady state infiltration with corresponding pressure heads for location 1 in the sites of SV 26, Goodale, and Preston, respectively. The infiltration rate decreases with the decrease in pressure head due to exclusion of larger diameter pores and number of pores.

Similar to water infiltration, steady state infiltration of ethanol decreased with the increase in ethanol head. All 3 sites showed a higher steady state infiltration rate of ethanol than that of water (Fig. 9). This is mainly due to the effect of SWR on the infiltration. Infiltrating 95% ethanol instead of water will give us an intrinsic infiltration rate, which is the infiltration not influenced by repellency of pores in soil. When the steady state infiltration rate is the same for both water and 95% ethanol, repellency has no effect on the infiltration as equivalent pores are filled under both liquids.

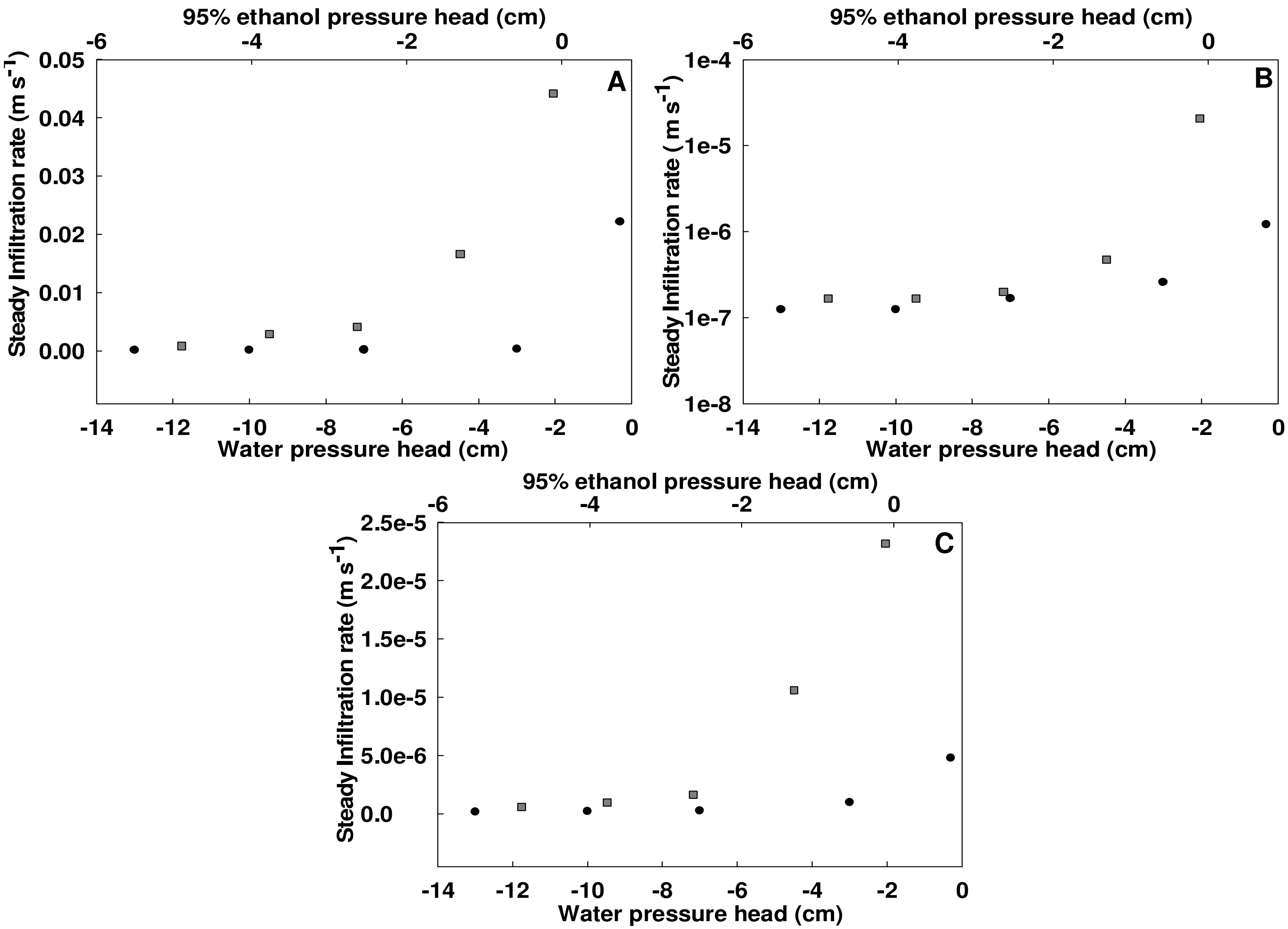

Fig. 9. Steady-state infiltration rate (x 10-2 m s-1) from location 1 on a) SV 26, b) Goodale, c) Preston; calculated from water pressure head (cm) on the bottom axis, and equivalent 95 % ethanol head (cm) on top axis. Water (●), Ethanol (■)

The liquid flux at a defined pore diameter range was calculated from subtracting the liquid flux at an upper constrained pressure head with the liquid flux at a lower constrained pressure head. For example at -0.3 cm of pressure head the water flux was subtracted from the pressure head of -3.0 cm which give us the water flux contribution from 1.0 x 10^{-2} to 1.0 x 10^{-3} m pore sizes. Water fluxes are subtracted with decreasing water pressure head from -0.3 to -3.0 cm, -3.0 to -7.0 cm, -7.0 to -10.0 cm, and -10.0 to -13.0 cm. This corresponds to the water flux through pores diameter ranges of 1.0 x 10^{-2} to 1.0 x 10^{-3}, 1 x 10^{-3} to 4.2 x 10^{-4}, 4.2 x 10^{-4} to 3.0 x 10^{-4}, 3.0 x 10^{-4} to 2.3 x 10^{-4} and ≤ 2.3 x 10^{-4} m equivalent pore diameters. Since the water flux compared to 95% ethanol flux was lower, I assumed that the 95% ethanol flux is our liquid flux in soil not affected by repellency (surface properties). This is true when soil water contents are higher than its critical water content. In SV 26 site for location 1, 2, 3, and 5, most of the liquid flow occurred at 1.0 x 10^{-2} to 1.0 x 10^{-3} m pore diameter range and for location 4 at the 1.0 x 10^{-2} to 4.2 x 10^{-4} m diameter range (Fig. 8a). In the Goodale site, most of the five locations show that liquid flows through the 1.0 x 10^{-2} to 1.0 x 10^{-3} m, with the exception of location 3 as some of the liquid flow went through the pores with a diameter ≤ 2.3 x 10^{-4} m (Fig. 8b). For the Preston site, most of the liquid flowed through the pores with a diameter between 1 x 10^{-2} to 1 x 10^{-3} m at locations 1, 3, and 4, while at locations 2 and 5 it flowed through the pores with diameter between 1.0 x 10^{-3} to 4.2 x 10^{-4} m (Fig. 8c). Since more liquid flows through larger diameter pores than through small diameter pores, the large diameter pore ranges contribute more to the total liquid flux.

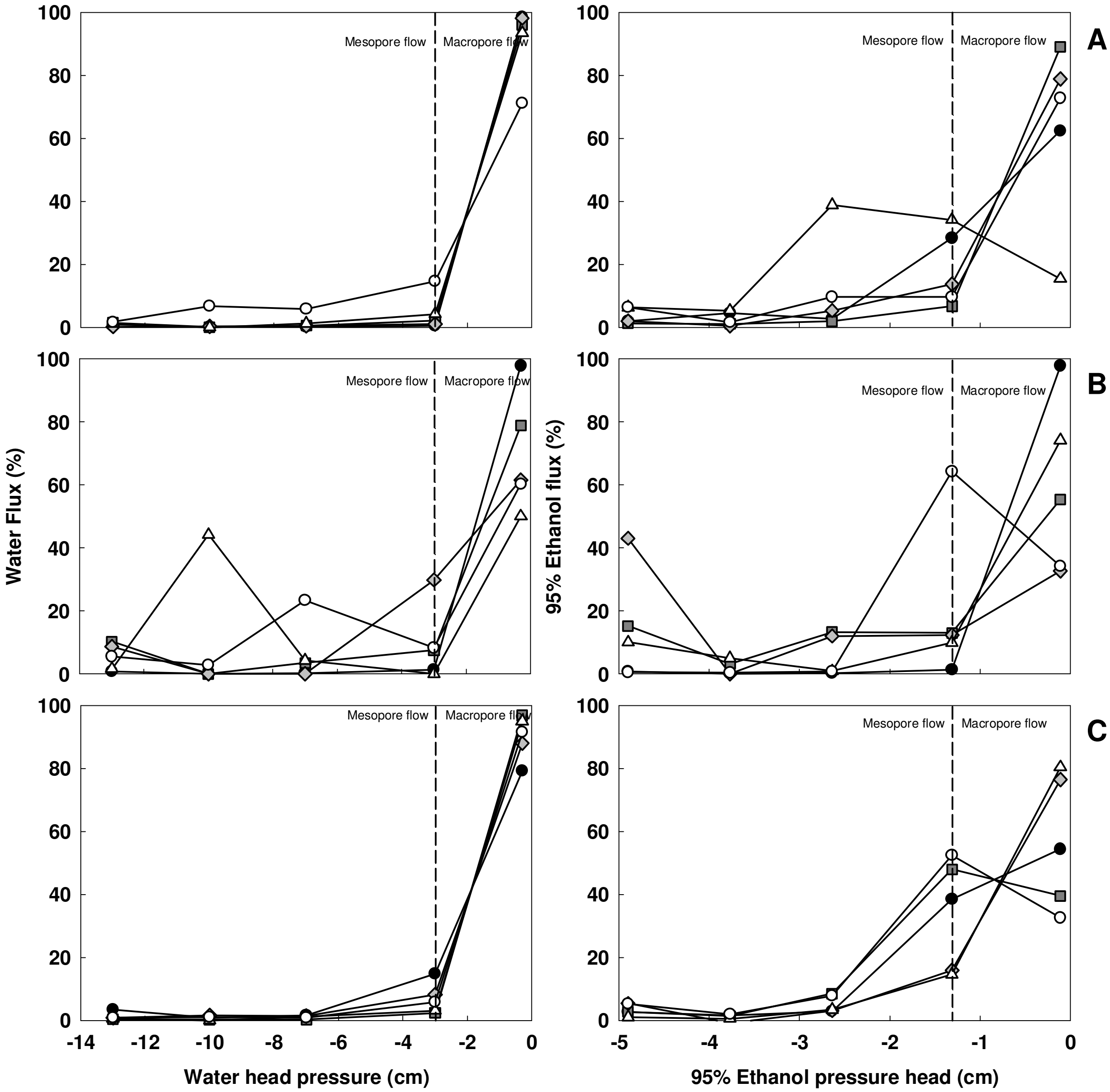

Fig. 8. The water and 95% ethanol flux contribution (% of total volume) as a function of the water pressure head (cm) and ethanol pressure head (cm) respectively for the five locations on a) SV 26, b) Goodale, and c) Preston. Macropore and mesopore contributions to liquid flux (%) are separated at -3 cm pressure head. Location 1 (●), 2 (■), 3 (◆), 4 (△), and 5 (○).

Under the same tension, with 95% ethanol and water, we can calculate the pore size range that is involved in the water conducting process. For example under the 13 cm and 4.9 cm of water and ethanol tension respectively, the pores that are involved are >2.3 x 10^{-4} m. However if repellency affects a portion of pores, then a significant portion of water conducting is lost due to change in CA in pores preventing or limiting the conduction in soil. Slightly water repellent soil have lower actual pore radii conducting flow compared to potential pore radii (The actual pore radii available for flow taking into account reduced wettability) (Beatty and Smith, 2013). The conducting porosity (calculated as the % of the total volume) in soil was generally higher under 95% ethanol infiltration than under water infiltration for all the locations in SV 26, Goodale and Preston sites at each pore diameter range. This resulted in an overall higher conducting porosity under 95% ethanol compared to water (Table 4). The difference is most likely caused by water repellency restricting flow through certain pores. This also supports the idea that all soils show some severity of repellency, therefore causing the difference in calculated conducting porosity. When examining water repellent soils, the larger the change in conducting porosity the higher the severity of SWR present. As SWR does not affect 95% ethanol infiltration, the lower conducting porosity in water than in ethanol would also indicate the presence of water repellent pores. The 95% ethanol infiltration was also used to determine the intrinsic conducting porosity (Lamparter et al., 2010).

Table 4. Estimation of the water conducting porosity (WCP) and intrinsic (95% ethanol) conducting porosity (ECP) (% of total soil volume) for pore diameter of 0.0231 to 1 mm for the five locations in each site

Pore Diameter	Location 1		Location 2		Location 3		Location 4		Location 5	
(a-b), cm	(WCP)	(ECP)	(WCP)	(ECP)	(WCP)	(ECP)	(WCP)	(ECP)	(WCP)	(ECP)
	%		%		%		%		%	
SV 26	3.04×10^{-3}	5.76×10^{-2}	5.20×10^{-3}	1.62×10^{-2}	4.29×10^{-3}	1.70×10^{-2}	5.65×10^{-3}	4.93×10^{-2}	1.11×10^{-3}	2.17×10^{-2}
Goodale	1.25×10^{-4}	1.38×10^{-3}	4.40×10^{-4}	1.02×10^{-3}	5.78×10^{-6}	4.19×10^{-4}	1.54×10^{-4}	1.18×10^{-3}	6.37×10^{-5}	3.81×10^{-2}
Preston	4.61×10^{-4}	6.39×10^{-3}	4.83×10^{-3}	6.77×10^{-3}	6.19×10^{-4}	6.06×10^{-3}	1.12×10^{-3}	2.74×10^{-2}	1.25×10^{-3}	6.83×10^{-3}

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At the pore diameter ranges of 2.3 x 10^{-4} – 1.0 x10^{-2} m, locations are ranked in order of decreasing total water conducting porosity; Location 4, 2, 3, 1, and 5 for SV 26; Location 2, 1, 4, 5, and 3 for SV 26; Location 2, 5, 4, 3, and 1 for SV 26 (Table 4). For the intrinsic (95% ethanol) conducting porosity in the pore diameter range of 2.3 x 10^{-4} – 1.0 x10^{-2} m, locations were ranked in terms of decreasing intrinsic total conducting porosity; Location 1, 4, 5, 3, and 2 for SV 26; Location 1, 4, 2, 3, and 5 for Goodale site; Location 5, 1, 4, 2 and 3 for Preston (Table 4). This difference in rank between water and 95% ethanol supports that RI measured under each pressure head varies in each location as the intrinsic total conducting porosity order has changed. The changes in the conducting porosity for all five locations for each of the 3 sites are shown Fig 8. The SWR had more of an effect on location 1, 4, as compared to 2, 3, and 5 because the change (2.3 x 10^{-4} – 1.0 x10^{-2} m diameter range) between the water and intrinsic conducting porosity was greater at location 1 and 4 than at location 2, 3, and 5 in site SV 26 (Fig. 11a). This is mainly due to the more pronounced effect of SWR on the water conducting porosity at the pore diameter ranges of 1.0 x 10^{-3} – 1.0 x10^{-2} m and 4.2 x 10^{-4} – 1.0 x10^{-3} m which constitutes 62% and 28% respectively of the liquid flux for location 1 in site SV 26 (Fig. 8a). At location 4, there is more mesopore contribution to water flow due to the higher bulk density (Table 3). This suggests that a large percentage of the total liquid flux in this location was contributed from the mesopores (85% of total flux came from diameters $\leq$ 1.0 x 10^{-3} m). Therefore, the change in the conducting porosity in location 4 is larger than locations 2, 3 and 5 in the SV 26 site.

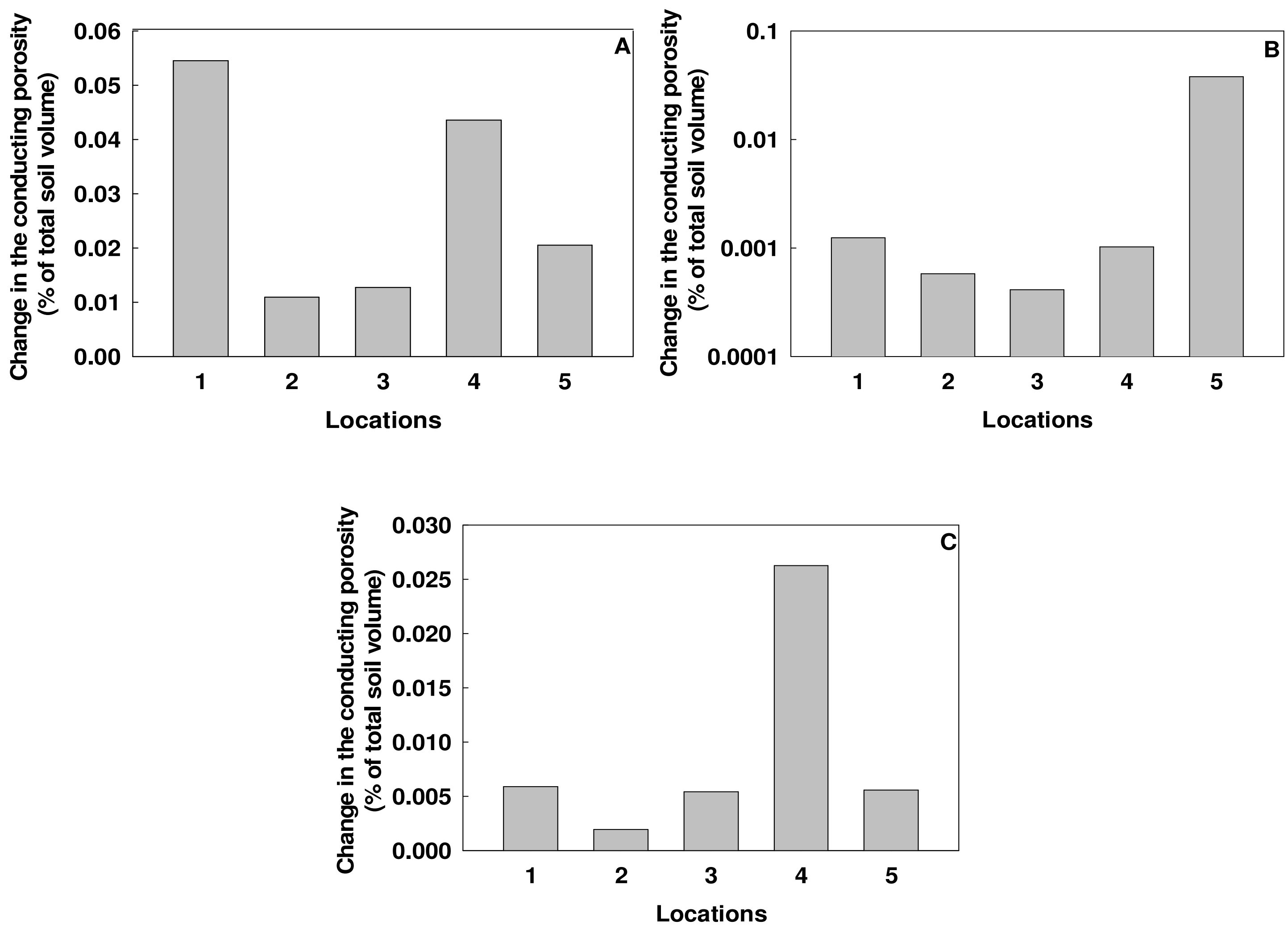

Fig. 11. The change in the estimated conducting porosity (95% ethanol conducting porosity – water conducting porosity) (% of total volume) in the five locations on a) SV 26, b) Goodale, and c) Preston.

In the Goodale and Preston site, the largest change in conducting porosity was observed in location 5 and location 4 respectively (Fig. 11b, 11c). In the Goodale site, Location 5 had only 34% of the total liquid flux flowing through 1.0 x 10^{-2} to 1.0 x 10^{-3} m pore diameter range, compared to Location 1 having 98% (Fig. 10b). However at 1.0 x 10^{-3} to 4.2 x 10^{-4} m pore diameter ranges, 64% of the total liquid flux flowed through these pores in location 5. As such the high RI observed at early time and steady state conditions in location 5 results in a larger change in the conducting porosity as compared to all locations in the Goodale Site (Fig. 11b). At location 4 in the Preston site, 80% of the total liquid flux occurred through1.0 x 10^{-2} to 1.0 x 10^{-3} m pore diameter sizes (Fig. 10c). With larger RI observed at early time than from steady state, this resulted in the largest change in conducting porosity compared to other locations in the Preston site (Fig. 10c).

Examining location 2 and 3 in SV 26, Goodale, and Preston sites, we observe lower levels of repellency as compared to all other locations in each of the 3 sites at all pressure heads (or all the pore sizes) except for location 2 in the Preston site (Fig. 8). Location 2 in the Preston site had a large repellency index at the most negative pressure head (pore diameter size ≤ 2.3 x 10^{-4}). However at this pressure head the liquid flowing through this pore diameter range did not contribute much to the total liquid flux. This resulted in not much change to the conducting porosity. In all the sites, Location 2 and 3's water conducting porosity between the pore diameter ranges of 1.0 x 10^{-3} – 1.0 x10^{-2} m and 4.2 x 10^{-4} – 1.0 x10^{-3} m did not change much with the intrinsic conducting porosity. This indicates that SWR will have less of an effect on the total conducting porosity in these two locations as the pore diameters that contribute more to the total liquid flux had a smaller RI as compared to the other three locations. This also suggests the SWR effect on the water conducting porosity originated mainly from the reduced infiltration through the larger pores. The effect of SWR on the conducting porosity is directly related to how much the infiltration through these pores is reduced and how severe the repellency is in pore diameter ranges which contribute to majority of the total liquid flux.

4.6 Conclusions

This study revealed that ethanol infiltration through a tension infiltrometer can be used to determine the intrinsic conducting porosity (unaffected by surface properties) in water repellent soils. The obtained intrinsic conducting porosity is much higher than that from water conducting porosity if the soil is water repellent. We also determined how RI varies under different pressure heads. RI values obtained under different pressure heads are different due to all pore size ranges tested showing some degree of repellency. This suggests that some pores are not being filled under certain pressures at equilibrium. This would suggest that water retention in soil would be affected by water repellency since some pores are not being filled under certain pressure conditions. The effect of RI on the water conducting porosity is related to how many and how much of each equivalent pore is filled under a certain pressure head to the total liquid flux. Large diameter pores contribute more to the liquid flux than smaller sized pores. As a result a high RI in the macropores in contrast to high RI at the mesopore ranges will result in a decrease in the water conducting porosity. The determination of the RI under different pressure heads gives a better indication of how SWR will affect water flow through different pore diameters. Additionally, determining RI using a tension which includes the pore diameters that contribute more to the total liquid flux would give a better indication of the role repellency plays in soil water transport.

5. A NOVEL METHOD FOR IDENTIFYING HYDROPHOBICITY ON FUNGAL SURFACES: CONTACT ANGLES[3]

5.1 Preface

Fungal surface hydrophobicity has many ecological functions and is speculated to be a major cause of SWR (White et al., 2000; Feeney et al., 2006). Water contact angles are a direct and simple approach for characterization of SWR and fungal surface properties (Wessel, 1988; Bachmann et al., 2000; Letey et al., 2000). However due to roughness of the surface of soil and fungi, water contact angles are often obscured and hard to measure (Smits et al., 2003). With advances in contact angle measurements, new fitting schemes have been developed to fit contact angle on obscured surfaces such as fungi and soil (Stalder et al., 2010). The objective of this study was to evaluate if utilization of *in-vitro* growth conditions coupled with versatile image analysis allows for more accurate fungal contact angle measurements. Fungal strains classified by the surface properties were utilized in Chapter 7 for assessment of the change in SWR and infiltration rate in soil.

5.2 Abstract

Fungal surface hydrophobicity has many ecological functions and water contact angles measurement is a direct and simple approach for its characterization. The objective of this study was to evaluate if *in-vitro* growth conditions coupled with versatile image analysis allows for more accurate fungal contact angle measurements. Fungal cultures were grown on agar slide media and contact angles were measured utilizing a modified microscope and digital camera

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[3] This work has been previously published in Chau, H.W., Si, B.C., Goh, Y.K., and Vujanovic, V. (2009). A novel method for identifying hydrophobicity on fungal surfaces. Mycological Research. 113:1046-1052. Minor modifications have been made for consistency.

setup. Advanced imaging software was adopted for contact angle determination. Contact angles were observed in hydrophobic, hydrophilic and a newly created chronoamphiphilic class containing fungi taxa with changing surface hydrophobicity. Previous methods are unable to detect slight changes in hydrophobicity, which provide vital information of hydrophobicity expression patterns. Our method allows for easy and efficient characterization of hydrophobicity, minimizing disturbance to cultures and quantifying subtle variation in hydrophobicity.

5.3 Introduction

Microbial surface characteristics via the cell wall interactions have important implications in a variety of processes (Dague et al., 2007). It is one of the surface properties influencing microbial interactions at the fungal interface (Van Loosdrecht et al., 1987; Gannon et al., 1991). Key functions include supporting internal turgor pressure, appressorium formation in the cells and also providing structure, shape, adhesion and aggregation (Lee and Dean, 1994; Dague et al., 2007). Microorganisms and their interactions at the interfaces are known to be controlled by physicochemical properties of the cellular surface (Smits et al., 2003). Research suggested that the presence of hydrophobic moieties causes the fungal cell surface to exhibit hydrophobic properties (Hazen, 1990; Hazen et al., 1990; Lopez-Ribot et al., 1991; De Vries et al., 1993). Spore dispersal, adhesion, pathogenesis and breaking surface tension have been linked to fungal hydrophobic moieties, which were identified as a class of cysteine rich proteins called hydrophobins (Hazen, 1990; Stringer et al., 1991; Wessels et al., 1991; Wessels, 1992; Bidochka et al., 1995a; b). The relationship between hydrophobins and fungal hydrophobicity is well understood (Wösten and de Vocht, 2000), but how the expression of these proteins affects contact angle measurement of fungal surfaces has not been addressed. It is known that changes in the developmental stages of a fungus cause expression of different hydrophobins, which is likely one of causes of change in fungal surface hydrophobicity (Peñas et al., 1998). Besides hydrophobins, secondary metabolites such as despides and depsidones were also found to contribute to surface hydrophobicity in lichen mycobionts (Lange et al., 1997; Huneck, 2003; Lumbsch et al., 2006). Other fungal metabolites and repellents including mycotoxins and free radicals may also contribute to fungal hydrophobicity (Teertstra et al., 2006; Orciuolo et al., 2007; Peiris et al., 2008). Examinations into the causes of fungal surface hydrophobicity should also encompass compounds beside hydrophobins.

Current methods employed to characterize/assess microbial cell surface hydrophobicity include binding to aliphatic acids, hydrocarbons, microsphere assay, colony imprints, dielectric permittivity, hydrophobic interaction chromatography, imprint assay, rolling drop assay, salt aggregation test and two phase partitioning (Doyle, 2000). These methods are subjected to criticism as they are indirect methods to quantify hydrophobicity of the microbial cell surfaces. Time, temperature, pH, ionic strength, and interaction species concentration are factors that affect these methods especially when dealing with adhesion techniques (Ofek and Doyle, 1994). Microbial adhesion methods involve both electrostatic effects and hydrophobic interactions to bind, which suggest other interactions may influence the results (Geertsema-Doornbusch et al., 1993; Doyle, 2000). Current methods involve manipulation of the specimen (washing, staining, extraction, adhesion and drying), which may drastically impact the hydrophobicity assessment.

Behaviours such as excretion of exudates, resistance to flooding, production of aerial mycelia, spores and fungal growth are the basis of some of methods for indirect differentiation between hydrophobic and hydrophilic fungal surface properties (Stenström, 1991; Unestam and Sun, 1995). The way that fungi and microorganism in general grow and develop, will determine the method employed (Hazen, 1990). In an effort to maintain consistency and reproducibility, much of the research requires sample manipulation to ensure standard conditions. Manipulation of the sample will undoubtedly affect the hydrophobicity, resulting in incorrect measurements. This is more prevalent when a change is made to hydrophobic samples such as fragile fungal cultures. Due to its delicate and multifaceted nature of fungal growth, they have been largely excluded from hydrophobicity measurements (Hazen, 1990; Smits et al., 2003). Unestam (1991) proposed a simple method which required placing small droplets (≤0.01 μl) on fungal structures and observing water drop penetration for at least 2 h at 20 °C. This method allows for direct characterization of hydrophobicity with minimized disturbances to fungal surfaces, but is time-consuming, subjective and does not provide information of surfaces energies (or degree of hydrophobicity).

An important parameter in surface science is contact angles; defined as the angle formed between liquid–vapour and the liquid–solid interfaces, at solid–liquid–vapour three phase contact area (Lam et al., 2002). Contact angles are a common measure of the degree of surface hydrophobicity, as well as surface energies, heterogeneity and roughness (Lam et al., 2002). Measurements are simple and result in high confidence level in assessing hydrophobicity (Doyle and Rosenberg, 1990; Doyle, 2000), making it one of the most widely used techniques (Lam et al., 2002). However acquiring consistent contact angles is a challenge because the effectiveness of contact angle measurement relies on the quality of the sample surface (Lam et al., 2002). Traditional methods for contact angle acquisition such as the goniometry, measure the angle by placing a tangent to the water drop at its base (Lam et al., 2002). However, due to background discrepancy and roughness of the sample surface, distinct baselines may not always be visible (Duncan et al., 1995; Stalder et al., 2006; Goclawski and Urbaniak-Domagala, 2007; Hauck et al., 2008).

To address the limitations of the goniometer method on fungal surfaces, Smits et al. (2003) measured contact angles on fungal cultures overgrown on filter paper, which was previously done on bacteria cells (Absolom et al., 1983). However, the amount of fungal accumulation on the filter paper may also affect the result (Van der Mei et al., 1991). In addition, the contact angle on filter grown with fungal cultures requires manipulations of sample (washing and air drying filter), which may have a direct influence on fungal surface hydrophobic properties. A simple method that utilizes an undisturbed sample and can accurately measure the subtle variations in both the persistence and degree of hydrophobicity is required.

The objective of this study was to develop and apply a simple and rapid method for assessing water contact angles on fungal cultures grown on slide media using a modified microscope. I hypothesized that this method can measure water contact angles on fungal cultures. With this method, we can evaluate both the contact angle (degree of hydrophobicity) and the water drop penetration (persistence of hydrophobicity) in effort to determine fungal hydrophobic properties. Contact angles at different growth stages can be compared to analyze how the age of the culture affects hydrophobicity. To assess if this method is successful in quantifying fungal surface property, we compared our results to fungal strains previously analyzed.

5.4 Materials and Methods

5.4.1 Fungal Inocula

Eleven fungal strains from phyla Ascomycota, Basidiomycota and Zygomycota were selected for quantifying fungal surface properties. Some of these fungal strains were previously analyzed by Smits et al. (2003) and Unestam (1991) and Unestam and Sun (1995). Cultures of *Alternaria sp.* SMCD 2122, *Penicillium aurantiogriseum* SMCD 2151, *Cladosporium* *cladosporioides* SMCD 2128, *Cladosporium minourae* SMCD 2130, *Suillus tomentosus* UAMH 9089/SMCD 2263, *Cenococcum genophilium* (Strain UAMH 5512) SMCD 2264, *Trichoderma harzianum* SMCD 2166, *Mortierella hyalina* SMCD 2145, *Laccaria laccata* UAMH 10033/SMCD 2265 and *Laccaria trichodermophora* SMCD 2267 were obtained from the Saskatchewan Microbial Collection and Database (SMCD) and the University of Alberta Microfungus Collection and Herbarium (UAMH). These strains were maintained on Oxoid potato dextrose agar (PDA) plates (39 g potato dextrose agar in 1 L distilled H_2O) or Modified Melin Norkrans (MMN) Media (UAMH Recipe) in plastic Petri dishes at 22.5 °C.

5.4.2 Preparation of Slide Media

Pre-cleaned microscope slides (76.2 mm x 25.4 mm) were aseptically sterilized by dipping in 95% ethanol solution and flaming. The sterilized slides were then aseptically transferred to sterilized petri dishes. Approximately 2 mL of the PDA or MMN media was spread uniformly on the slide using a micropipette. Then the slide media was allowed to harden.

5.4.3 Fungal Slide Culture

Under aseptic conditions, a small scraping of fungi from an active growing culture was inoculated on the center of the slide media. The slide was then incubated in the dark at 22^{o}C, and examined every day until adequate growth occurred. The slides were then taken from the sterile plates and placed on sample stage. Contact angles of 5 to 6 water droplets of 10μL were measured from one edge of the slide to the other edge on two replicate slides. Additional measurements on the PDA slide were preformed to assess reproducibility of contact angles of the strains.

5.4.4 Contact Angle Acquisition

The experimental apparatus for contact angle measurements consisted of three main parts: sample stage, image observation and acquisitions device, and contact angle analysis software. Sample stage was a biochem* support jacks Brand tech (16cm (L) x 13 cm (W) x 27.5 cm (H)). It allowed for easy movement of a slide for image viewing and manipulations. Slide samples were viewed at a maintained temperature of 25^{o}C and at 30% relative humidity. Viewing and acquisition of contact angle images was conducted using a modified stereo microscope with a horizontal light path (Fig. 12). A Zeiss SV 6 Stereomicroscope was modified by moving the view from a vertical direction to a horizontal direction. Coupled with Nikon Cool Pix 8400 camera with 3264 x 2448 resolutions contact angles were viewed through the microscope and images were captured with the camera (Fig. 12). The images were then transferred to a computer that contains imaging software.

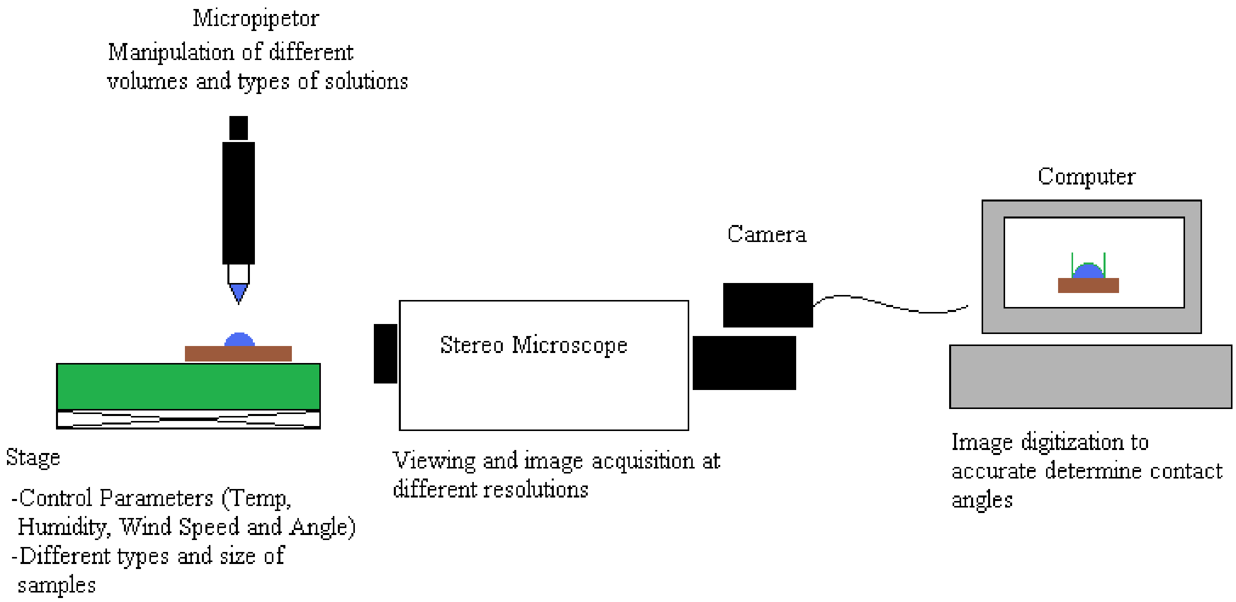

Fig. 12. Diagram of the modified microscope setup for image acquisition and contact angle measurement.

5.4.5 Contact Angle Measurement

The software utilized in this study is the open source multi-platform java image processing program ImageJ, which is publicly available at http://rsb.info.nih.gov/ij/. Measurement of contact angles was obtained by using the Low Bond Axisymmetric Drop Shape Analysis Model of Drop Shape Analysis (LB_ADSA) plug in, which is available online for free at http://bigwww.epfl.ch/demo/dropanalysis/. This model utilized image gradient energy and cubic spine interpolation to obtain high precision contact angle image measurements (Stalder et al., 2006). This global model utilized first order perturbation solution of the Laplace equation for measuring axis-symmetric drops while fitting the whole drop profile (Stalder et al., 2006). Fitting of the whole drop profile allows for contact angle determination on fungal cultures obscured due to aerial mycelia by overcoming the baseline issues of the goniometer approach. Stalder et al. (2006) suggested that this computational solution was more efficient than the numerical integration and allowed for more accurate contact angle measurements (ADSA approach).

5.5 Results and Discussion

5.5.1 Hydrophobic / Hydrophilic Fungi

Alternaria sp., *Cladosporium cladosporioides*, *Cladosporium minourae* and *Penicillium aurantiogriseum* illustrated hydrophobic surface properties due to contact angles measurement >90° (Table 5, Fig. 13). Fungal strains such as *Alternaria sp.*, *C. cladosporioides*, *C. minourae* exhibited uniform colony growth on slide media, and allowed for characterization of growth rate (cm of growth/time). Water contact angles were evaluated from the point of inoculation to the end of the slide to assess how hydrophobicity changes with time (Fig. 13a). We observed only a slight difference in the contact angles assessed with the four hydrophobic strains as the standard deviation was low (Table 5). Fig. 13b shows similar water contact angles from 10 µl water droplets deposited onto the surface of *C. cladosporioides*. *Cladosporium* strains showed typically higher contact angle values, but were still within the same hydrophobicity classes (Table 5). The lower values previously recorded from the fungal filter technique (Smits et al., 2003) may be attributed to lack of fungal cell accumulation in the filter or that washing/drying the filter membrane may have reduced the hydrophobicity. For example, excretions of exudates and spores formation may be washed off the surface, which explains why smaller contact angles were observed from manipulative methods than from the proposed contact angle method.

Table 5. Comparison of contact angles obtained from the modified microscope approach on fungal slide cultures with similar fungal species from literature.

Fungus Culture	Time (Days)	PDA† θ_w	MMN‡ θ_w	Fungal Surface Classification	Literature Fungal surface classification
Cladosporium cladosporioides	10	142 ° ± 1 (106±2) §	135 ° ± 1 (100±3) §	Hydrophobic	*Cladiosporium sp.* Hydrophobic (Smits et al., 2003)
Cladosporium minourae	10	142 ° ± 5 (106±2) §	141 ° ± 1 (100±3) §	Hydrophobic	*Cladiosporium sp.* Hydrophobic (Smits et al., 2003)
Penicillium aurantiogriseum	10	128 ° ± 1	124 ° ± 1	Hydrophobic	n/a
Alternaria sp.	5	122 ° ± 1	124 ° ± 2	Hydrophobic	n/a
Suillus tomentosus	30	89 ° - 134 °	96 ° - 118 °	Chronoamphiphilic	*Suillus tomentosus* Hydrophobic (Unestam, 1991)
Trichoderma harzianum	3	61 ° - 117 ° (27±3) §	43 ° - 108 ° (25±3) §	Chronoamphiphilic	*Trichoderma harzianum* Hydrophilic (Smits et al., 2003)
Cenococcum geophilum	30	68 ° -133 °	74 ° - 81 °	Chronoamphiphilic	*Cenococcum geophilum* Hydrophilic (Unestam and Sun, 1995)
Laccaria laccata	30	0 °	0 °	Hydrophilic	*Laccaria laccata* Hydrophilic (Unestam and Sun, 1995)
Laccaria trichodermophora	21	0 °	53 ° - 82 °	Hydrophilic	*Laccaria sp*. Hydrophilic (Unestam and Sun, 1995)
Mortierella hyalina	7	59 ° ± 1	31 ° - 51 °	Hydrophilic	n/a

† Potato Dextrose Agar Media
‡ Melin Norkrans Media
§ Referenced Values
¶ No measured contact angles

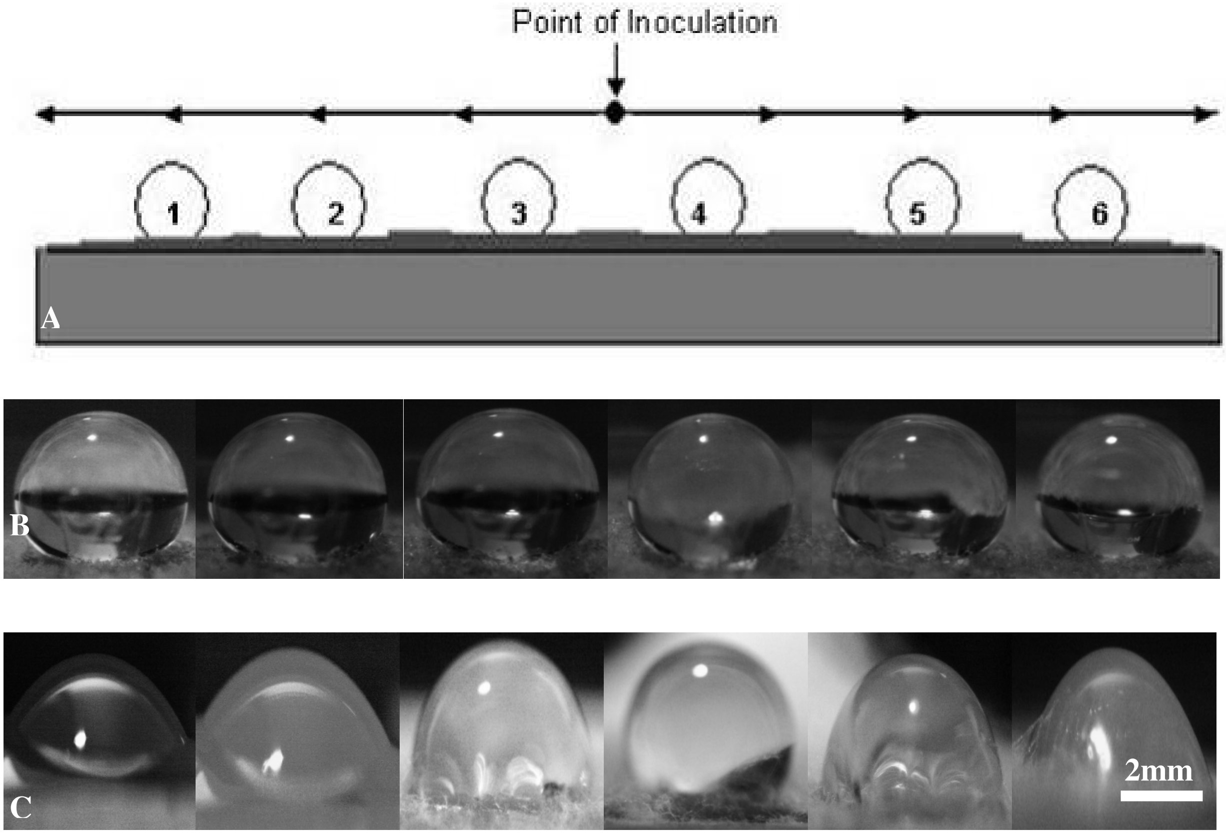

Fig. 13. a) Diagram of placement of 10uL water droplets in respect to the point of inoculation. b) Images of 10 µl water droplets on the surface of *Cladosporium cladosporioides* grown on a PDA slide. c) Images of 10 µl water droplets on the surface of *Suillus tomentosus* grown on a PDA slide media.

Mortierella hyalina, *Laccaria laccata* and *Laccaria trichodermophora* had water contact angle readings <90° and thus were classified as hydrophilic (Table 5). *L. trichodermophora* and *M. hyalina* did however show an increase in the contact angle as the placed droplets approached the area of inoculation. Fig. 13c shows lower contact angles further away from the point of inoculation from water droplets placed on the surface of *Suillus tomentosus*. Busscher et al. (1984) suggested this change in contact angle was due to denser colonization at the zone of inoculation. However, Smits et al. (2003) found no morphological differences between the two zones of colonization using scanning electron microscopy and thus concluded that it must be caused by an increase in hydrophobicity. We agreed with Smits et al. (2003), but proposed that the increase in hydrophobicity is due to a change in growth state from the point of inoculation to the edge of the slide. Our observations and results from Smits et al. (2003) suggested that the possible accumulation of hydrophobic substances as fungi aging might have caused the shift in hydrophobicity due to age and stability of fungi hydrophobicity after a certain time period. Therefore, to obtain consistent and stable water contact angle readings require stability of fungal growth. Understanding fungal life cycle, gene expression and metabolism may also provide insights into how fungal surface properties change due to external factors. *C. cladosporioides* and *C. minourae*, *P. aurantiog*riseum and *Alternaria* sp. showed little difference between contact angles on the two types of media, while *L. trichodermophora* showed the highest change (Table 5). It was also noted that the increase in contact angle was not large enough to change the classification of the strain. Depletion of carbon source in MMN medium as compared to PDA medium may have resulted in the change in contact angles. Smits et al. (2003) and Nielsen et al. (2001) noted that depletion of carbon sources may potentially shift the metabolism of a particular fungus, resulting in changes in surface property. Moreover, Vergara-Fernández et al. (2006) found that type of carbon source and cultivations conditions affect the surface hydrophobicity of *Fusarium solani*. Glucose- and nitrogen-depleted media were also observed to show high or increased in expression of certain hydrophobin-encoding genes in *Trichoderma reesei* (Nakari-Setälä et al., 1997).

5.5.2 Chronoamphiphilic

S. tomentosus and *T. harzianum* had a contact angle $> 90^{o}$ at the point of inoculation, but contact angles became smaller further away from the point of inoculation. At the end of growth, contact angles were $< 90^{o}$ on the slide (Fig. 13c). Therefore, these two strains showed hydrophobic characteristics at the point of inoculation, but growth further away from the point of inoculation showed hydrophilic characteristics. However, *S. tomentosus* was classified as hydrophobic by Unestam (1991) and *T. harzianum* was described as hydrophilic by Smits *et al.* (2003). Unestam (1991) also speculated that hydrophobic fungus must also have hydrophilic structures to aid in uptake of water which might be the case in *S. tomentosus*.

The presence of sporulation, and change in life cycles may also result in a shift in hydrophobic property (Smits et al., 2003). For example, after approximately 3 days of growth on PDA and MMN, *T. harzianum* started producing green spores as opposed to the visible white mycelia growth (Smits *et al*. 2003). Conidia were collected from 1st, 3rd, and 5th days of *T. harzianum* PDA grown cultures according to the procedures outlined in Whiteford and Spanu (2001). Amount of conidia was counted with haemacytometer and day 5 had the highest average number of conidia (1.6×10^6 cells mL^{-1}) as compared to day 1 and day 3 (1.7×10^4 cells mL^{-1} and 4.0×10^5 cells mL^{-1} respectively). Fig. 14a and 14b visualizes a substantial increase in spore number from the zone of older growth when compared to the younger growth on the same plate. As *T. harzianum* aged in culture, generation of hydrophobic spores can further affect water contact angle (Smits et al., 2003). Nakari-Setälä et al. (1997) discussed that most of the hydrophobins are found in fungal aerial structures, such as spores or conidia and these cells are highly hydrophobic. Furthermore, production of suitable substrates and moisture could drastically increase the spore production in *Trichoderma* species, which would undoubtly affect its surface properties (Cavalcante et al., 2008). We recognize that temporal changes in surface hydrophobicity can occur and proposed a new *Chronos (Greek: time) – amphiphilic (Greek: loving both)* class containing fungal taxa with shifting hydrophobicity over time and space. With the additional of a new class, emerging hydrophobicity expression patterns within the same species can be quantified more accurately throughout growth chronosequences, time and space scales. This provides further insights into the functional significance of fungal surface properties.

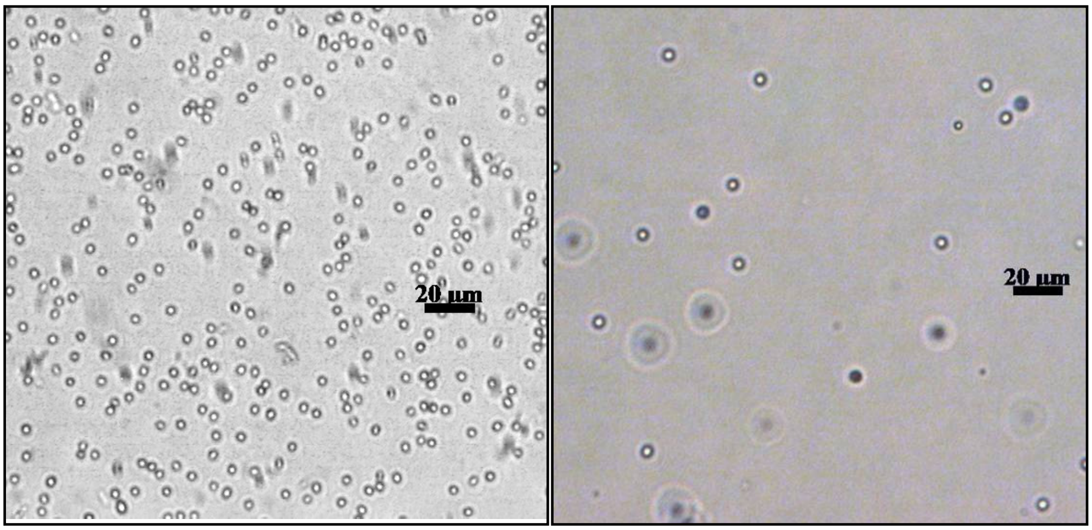

Fig. 14. Wet mount of spores extracted from *Trichoderma harzianum* from two areas from the same fungal cultures: a) Older growth (green area/centre portion) and b) Younger growth (white area/outer region). * Drops of 10µl sterile dH_2O were spotted onto fungal cultures of both centre and outer portions. Water droplets were pipetted few times and transferred to slides for microscopy observation.

With the proposed method, we can assess variations in fungal hydrophobicity and test shifts in surface properties of actively growing fungi. To perform the latter assessment and tests, the microscopic slide can be exposed to varying environmental parameters, e.g. temperature, humidity, oxygen concentrations and/or UV radiation. Since agar is the support medium, the nutrient content of the support media can also be modified in order to analyze behavioural changes of particular fungal strain (Fig. 14. *T. harzianum*). Inhibitory compounds can further be used to evaluate induced changes in fungal hydrophobicity.

Finally, our study proposes a more efficient method for monitoring fungal hydrophobicity— taking into account variations in environmental factors influencing fungal surface properties. Therefore, this novel method is particularly important for understanding fungal biological cycles and ecological functions, which are closely related to surface properties.

6. ASSESSMENT OF ALCOHOL PERCENTAGE TEST FOR FUNGAL SURFACE HYDROPHOBICITY MEASUREMENT[4]

6.1 Preface

Measurement of contact angles on fungal surfaces and soils are often difficult to measure due to obscured measurement due to fungal mycelia and roughness of soil particles (Chapter 5). Alternative measurement techniques have been developed to measure water repellency on soil (Roy and McGill, 2002). Utilizing different concentration of ethanol solutions (molarity of ethanol method or alcohol percentage test) with different surface tension and measuring the lowest concentration of water/ethanol solution that resist wetting, this provides an indication of water repellency in soils. My aim was to extend this method further and apply it on fungal surfaces for characterization of fungal hydrophobicity. The objective of this study was to determine whether assessing the penetration of solutions with different concentrations of ethanol (Alcohol Percentage Test: APT) on fungal surfaces is effective in characterization of hydrophobicity on fungal surfaces. Results were also validated against contact angle measurements obtained in Chapter 5.

6.2 Abstract

The aim of this study was determine whether assessing the penetration of solutions with different concentrations of ethanol (Alcohol Percentage Test: APT) on fungal surfaces is effective in characterization of hydrophobicity on fungal surfaces. Alcohol percentage test and contact angle measurements were conducted on nine hydrophobic and two hydrophilic fungal strains from the

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[4] This work has been previously published in Chau, H.W., Goh, Y.K., Si, B.C., and Vujanovic, V. (2010). Assessing ethanol sorptivities on fungal surfaces: a measure of the degree of hydrophobicity. Letters in Applied Microbiology. 50:295-300. Minor modifications have been made for consistency.

phyla of Ascomycota, Basidiomycota and Zygomycota. There was a strong positive correlation (R^2=0.95) between the APT test and contact angle measurements from eight out of the nine hydrophobic stains (four pathogenic and mycotoxigenic *Fusarium* taxa, one melanosporaceous biotrophic taxon, *Alternaria* sp, *Penicillium aurantiogriseum, and Cladosporium cladosporioides).* Hydrophilic control strains, *Mortierella hyalina* and *Laccaria laccata* had contact angles < 90^o and no measurable degree of hydrophobicity using the APT method. The APT method was effective in measuring the degree of hydrophobicity and can be conducted on different zones of fungal growth. Characterization of fungal surface hydrophobicity is important for understanding of its particular role and function in fungal morphogenesis and pathogenesis. The APT is a simple method that can be utilized for fungal hydrophobicity measurements when CA cannot be measured due to obscured view from aerial mycelia growth.

6.3 Introduction

Microbial cell walls and hydrophobicity of cell surfaces have recently been recognized for their importance in ecology, medicine, food industry, chemistry and biology. Surface hydrophobicity is crucial for several key functions, such as appressorium formation in the cells and also providing structure, shape, adhesion and aggregation (Lee and Dean, 1994; Dague et al., 2007). These key functions may increase the virulence and pathogenicity of human and plant pathogenic microorganism. Research into fungal surface hydrophobicity has increased dramatically in recent years due to the discovery of hydrophobins. Hydrophobins are proteins that are ubiquitous to filamentous fungi and are often implicated as one of the metabolites that contribute to fungal surface hydrophobicity (De Vries et al., 1993; Wessels, 1994). These proteins are usually found and secreted on the outer surfaces of conidia, spores, aerial hyphae, infection structures and fruiting bodies (Wessels, 1996; Kershaw and Talbot, 1998; Wösten, 2001; Linder et al., 2005). They also have important functions such as allowing the escape from aqueous environments, which then allows fungi to produce aerial mycelia and spores (Schuren and Wessels, 1990; Linder et al., 2005).

Hydrophobicity mediates fungal attachment processes on other hydrophobic surfaces, which is an important step of fungal pathogenesis initiation (Howard and Valent, 1996; Linder et al., 2005). Inhibition of this surface hydrophobicity might hinder fungal pathogenesis initiation (Tucker and Talbot, 2001) and decrease aerial spore production, attachment, spread, pathogenicity, and symbiotic association with a host (Talbot et al., 1993; Tucker and Talbot, 2001). The degree of fungal hydrophobicity also has important implications for human health. The relationship between surface hydrophobicity and human pathogenic fungi is well documented in *Candida albicans* and *Aspergillus fumigatus* (Karkowska-Kuleta et al., 2009). Tighter adherence to epithelial cells, endothelial cells and extracellular proteins in *C. albicans* as well as adhesion to albumin and collagen in *A. fumigatus* are the result of hydrophobic surface properties (Karkowska-Kuleta et al., 2009). Due to hydrophobic phenomena on fungal surfaces, characterization of this property is important for understanding its functions and role in the environment as well as the host.

Despite the noted importance of fungal surface hydrophobicity, characterization methods are limited to the manner in which the fungus grows. The majority of methods are based on indirect observations, such as growth behaviour on plates and broth cultures, excretions of hydrophobic compounds and aerial growth of mycelia and spore formation (Smits et al., 2003). Techniques used to assess microbial hydrophobicity are also subject to criticism, as most of these approaches are based on adhesion properties (Chau et al., 2009). Microbial adhesion techniques involve both electrostatic effects and hydrophobic binding interactions, which led to the conclusion that other factors may influence the results (Geertsema-Doornbusch et al., 1993; Doyle, 2000). Also, adhesive methods involve manipulation of the specimen (washing, staining, extraction, adhesion and drying), which may drastically affect the hydrophobicity assessment through degradation of fungal cultures. In order to ensure accurate hydrophobicity assessment, the techniques employed must be direct and offer minimal disturbance to the culture.

Two simple techniques that are currently employed for characterization of hydrophobicity are the water drop penetration time (WDPT; Letey (1969)) and contact angle (CA) methods (Letey et al., 1962). WDPT measures the time taken by a water droplet to either penetrate or spread on a surface. Typically WDPT is assessed on porous surfaces, but it is also applicable on planar surfaces by means of absorption (Unestam, 1991). The WDPT is a measure of the persistence of hydrophobicity on a particular surface. Contact angles refer to the angle of the liquid/vapour interface where a particular liquid meets the surface. It is also commonly known as a measurement of the degree of hydrophobicity. The acquisition of contact angles is affected by the surface smoothness and uniformity, properties of the measuring liquids and the methodological approach. Unfortunately, due to the ability of fungi to produce aerial mycelia, contact angles may be difficult to measure as the angle and baseline where it is measured is often obscured. In these certain instances, an additional method is required.

Contact angle measurements and WDPT have been employed extensively in analyzing surface hydrophobicity and SWR (Letey et al., 2000). However, Unestam (1991) proposed a technique similar to WDPT on fungal surfaces, by observing the absorption of 0.01 μl water droplets on ectomycorrhizal short roots, mycelia, rhizomorphs and mats. This allowed for a direct measure of persistence of hydrophobicity on fungal surfaces.

A liquid surface tension that has a surface CA of 90° was proposed as an index of water repellency by Watson and Letey, (1970). This procedure is predicated on the assumption that a liquid can only completely wet a surface if the CA is less than 90°. For surfaces with a CA less than 90° the surface tension of the droplet is assumed to be less than that of the surface. When the CA is greater than 90°, surface tension of the droplet (liquid) is greater than that of the surface and thus will prevent wetting of the surface. Theoretically, a series of solutions providing various surface tensions can be prepared and placed onto a hydrophobic surface. The solutions with higher surface tension will tend to reside on the surface and contribute to a higher degree of hydrophobicity, while solutions with lower surface tension will either penetrate or spread on the surfaces due to a lower degree of hydrophobicity. As discussed earlier, surface tension with a CA equal to 90° is the surface tension of a solution where there is a transition from wetting, to repelling on the surface. Fluids with low surface tension can be mixed with miscible fluids that have high surface tension to create a series of solutions with varying surface tensions. Alcohol percentage test (APT) (Dekker and Ritsema, 1994), more commonly referred to as the Molarity of Ethanol method (MED) (Watson and Letey, 1970) was developed based on the fact that ethanol has a smaller surface tension (0.0219 N m^{-1}) than water (0.0719 N m^{-1}). The APT uses aqueous ethanol solutions with different concentrations to determine the lowest concentration of ethanol solution that absorbs or wets the surface (Watson and Letey, 1970). The higher the ethanol concentration that wets the surface, the more severe the degree of hydrophobicity. For soil test, five and 10 s are commonly utilized as the reference time for absorption or wetting (Letey et al., 2000). Application of this technique on fungal surfaces has yet to be assessed and may offer another direct measure of the degree of hydrophobicity. However, this approach on fungi may present some challenges due to the delicate nature of fungi as it may cause degradation of its hydrophobicity. As such, a reference point of approximately 5 s or less is more reasonable for assessing hydrophobicity on fungal surfaces due to the effect of hydrophobicity degradation (Crockford et al., 1991). The objective of this study was to determine if APT is an effective approach to characterize the hydrophobicity on fungal surfaces. I hypothesized that APT can be used to measure the hydrophobicity on fungal surfaces.

6.4 Materials and Methods

6.4.1 Fungal Strains

Eleven fungal strains from phyla of Ascomycota, Basidiomycota and Zygomycota were selected for assessing the application of the APT method. Four *Fusarium* strains (*F. avenaceum*, *F. oxysporum*, 3- and 15-Acetyldeoxynivalenol-producing *F. graminearum* chemotypes), one biotrophic mycoparasite - *Sphaerodes mycoparasitica* SMCD 2020, *Alternaria* sp. (Kunze) Wiltshire SMCD 2122, *Penicillium aurantiogriseum* Dierckx SMCD 2151, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries SMCD 2128, *Cladosporium minourae* Iwatsu SMCD 2130, *Mortierella hyalina* (Harz), W. Gams SMCD 2145 and *Laccaria laccata* Scop & Cooke UAMH 10033 /SMCD 2265, were obtained from Saskatchewan Microbial Collection and Database (SMCD) and University of Alberta Microfungus Collection and Herbarium (UAMH). The fungal isolates were maintained on potato dextrose agar (PDA) (Difco) supplemented with antibiotics (100 mg L^{-1} streptomycin sulphate and 12 mg L^{-1} neomycin sulphate) (Sigma-Aldrich) prior to the experiments to prevent contamination.

6.4.2 Contact Angles

Fungal cultures were inoculated onto slide media (Chau et al., 2009) and were incubated in the dark at 23°C. Growth was assessed daily until complete coverage of the glass slide was observed (Table 6). Approximately five to ten, 10 µl droplets of water were deposited onto fungal surfaces. Pictures were taken immediately following deposition of the droplets. Contact angles of the droplets were measured by obtaining the images using a modified microscopy apparatus and fitting a drop profile using Low Bond Axisymmetric Drop Shape Analysis Model of Drop Shape Analysis (LB_ADSA) (Stalder et al., 2006; Chau et al., 2009). Fungal plates were prepared in triplicates, while the experiment was repeated twice. Contact angles obtained on slide cultures were used to validate the APT method by linear regression.

Table 6. The age of cultures for complete coverage of slide media and contact angles obtained from surface measurements.

Fungus Cultures	Time (Days)	PDA* (θ_w)
Fusarium avenaceum	7	108 º ± 3
Fusarium oxysporum	7	116 º ± 1
3-Acetyldeoxynivalenol Fusarium graminearum	7	124 º ± 1
15-Acetyldeoxynivalenol Fusarium graminearum	7	125 º ± 2
Sphaerodes mycoparasitica	7	125 º ± 1
Cladosporium cladosporioides	10	142 º ± 1
Cladosporium minourae	10	142 º ± 5
Penicillium aurantiogriseum	10	128 º ± 1
Alternaria sp.	5	122 º ± 1
Laccaria laccata	30	0 º ± 0
Mortierella hyalina	7	59 º ± 1

* Potato Dextrose Agar Media

6.4.3 Alcohol Percentage Test

A series of aqueous ethanol solutions were prepared in 5% increments starting from 0 to 100 % ethanol (Dekker and Ritsema, 1994). Conversions of alcohol percentages to molarity, or surface tension, can be performed by using the relationship illustrated in Watson and Letey (1970) or refer to previous published data in Butler and Wightman (1932) and Roy and McGill (2002). The APT/MED protocol described by Watson and Letey (1970) and Crockford et al. (1991) was used. Four microlitre droplets of ethanol solutions were applied on the surface of fungal colonies and the time interval used for infiltration of the solution droplets was <5-s. This short penetration time was vital to ensure that hydrophobicity decay did not affect our results (Crockford et al., 1991). Inner and outer zones of fungal colony growth were defined by observations of two distinct zones, with differences in color, structure and aerial mycelia. Replicates of three droplets on each zone were assessed on three replicates of fungal cultures.

6.5 Results and Discussion

L. laccata and *M. hyalina* were the control strains and showed no hydrophobicity with CAs less than 90°, resulting in an APT reading of zero (Fig. 15a). Strains with the highest CA were *Cladosporium* (> 140°), while *F. avenaceum* strains had the lowest CA (108°) among all hydrophobic fungal strains (Fig. 15a). This APT reading (degree of hydrophobicity) was due to surface tension of the fungus (> 0.0719 Joules m^{-2}) being greater than that of any of the aqueous ethanol solutions.

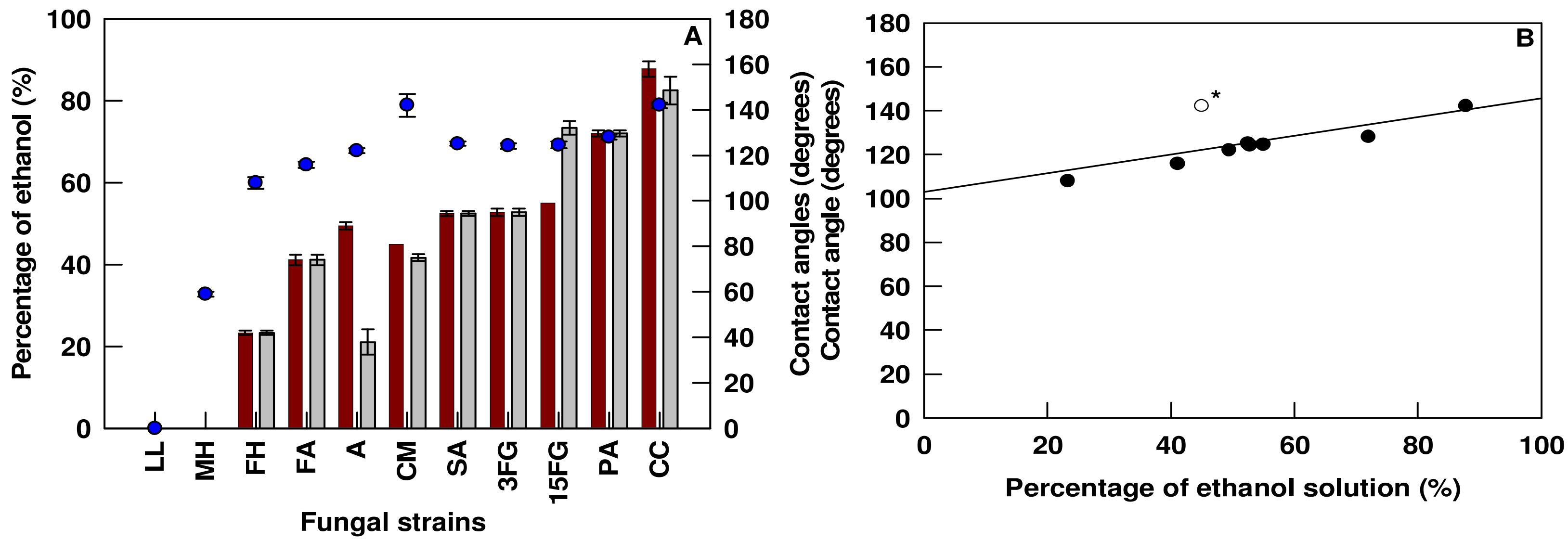

Fig. 15 a) Plot of fungal strains versus the alcohol percentage (ethanol) absorbed into fungal surfaces on PDA grown cultures and contact angles of water droplets on the fungal inoculated PDA agar slides (Chau et al., 2009): (●) Contact Angles, (■) Inner zone APT (%) and (■) Outer Zone APT (%). Note: Strains designated as *L. laccata* (LL), M. *hyaline* (MH), *F. avenaceum* (FH), *F. oxysporum* (FA), *Alternaria sp.* (A), *C. minourae* (CM), *S. mycoparasitica* (SA), *3-Acetyldeoxynivalenol F graminearum* (3A-FG), *15-Acetyldeoxynivalenol F. graminearum* (15A-FG), *P. aurantiogriseum* (PA) and *C. cladosporioides* (CC). b) The relationship between the ethanol percentage method and water contact angle measurements on agar inoculated hydrophobic fungal cultures (fitted by linear regression R^2= 0.95): (●) Fungal strains and (○) *C. minourae*.

*Removed from linear correlation due to interaction effects from ethanol solutions.

Through characterization of hydrophobicity using the APT method, we found that *Alternaria* sp., *C. cladosporioides*, *C. minourae* and 15-Acetyldeoxynivalenol-producing *F. graminearum* chemotype exhibited two zones of hydrophobicity. It was observed that mycelial growth further from the point of inoculation showed growth into the media; while in the center aerial mycelia were being produced. However, it was noted that 15-Acetyldeoxynivalenol-producing *F. graminearum* chemotype showed higher degrees of hydrophobicity on white/aerial mycelia (APT = 73±3%) than the red pigment growth in the center (APT = 55±0%) (Fig. 15a). The red pigment growth eventually replaced the growth of the white mycelia.

Linear relationships between CA and APT between hydrophobic strains were low (R^2 =0.50) and mainly due to one of the fungal strains, that is, *C. minourae*. Indeed, removal of this strain resulted in a better correlation (R^2 = 0.95) (Fig. 15b). APT values for *C. minourae* of 45% may be attributed to some interaction between the ethanol and fungal surfaces or the effect of ethanol on fungal cultures.

6.6 Conclusions

Results presented in this study revealed differential expressions of fungal hydrophobicity, which may be based on the age of mycelia growth. Understanding the expression pattern of fungal hydrophobicity may further provide insight into its implications, such as pre-pathogenesis in plant pathogenic fungi and attachment to host in human pathogenic fungi (Tucker and Talbot, 2001; Karkowska-Kuleta et al., 2009). Wessel et al., (1991) found that accumulation of hydrophobins on older mycelia growth as compared to younger growth may cause a decrease in hydrophobicity assessment at the location further away from the point of inoculation. Differences in the zones of growth may also be due to depletion of nutrients at the point of inoculation, resulting in a shift from primary to secondary metabolism (Smits et al., 2003). As we know, submerged and aerial hyphae have been shown to have differences in functions and surface hydrophobicity (Wösten and Willey, 2000). Fungi tend to acquire their nutrients by hydrophilic means (Unestam, 1991). However, when nutrients are depleted, formation of hydrophobic aerial structures and spore productions will occur. Distinguishing the differences in aerial and submerged hyphae hydrophobicity may aid in understanding spore dispersal patterns of certain types of fungi (Wösten and Willey, 2000). The APT method allows for the assessment of hydrophobicity on these two morphologically or chemically different regions because this method is a direct measurement and requires very little solution (< 10 μl).

The 15-Acetyldeoxynivalenol-producing *F. graminearum* chemotype showed a higher degree of hydrophobicity on white/aerial mycelia than the red growth in the center. This could be due to the generation of more soluble toxin or pigment compounds when compared to white aerial mycelia growth. In several previous studies, few *Fusarium* mycotoxins were found to be more hydrophobic than others (Yoshizawa and Morooka, 1973; Elosta et al., 2007). Red pigmentation is related to melanin or aurofusarin production and might offer protection against desiccation stress (Butler and Day, 1998). However, Prota (1992) found that melanin contains large amounts of water to preserve the structure of the pigment. The ability of melanin to store water and ions may suggest lower degrees of hydrophobicity as compared to the aerial mycelia (White, 1958). More research is needed to better understand the importance of morphological and hydrophobic regions as well as their relationship with *Fusarium* function and pathogenicity.

Furthermore, additional research is needed to examine how hydrophobicity will change due to alteration of growth conditions, such as nutrient status, pesticide and chemical treatment, and the presence of host plant and biocontrol organisms such as bacteria. In the case of altering growth conditions, there is the potential for alteration of fungal surface hydrophobicity in response to the changes (Smits et al., 2003; Feeney et al., 2006; Chau et al., 2009). It is also possible that any changes that may occur will result in a decrease in pathogenicity of plant pathogens (Talbot et al., 1993; Kazmierczak et al., 2005).

Hydrophilic classification of fungi is not suitable from APT measurements, due to the fact that surface tensions of fungal surfaces are always lower than the tension of aqueous ethanol solutions. Therefore, the result is an instantaneous infiltration or spreading of the solution. With the current limitations of the contact angle approach, related to its subjective nature and the possibility of obscured views, the APT method may offer an alternative approach for characterization of fungal hydrophobicity. This study has shown that the APT method is useful for analyzing the degree of hydrophobicity of hydrophobic fungal strains through comparison of CA measurements on the fungal surfaces. The APT measurements were also useful for characterization of different zones of hydrophobicity on the same culture. This will aid in providing a better understanding of the expression patterns of hydrophobicity in zones of colour changes and aged growth due to different morphological, chemical, or metabolic reasons. Given the advantages of the APT method such as the reproducibility and simplicity, it should be considered as one of the methods for quantifying the degree of hydrophobicity on fungal surfaces.

7. WETTING PROPERTIES OF FUNGI MYCELIUM ALTER SOIL INFILTRATION AND SOIL WATER REPELLENCY IN A γ STERILIZED WETTABLE AND REPELLENT SOIL[5]

7.1 Preface

Reduced infiltration, increased runoff, increased leaching and preferential flow, reduced plant growth and seed germination and increased soil erosion are some of the common issues with soil water repellency (SWR). Previous management strategies to remediate water repellency in soils such as clay addition, surfactant treatment and intensive irrigation are unfeasible due to the lack of clay material, toxicity of surfactants, and cost and access to water (DeBano, 2000; Franco et al., 2000). Alternative strategies are preferred (McKenna et al., 2002). Hydrophobic fungal structures and exudates have been known to be a major contributor in changing the soil water relationship in natural soils (Unestam, 1991; Unestam and Sun, 1995). Effective characterization of fungal strains that may cause and suppress SWR has not been examined to date. However, with results from Chapter 5 and 6, we were able to classify fungi based on its surface property. The surface property of fungi is one of the mechanism in which fungi can change the water repellency in soil. The ability of fungi to alter the SWR and enmesh soil particles can result in changes to the infiltration dynamics in soil. The objective of this study was to determine whether SWR and infiltration could be manipulated through inoculation with classified fungi based on surface properties. This study would improve our understanding of how fungi can change the SWR in soil and alter the infiltration rate. Also fungal inoculation or

[5] This work has been previously published in Chau, H.W., Goh, Y.K., Si, B.C., and Vujanovic, V. (2012). Surface properties of fungi mycelium alters soil infiltration and soil water repellency in a γ sterilized wettable and repellent soil. Fungal Biology 116:1212-1218. Minor modifications have been made for consistency.

stimulation of native fungal species could be explored as a suitable remediation strategy for SWR. Fungal strains selected for this study were previously characterized for fungal hydrophobicity (Chau et al., 2009). The suitability for inoculation in field must take into account fungal pathogenicity and virulence.

7.2 Abstract

Soil water repellency (SWR) has a drastic impact on soil quality resulting in reduced infiltration, increased runoff, increased leaching, reduced plant growth and increased soil erosion. One of the causes of SWR is hydrophobic fungal structures and exudates that change the soil water relationship. The objective of this study was to determine whether SWR and infiltration could be manipulated through inoculation with fungi. The effect of fungi on SWR was investigated through inoculation of three fungal strains (hydrophilic-*Fusarium proliferatum*, chrono-amphiphilic-*Trichoderma harzianum* and hydrophobic-*Alternaria* sp.) on a water repellent soil (WR-soil) and a wettable soil (W-soil). The change in SWR and infiltration was assessed by the water repellency index and cumulative infiltration respectively. *F. proliferatum* decreased the SWR on WR-soil and slightly increased SWR in W-soil, while *Alternaria* sp increased SWR in both the W-soil and the WR-soil. Conversely *T. harzianum* increased the SWR in the W-soil and decreased the SWR in the WR soil. All strains showed a decrease in infiltration in W-soil, while only the *F. proliferatum* and *T. harzianum* strain showed improvement in infiltration in the WR-soil. The ability of fungi to alter the SWR and enmesh soil particles results in changes to the infiltration dynamics in soil.

7.3 Introduction

Soil water repellency (SWR) is a worldwide issue that affects soil quality, resulting in reduced water infiltration, increased runoff, leaching of nutrients and pesticides, reduced plant available water and increased soil erosion (Bauters et al., 1998; Doerr et al., 2000; Rillig, 2005). SWR may be beneficial through contribution to water stable aggregates; this has a positive influence on soil structure and carbon storage (Piccolo and J.S.C., 1999; Spaccini et al., 2002). SWR is primarily caused by hydrophobic compounds coating the mineral surfaces of soil particles (Diehl and Schaumann, 2007). There are many factors further exacerbating SWR including soil moisture, organic matter, fire, soil texture and microorganism interactions (Rillig, 2005). In addition, fungal biomass and exudates are also known to increase SWR. This phenomenon can be seen in the highly localized water repellency of fairy rings caused by basidiomycetous fungi (York and Canaway, 2000).

Hallett and Young (1999) found that stimulating the microbial biomass with nutrients can greatly enhance repellency in soils. As well, Feeney et al. (2006) reported a strong relationship between fungal biomass and SWR. Furthermore, Hallett et al. (2001a) selectively inhibited either fungi or bacteria on a sandy soil with biocides to separate the influence of each group on SWR. Inhibition of fungal growth decreased the development of SWR after 10 days of incubation in a nutrient amended soil. By inhibiting bacterial proliferation, SWR was greatly enhanced, possibly because bacteria can degrade hydrophobic compounds and/or the native fungi experienced less competition (Hallett et al., 2001b). Research to date into sandy soils has identified fungi as the dominant microbial group that causes water repellency, while bacteria may decrease repellency (Roper, 2004). However, certain fungal strains do not express hydrophobic wetting properties; instead they express hydrophilic wetting properties (Unestam and Sun, 1995). Little is known about how these wetting properties from fungi can alter SWR.

Hydrophobins, a recently discovered class of small amino acids which is a ubiquitous protein found in filamentous fungi (Wessels, 1996) has created interest in its connection to SWR

(Rillig, 2005). Linder (2009) found that the increase in hydrophobic wetting properties is related to the amount of hydrophobins produced on fungal surfaces. Rillig et al. (2010) was the first to report a causal relationship between the growth of Arbuscular mycorrhiza fungal mycelia and SWR. This relationship is due to the presence of a hydrophobin-related protein; glomalin. They both speculated the hydrophobins and glomalin-related soil proteins (GRSP) on fungal surfaces might be the cause of the increased SWR (Rillig, 2005). However, conflicting evidence suggests that hydrophobins and GRSP in some fungal strains does not necessarily confer hydrophobic wetting properties (Feeney et al., 2006). As such, the cause of SWR due to fungi remains inconclusive. However, characterization of water repellency from fungal wetting properties as a whole (Chau et al., 2009) and its effect on SWR may give a better indication on the role that fungi play in SWR (Spohn and Rillig, 2012).

The objective of this study was to determine whether SWR and infiltration in soil could be manipulated through inoculation with fungi. Three extensively researched species capable of exhibiting varying wetting properties were investigated, namely, *Alternaria* sp., *Trichoderma harzianum* and *Fusarium proliferatum* (Chau et al., 2009). I hypothesized that the wetting properties of fungi are a main determinant of SWR and that a change in the surface property of the soil will result in a change in SWR and infiltration behavior.

7.4 Materials and Methods

7.4.1 Fungal Cultures

Cultures of *Alternaria* sp. SMCD (Saskatchewan Microbial Community Database) 2122, *Trichoderma harzianum* SMCD 2166 and *Fusarium proliferatum* SMCD 2241 were maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) plates (39 g potato dextrose agar in 1 litre distilled H_2O) at 22.5 ^{o}C (room temperature) in darkness. The wetting properties of the fungal surface mycelium for *Alternaria* sp. *T. harzianum* and *F. proliferatum* were determined by measuring the water droplet contact angle on growing PDA cultures following the procedure proposed by Chau et al. (2009). Fungi from an active growing culture were inoculated on the center of the slide PDA media. The slides were replicated three times. The slides were then incubated in the dark at 22 °C until complete coverage of the slide media was obtained. Contact angles of 5 water droplets of 10 µL were measured across the slide. In addition, *Alternaria* sp., *F. proliferatum*, and *T. harzianum* were also tested for wetting properties using the alcohol percentage test (APT) as described by Chau et al. (2010). A series of aqueous ethanol solutions were prepared in 5% increments starting from 0 % to 100 % ethanol (Chau et al., 2010). Ethanol solutions (4 µl) were applied on the surface of fungal colonies. Three droplets were assessed on three replicates of fungal cultures with a time interval used for infiltration of less than five seconds. *Alternaria* sp. had contact angles of 122°±1 and an alcohol percentage value of 50%, therefore it was classified as hydrophobic, while *F. proliferatum* had contact angle of 0° and thus classified as hydrophilic. *Trichoderma harzianum* had contact angles that ranged from 61-117°, and was classified as chrono-amphiphilic (change in wetting properties with time). *T. harzianum* and *F. proliferatum* could not be measured using the APT due to having contact angles below 90°, which resulted in an APT reading of 0%.

7.4.2 Soil Water Repellency

The soils for this study were taken from a study area located in the Canadian Boreal Forest Regions, within the Central Mixed wood sub region (Natural Regions Committee, 2006). This area is characterized by a continental boreal climate with long and cold winters and short cool summers. The average annual precipitation is 455 mm, with mean daily temperatures of

-18.8°C in January and 16.8°C in July (Environment Canada, 2002). Approximately 20% of the land in the region is comprised of coarse textured glacial fluvial and eolian deposits on which Brunisolic soils (Inceptisols) have developed. These soils support a variety of ecosite types described by Beckingham and Archibald (1996) and are of critical importance for reclamation practices of the Athabasca Oil Sands industry. Ecosites are classified ecological units developed under similar environmental conditions (climate, moisture, and nutrient regime) (Beckingham and Archibald, 1996). The poor water storage property of these coarse textured soil materials with the additional concerns of SWR makes reclamation of these ecosites difficult. However, mitigating the influence of SWR on soil will improve the outcome of these reclamation scenarios (Müller and Deurer, 2011).

Laboratory experiments were performed on coarse textured, Brunisolic, sandy soil materials typical of this region. Two sandy soils were selected from sites previously observed to show the presence of SWR by Hunter et al. (2011). The first site,(ALFH) was a constructed of 0-10 cm of LFH (litter, fermentation and humus) overlaid onto 10-100 cm peat mineral mix soil located on a southeast facing complex slope of a saline sodic overburden. The second site (CPA) was a disturbed open pit mining site, which was comprised of Brunisolic soils typical of the region. Soil texture was determined using a Laser Scattering Particle Size Distribution Analyzer (Horiba LA - 950, Horiba Instruments Inc., Irvine, CA) after air-drying and sieving to 2 mm. Total C and N contents were also determined using a LECO CNS-2000 analyzer (LECO Corp., St. Joseph, MI). To test for SWR in the soil initially, contact angles were determined using the sessile drop method and the Low Bond Axisymmetric Drop Shape Analysis (Bachmann et al., 2000; Stalder et al., 2010). The soil from the first site (57°04`N, 111°30`W) was coarse-textured (93% sand, 5 % silt and 2% clay), nutrient poor (carbon 0.96 g g^{-1}, nitrogen 0.05 g g^{-1}) and had a

WDPT (water drop penetration time) after 2 hrs with an initial contact angle of 128°±2. This soil would be classified as extremely water repellent (WR-soil) based on the description from Bauters et al. (2000). The soil from the second site selected (57°19`N, 111°32`W) was also coarse textured (98 % sand, 2% silt and 0% clay) and nutrient poor (Carbon 0.08 g g^{-1}, Nitrogen 0.015 g g^{-1}), however it was found to be non-water repellent due to instantaneous infiltration of water droplets (WDPT=0) and with an initial 0° contact angle. Therefore, this soil was classified as wettable soil (W-soil) (Bauters et al., 2000).

7.4.3 Preparation of Soil for Inoculation

The soils were air-dried for a week and were subsequently sieved through a 2 mm sieve to remove any large debris and plant material. The soils were then subject to gamma sterilization using γ – irradiation (562.32 Gy/hr for 3 days and 5 hours) in sealed polyethylene cylinders (Trevors, 1996). After irradiation, the soils were frozen to decrease the change in soil prior to experimentation and were maintained under sterile conditions (Trevors, 1996). Serial dilutions were performed on irradiated soils and no colony forming units were present on LB (lysogeny broth) and PDA media incubated at 30°c and 23°C in the dark respectively. Soil was loosely packed into glass petri dishes with dimensions of 5.3 cm diameter x 1.3 cm height for fungal inoculations.

7.4.4 Preparation of Fungal Inocula

Two fungal plugs (1 cm x 1cm) from each of the three strains from actively growing plates were aseptically transferred to separate 100 mL of potato dextrose broth (PDB) (24 g of Difco PDB in 1 litre of distilled water) solutions. Uninoculated 100 mL PDB served as controls. The fungal cultures were incubated for 7 days (23^{o}C, in darkness and 250 revolutions per minute (RPM) on an orbital shaker). After 7 days, fungal suspensions were filtered through sterile cheese cloth to remove the spent media, and the fungal mass was weighed. Weighted fungal mass of 1.6 grams was transferred to sterile 50 mL Falcon tubes (Falcon Plastics, Los Angeles, California). Additionally, fresh PDB with sterile glass beads was added to falcon tubes. The tubes were then vigorously agitated by shaking and using the vortex until a uniform suspension of fungal mycelia was obtained. The suspension was then filtered through a layer of sterile cheese cloth to remove the glass beads. The fungal suspension was then aseptically transferred to a 150 mL conical flask and PDB was added to make the final concentration of fungus/PDB (1.6 g 80 mL^{-1}). Five mL of PDB-fungal suspension (*Alternaria* sp, *T. harzianum* and *F. proliferatum*) was applied to the soil. Un-inoculated PDB solutions served as controls. The procedure discussed above was repeated for inoculation of the WR-soil. In addition, 5 mL of distilled water was mixed into the WR-soil, to increase soil above its critical water content (water content at which soil changes from repellent to wettable) (Dekker and Ritsema, 1994) and facilitate wetting of the soil fungal suspension. Without increasing the water content of WR-soil prior to inoculation, the fungal suspension would not infiltrate the soil surface and colonize the soil. Addition of water does not change the characteristics of SWR in our soils as drying the sample prior to experimentation resulted in reestablishment of SWR. Each inoculation was replicated three times with five sampling times, giving a total of 18 inoculated soils for each two soil conditions (wettable and water repellent). Soils were then incubated in a closed plastic chamber in the dark at 25°C with a relative humidity of 80-85%. The W-soil was tested on days 0, 3, 5, 7 and 21, while the WR-soil was tested on day 0, 6, 12, 18, 21. The WR-soil was tested at different days as fungal colonization was slower in the WR soil as compared to the W-soil. Soils were taken out and subsequently dried at 45°C for 24 hrs to maximize SWR and provide uniform water content between all the samples before the infiltration measurement (Hallett and Young, 1999).

7.4.5 Water Repellency Index

To obtain the small scale hydraulic properties, a miniaturized infiltrometer (Fig. 16) was constructed, modified from the design of Leeds-Harrison et al. (1994). The infiltrometer tip had a contact radius of 2 mm, which is standard for typical miniaturized infiltrometers. The design consisted of glass tubing, a 250 mL plastic bottle and a 200 uL plastic pipette tip cut to 2mm verified with a digital calliper under a conventional stereo microscope. Porous sponge (Leeds-Harrison et al., 1994) and fibers used by Hallett et al. (2004) were not suitable as ethanol caused the cellulose to harden and not allow adequate liquid flow through the tip and also did not restrict the air entry. Instead the tip was modified by affixing nylon mesh to the end of the infiltrometer using cyanoacrylate (superglue) adhesive. The nylon mesh had an air entry value of about -3.0 cm (-3 to -3.2 kpa) water pressure head. This worked best as it did not impede liquid transport and it restricted the air entry (Hallett et al., 2004). The amount of liquid entering the soil was measured by recording the weight loss in reservoir as a function of time. The infiltrometer was set at 2 cm pressure head to minimize the influence of macropores. Cumulative infiltration from both 95% ethanol and water was plotted against the square root of time ($t^{1/2}$) to obtain the early time infiltration curve. Early time sorptivity was determined for both liquids by using the formula, described by Philip (1954) (Eq. 7.1);

$$I = St^{1/2} \qquad \text{Eq. 7.1}$$

where, I is the cumulative infiltration, ($L\ T^{-1}$), for each measured pressures, S is sorptivity, ($L\ T^{-1}$), of infiltrating liquid and t is the time, (T).

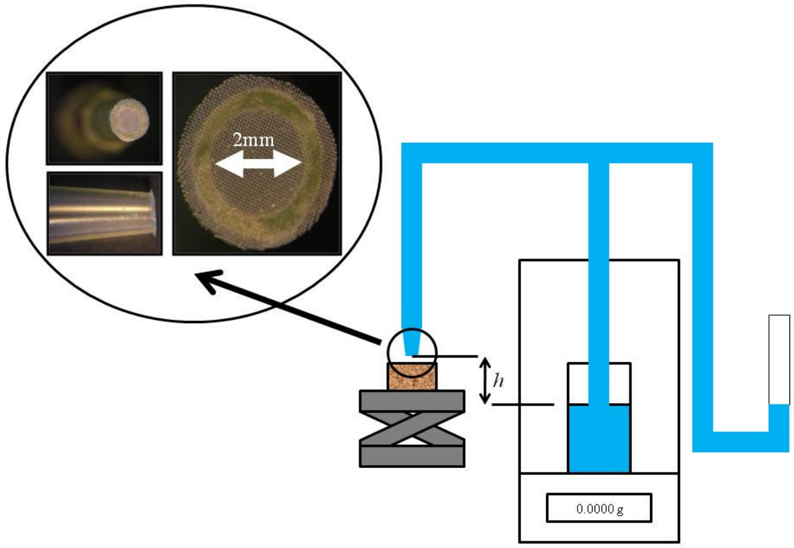

Fig. 16. Miniaturized infiltrometer to measure the infiltration of two liquids (water and 95% ethanol) in a small circular surface area. Caption: modified infiltrometer tip using nylon membrane affixed to 200 ml pipette tip. Inner diameter measures 2 mm.

Sorptivity values determined from water and 95% ethanol infiltration into soil from early time infiltration curve were used to determine the change in SWR. Water repellency index (RI) was determined by (Eq. 7.2):

$$RI = 1.95\left(\frac{S_E}{S_W}\right) \qquad \text{Eq. 7.2}$$

where S_E and S_W are the sorptivity measurements of 95% ethanol and water, respectively. (Tillman et al., 1989). The constant, 1.95 accounts for the differences in the liquid's surface tension and viscosity. An RI value that is greater than one implies the presence of SWR in soil.

7.5 Results and Discussion

Uninoculated W-soil showed no initial water repellence (RI=1) and no change in RI during the 21 day period of incubation (RI=1) (Fig. 17a). The uninoculated WR-soil showed high initial water repellence (RI>1000) and no change in RI as a function of time (Fig. 17b). WR-soil had at least 1000 times (1000x) higher RI than W-soil (Fig. 17). The inoculated WR-soil showed more dense colonization on the top of the soil than W-soil. Moreover, visible mycelia growth was found later on the inoculated WR-soil, compared to the W-soil. The WR-soil was initially wetted, so that inoculation with the fungal suspension could occur. This led to temporary removal of SWR, which allowed for penetration of the fungi suspension into the soil. Re-establishment of SWR was evident after a drying event (Fig. 17b). There was no change to SWR as wetting the soil only altered the water content during the inoculation and incubation of the soil.

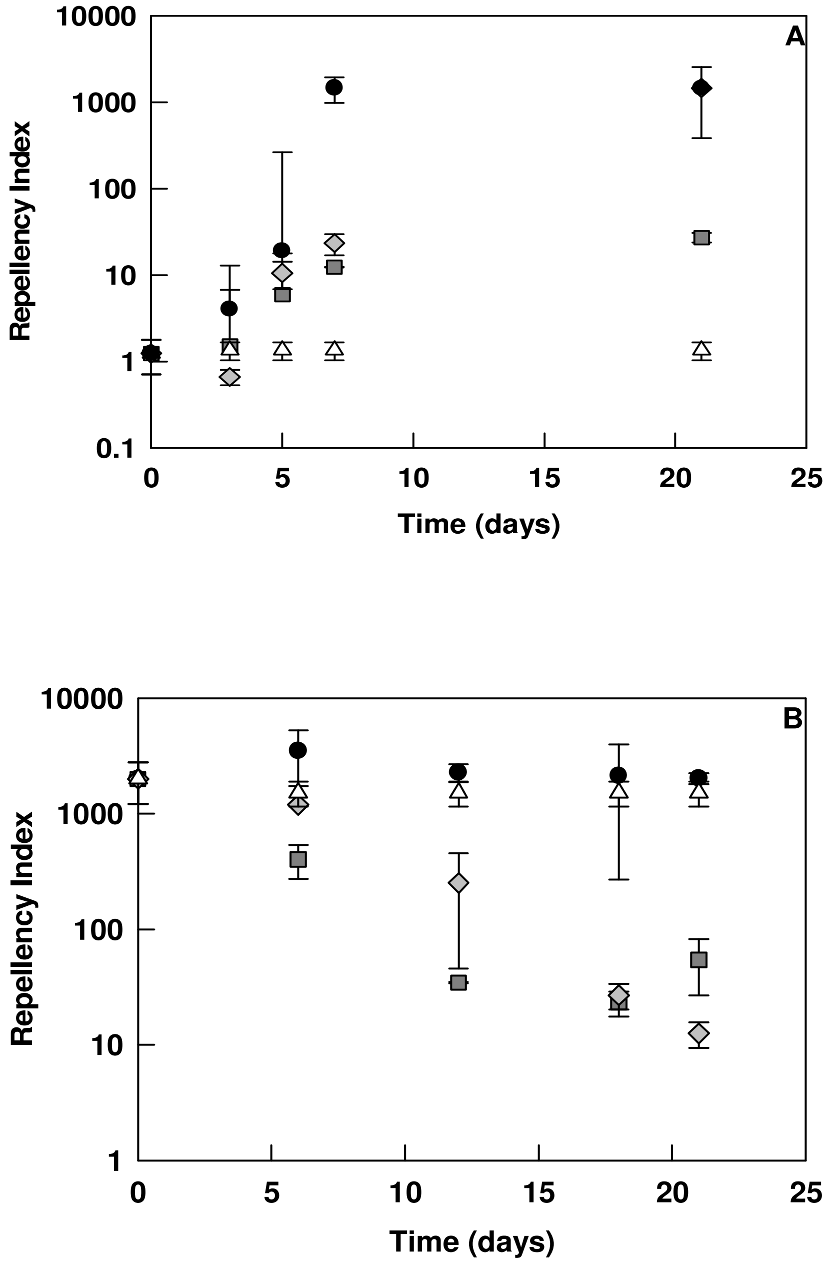

Fig. 17. Plot of the water repellence index as a function of time (days) for incubation of 3 strains of fungi and the control on a) wettable soil, and b) water repellent soil. Errors bars represent the standard error of the mean. (● *Alternaria sp*.-hydrophobic, ■ *F. proliferatum*-hydrophilic, ◆ *T. harzianum*-chrono-amphilic and △ control-uninoculated soil)

Water repellent characteristics of a soil inoculated with fungus are drastically affected by the fungal wetting properties, due to growth of the fungi around the soil particles (particle enmeshment) (Tisdall, 1994; Degens, 1997; Tisdall et al., 1997). *F. proliferatum* was classified to be hydrophilic due to 0° contact angle on the surface mycelium. After day 21 the RI value of *F. proliferatum*-inoculated W-soil was 10 times (10x) that of the uninoculated W-soil (Fig. 17a). In the WR-soil, inoculation of *F. proliferatum* decreased RI by 100 times (100x) after 21 days of incubation compared to the control. Using fungal strains to manipulate SWR might be effective in remediating extremely water repellent soils (Hallett et al., 2006).

Alternaria sp. was classified as hydrophobic with contact angles of 122°±1 and APT value of 50% on the surface of the mycelium. The *Alternaria* sp. in the W-soil increased the RI after 21 days of incubation. This increase in RI was 1000 times (1000x) greater than uninoculated W-soil (Fig. 17a). The inoculated *Alternaria* sp soil showed an increase by 2 times (2x) in RI in WR-soil compared to the uninoculated WR-soil (Fig. 17b). As the wetting properties of fungi are a main determinant of fungal derived-SWR, the repellency in this inoculated soil would never increase past levels observed on the fungal surface. The increase in SWR caused by hydrophobic fungal strains will have implications in water transport in soil, as increases in SWR are associated with preferential flow, runoff, erosion and decreased water storage (Morales, 2010). Hydrophobic fungi may be used as a bio-barrier Kim (2004) or bioclogging agent (Seki et al., 1998) to change the water flow characteristics by changing the repellency of certain type of soils. Furthermore, low levels of repellency may also slow down water movement (through altering soil hydraulic properties) (Hallett et al., 2004) in soil, therefore increasing water residence time for plant uptake. This is particularly useful in sandy soils where drainage and water retention properties are a major limiting factor.

The chrono-amphiphilic strain, *T. harzianum* changes its wetting properties as a function of time with contact angles from 61-117° (Chau et al., 2009). The most interesting results from this fungal strain, is that it's wetting properties also changes depending on the nutrient conditions it is under. *T. harzianum* strain increased the RI by 1000x at 21 days of incubation in W-soil (Fig. 17a). As well, the *T. harzianum* strain decreased the RI 100x at 21 days incubation in WR-soil (Fig. 17b.). Such a dynamic organism can possibly survive under both flooding and desiccation situations. The possibility of using *T. harzianum* for manipulating or remediating SWR should be examined further as this organism is also found to be useful as a bio-control agent (Grondona et al., 1997).

At 21 days of incubation, we observe the largest change in the RI for all the strains under both soil types. Cumulative infiltration at 21 days was higher for the W-soil than for the WR-soil (Fig. 18). Fig. 18a depicts the change in the cumulative infiltration (mm) as function of time (sec) from *F. proliferatum, Alternaria* sp*, T. harzianum* strains and uninoculated control on W-soil after 21 days of incubations. All strains showed a decrease in cumulative infiltration as compared to the uninoculated control (Fig. 18a). The smallest decrease in cumulative infiltration was found for the *F. proliferatum* inoculated sample, followed by the *T. harzianum* and the *Alternaria* sp. inoculated sample. Although *F. proliferatum* was classified as hydrophilic, some impedance to infiltration was observed. However, this was not to the same extent from the two more repellent strains. Due to colonization and enmeshment of soil particles from fungal mycelia, the sizes of pores would decrease resulting in a decrease in hydraulic conductivity and thus infiltration into soil is reduced (Ritz and Young, 2004). As such we can conclude fungal inoculation, irrespective of their wetting properties, will alter the infiltration in soil that is not repellent.

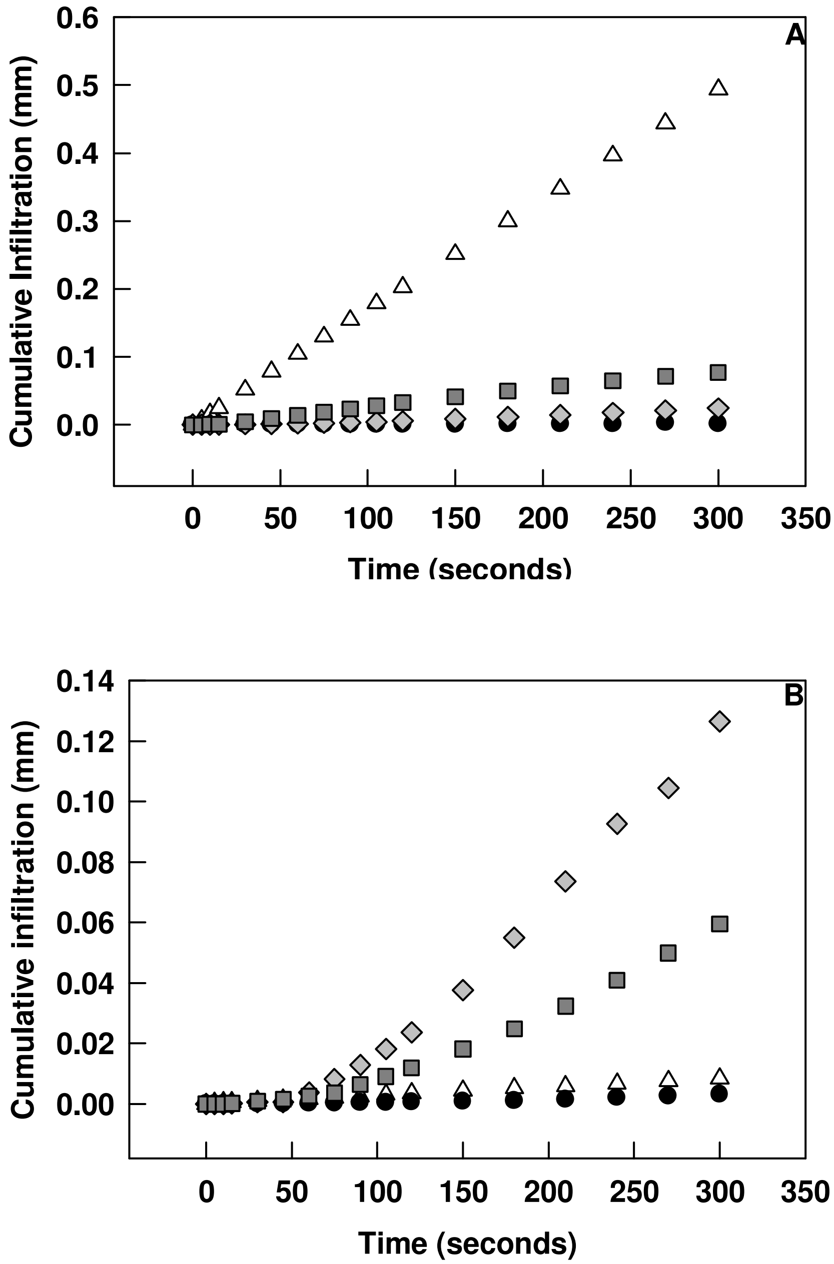

Fig. 18. Plot of cumulative infiltration as a function of time from 21 days of incubation for 3 strains of fungus and the control on a) wettable soil, and b) water repellent soil. (● *Alternaria sp*.-hydrophobic, ■ *F. proliferatum*-hydrophilic, ◆ *T. harzianum*-chrono-amphilic and △ control-uninoculated soil)

The change in the cumulative infiltration (mm) as function of time (sec) from *F. proliferatum, Alternaria* sp*, T. harzianum* strains and uninoculated control on WR-soil after 21 days is shown in Fig. 18b. Cumulative infiltration in the uninoculated control in the WR-soil was drastically less than the control W-Soil. *F. proliferatum and T. harzianum both* showed increase in cumulative infiltration as compared to the uninoculated control (Fig. 18b). However, *Alternaria* sp showed a decrease in the cumulative infiltration (Fig. 18b). The improvement in infiltration and water absorption in the WR-soil suggest enmeshment and colonization of fungi with hydrophilic wetting properties on the soil surface.

Using fungal strains to manipulate SWR (improving infiltration) might be effective in remediating extremely water repellent soils. However this bioremediation would require surfactants for the fungus to penetrate the soil as illustrated by our prewetting to reach the critical water content in our WR-soil. Also, some fungal strains have hydrocarbon degrading capabilities (Hallett et al., 2006). The use of them synergistically to degrade pollutants and then remediate the SWR caused by the pollutant may be a possible novel approach to improving soil hydrology in hydrocarbon contaminated soils. However the ability of fungi to enmesh water repellent soil and thus alter the SWR is not the mechanism that is occurring in these soils. Degradation of hydrophobic compounds in these hydrocarbon contaminated sites results in decrease in SWR related to the decrease in quantity of hydrophobic compounds. The shortcoming of this is a particular strain with the ability to degrade the hydrocarbon contaminants also increases the water repellent property of soil. This is mainly due to a fungal strain exhibiting its own hydrophobic wetting properties (Hallett et al., 2006). For the fungal strain to grow and uptake contaminants, some degree of hydrophobic interactions must occur

(Johnsen et al., 2005). It would be beneficial to select strains that have hydrocarbon degrading qualities as well as hydrophilic wetting properties.

Soil water repellency is commonly considered detrimental to soil quality, however the presence in soils around the world suggest a beneficial role not yet realized. Subcritical (low level) repellency is known to decrease the saturated hydraulic conductivity and increase the residence time of water due to bioclogging and decreasing the effective water conducting porosity (Morales et al., 2010). Further studies should encompass the role that fungi play in soils which have subcritical SWR (White et al., 2000).

7.6 Conclusions

This is the first demonstration of fungal effects on SWR and infiltration as a function of the wetting properties of the fungal strain added. The ability of fungi to enmesh soil particles and fill pore spaces allows for the alteration of SWR and infiltration. Under these conditions, the use of fungal strains to decrease the repellency can be proposed. However, in our setup, we focused only on the effect of fungal strains on γ-sterilized sandy soil. Further testing should be done to assess the interacting effect between strains from native species in a water repellent soil. Additionally further testing of these strains under stress and limited nutrient conditions should be performed, as the wetting properties in fungi might change due to sporulation.

8. SYNTHESIS AND CONCLUSION

Soil water repellency causes reduced infiltration, reduced soil water storage, enhanced runoff, increased preferential flow, and reduced ecosystem productivity. Improving the water dynamics in water repellent soils is difficult due to inconsistence in measurements, understanding in water flow through repellent soil and some of the causes of SWR. Although the presence of SWR has been documented in many soils (Doerr et al., 2000), the issue of water repellent pores has yet to be examined. Pore properties including tortuosity, surface roughness, discontinuity and dead end pores can impede water flow through soil, however less is known about the effect of SWR in soil pores.

Fungi are a major contributor to SWR, however there is a lack of knowledge of which fungal strains has the ability to cause SWR. Most studies focus on the ability of fungi to decrease SWR through decomposition of hydrophobic organic matter in soil (White et al., 2000; Feeney et al., 2006). Few studies examined the surface property as an indicator of fungi ability to cause SWR. Water contact angles are a direct and simple approach for characterization SWR and fungal surface properties (Unestam, 1991). However due to roughness of the surface of soil and fungi, water contact angles are often obscured and hard to measure (Busscher et al., 1984; Krishnan et al., 2005). With advances in contact angle measurements (Stalder et al., 2010), new fitting schemes have been developed to fit contact angle on obscured surfaces such as fungi and soil. Alternative surface tension measurements have been developed to measure water repellency on soil (Roy and McGill, 2002; Lamparter et al., 2010). Utilizing different concentrations of ethanol solutions with different surface tension and measuring the lowest concentration of ethanol solution that resist wetting, provides an indication of water repellency in soils. Extending this method further and applying on fungal surface for characterization of fungal hydrophobicity would be useful.

Previous management strategies to remediate water repellency in soils such as clay addition, surfactant treatment and intensive use of irrigation are unfeasible due to lack of clay material, toxicity of surfactants, and cost and access to water (Franco et al., 2000; Ritsema and Dekker, 2003; Hallett, 2008; Müller and Deurer, 2011). Hydrophobic fungal structures and exudates have been known to be a major contributor in changing the soil water relationship in natural soils (Unestam, 1991; Unestam and Sun, 1995; Sun et al., 1999). Effective characterization of fungal strains that may cause and suppress SWR has not been examined to date. The surface property of fungi is one of the mechanisms in which fungi can change the water repellency in soil.

The research presented in this dissertation addresses knowledge gaps on the characterization of SWR, understanding the causes of SWR and its effect on conducting porosity and infiltration. The objectives of this research was to understand the relationship between severity, persistence and CWC in water repellent soils, effect of SWR on pores and conducting porosity, to develop and modify methods for classification of fungal strains that cause SWR and to determine the effects of fungal hydrophobicity on SWR and infiltration in soil.

8.1 Summary of Findings

Soil water repellency is a key issue faced by farmers and land managers throughout the world. The issue with SWR measurement is there is little literature on the dynamic properties of SWR (Beatty and Smith, 2013). Often the measurement technique will influence the results. In addition, measurement techniques only focus on the severity or persistence as indication of the presence of repellency. Furthermore severity and persistence are done at one antecedent moisture content and this will likely influence the results of the measurement due to change in water content with time. Since SWR is a dynamic property affected by soil water content, our objective was to examine the severity as a function of persistence and as a function of water content (Chapter 3). The results demonstrate that severity is not always related to persistence in both an extremely repellent and subcritical repellent soil. Contact angles were not strongly correlated with WDPT and were not statistically significant ($r = 0.37$, $p > 0.05$) (Chapter 3). This is due to the nature of the hydrophobic material causing repellency in these soils. This also suggests that the role SWR plays in hydrological processes is more complex than what was previously thought (Chapter 3). For example, a soil with a high severity of SWR but low persistence would result in more runoff on slope and less infiltration (Cerdà and Doerr, 2007), but will subsequently become wettable, negating the influence of the water repellency. Differences between severity and persistence would be important when determining runoff scenarios considering the magnitude and frequency of the rainfall events (Beatty and Smith, 2013). The difference between contact angle and WDPT is due to wetting mechanism occurring at the surface of soil particles. Contact angles formed on the soil surface are caused by the difference in surface energy between water, soil surface and air. Although a soil surface may have a large surface energy compared to water, the rate at which surface energy changes is dependent on the composition of hydrophobic coating on the soil surface (Chen and Schnitzer, 1978). As such comparison between the severity and persistence measured by contact angles and WDPT between soils are much more complex considering they measure different properties for determination of SWR. In natural, reclaimed and agricultural soils, a high severity (Contact angle) of repellency does not necessarily denote long persistence (WDPT) or high CWC. Measurement of severity and persistence are related to the differences and changes in surface energy between water and the soil surface respectively. Although the CWC gives the water content at which above it SWR is negligible, the trend between contact angle and increasing water content proved to be more informative. The trends show how repellency changes as a function of water content (Fig. 7). A more rapid decrease in repellency (measured by contact angle) with increases in water content would indicate less severe repellency even though the initial severity may be high (Fig. 7a, 7b). A slow decrease or persistent repellency at low water contents would indicate more severe repellency in a site (Fig. 7c, 7d). As water content increases such as after a rainfall event, the soil will approach its critical water content. The expression of repellency is important to determine as the soil increases in water content. If the soil does not reach the critical water content, soil infiltrability in the soil matrix will be reduced, with an increased chance of preferential flow and runoff causing decrease in soil water storage in soil profile.

Additionally, it was found that the severity of repellency is not necessary related to critical water content as some of soils with high severity of repellency had low CWC (Table 2). Furthermore soils with low severity of repellency were also found to have high critical water content. This is most likely due to the composition of hydrophobic compound causing the repellency. Hydrophobic compounds in soils with high CWC indicate that the difference between the surface tension from water and the soil surface at low water contents is persistent. However when water is present and the soil reaches the CWC, the particles reorientate rapidly making the effect negligible and making SWR manageable (Doerr et al., 2000; Lehrsch and Sojka, 2011). While hydrophobic compounds in soils with lower initial severity and high critical water contents have more complex organic compounds which take substantially longer time to reorientate when subject to wetting. The complex nature of compounds that cause repellency further complicates measurement as differences in amount and type will determine the severity, persistence and CWC of repellency in the soil (Horne and McIntosh, 2000).

As the soil water content increases, the severity and persistence of SWR decreases. This phenomenon will influence the water flow in certain pores. Understanding how repellency affects water flow in certain pores is important (Chapter 4). This study revealed that ethanol infiltration through tension infiltrometer can be used to determine the intrinsic conducting porosity (unaffected by surface properties) in water repellent soils. The obtained intrinsic conducting porosity is much higher than that from water conducting porosity if the soil is water repellent. We also determined how RI varies under different pressure heads. RI values obtained under different pressure heads are different due to all pore size ranges tested showing some degree of repellency. This suggests that pores that will be filled with water in wettable soils under certain pressure, are not being filled under the same pressures at equilibrium due to water repellency in water repellent soils (Beatty and Smith, 2013). This would also suggest that water retention in soil would be affected by water repellency since some pores are not being filled under certain pressure conditions. The effect of RI on the water conducting porosity is related to how many and how much of each equivalent pore is filled under a certain pressure head to the total liquid flux. Large diameter pores contribute more to the liquid flux than smaller sized pores. As a result a high RI in the macropores in contrast to high RI at the mesopore ranges will result in a decrease in the water conducting porosity. The determination of the RI under different pressure heads gives a better indication of how SWR will affect water flow through different pore diameters. The RI under -3 cm of pressure was more representative of the total repellency in the soil as more contributing diameter pores to the water flux were included in the measurement. A high RI affecting larger diameter pores has more influence on the conducting porosity due to the contribution to the total liquid flux. To accurately determine the actual conducting porosity in soil, SWR must be taken into account. Additionally, determining RI using a tension which includes the pore diameters that contribute more to the total liquid flux would give a better indication of the role repellency plays in soil water transport.

One issue with measuring contact angles for determining water repellency in soils and hydrophobicity of fungal strain is surface roughness. This surface roughness is due to rough particle surfaces soil and the mycelia of fungi. How can we measure which fungal strains have the ability to change the SWR in soil? Firstly our objective was to develop a method to accurately characterized repellency on fungi surfaces (Chapter 5). Fungal cultures were grown on agar slide media and contact angles were measured utilizing a modified microscope and digital camera setup, with advanced imaging software for contact angle determination. Contact angles were observed in hydrophobic, hydrophilic and a newly created Chrono-amphiphilic class containing fungi taxa with changing hydrophobicity. Our study proposes a more efficient method for monitoring fungal hydrophobicity, taking into account variations in environmental factors influencing fungal surface properties. Therefore, this novel method is particularly important for understanding fungal ecological functions, which are closely related to surface properties and affect the SWR.

A method was developed using different concentration of ethanol solutions with different surface tension to measure the spread on soil surface or infiltration as indication of the severity (Roy and McGill, 2002). The objective of this study was to examine if the contact angles measured on fungal surface is related to the percentages of alcohol droplets (Chapter 6). This method would be suitable for measurement of fungal culture with large aerial mycelia which would obscure the view CA on fungi. APT and contact angle measurements were conducted on nine hydrophobic and two hydrophilic fungal strains from the phyla of Ascomycota, Basidiomycota and Zygomycota. There was a strong positive correlation (R^2=0.95) between the APT test and contact angle measurements from eight out of the nine hydrophobic stains (four pathogenic and mycotoxigenic *Fusarium* taxa, one melanosporaceous biotrophic taxon, *Alternaria* sp, *Penicillium aurantiogriseum, and Cladosporium cladosporioides).* Hydrophilic control strains, *Mortierella hyalina* and *Laccaria laccata* had contact angles < 90^o and no measurable severity of hydrophobicity using the APT method. This study has shown that the APT method is useful for analyzing the degree of hydrophobicity of hydrophobic fungal strains through a comparison of CA measurements on the fungal surfaces. APT measurements were also useful for characterization of different zones of hydrophobicity on the same culture. This will aid in providing a better understanding of the expression patterns of hydrophobicity in zones of colour changes and aged growth due to different morphological, chemical, or metabolic reasons. Given the advantages of the APT method such as the reproducibility and simplicity, it should be considered as one of the methods for quantifying the degree of hydrophobicity on fungal surfaces.

In terms of management scenarios, application of surfactants to reclaim repellent soils are costly and may have a negative impact to the environment (Franco et al., 2000; McKenna et al., 2002; Ritsema and Dekker, 2003; Hallett, 2008; Müller and Deurer, 2011). In Chapter 7 we examined the use of fungal strains or the stimulation of fungal strains to change the SWR in soil. The objective of this study was to determine whether SWR and infiltration could be manipulated through inoculation with fungi. The effect of fungi on SWR was investigated through inoculation of three fungal strains (hydrophilic-*Fusarium proliferatum*, chrono-amphiphilic-*Trichoderma harzianum* and hydrophobic-*Alternaria* sp.) on a water repellent soil (WR-soil) and a wettable soil (W-soil). The change in SWR and infiltration was assessed by the RI and cumulative infiltration respectively. This is the first demonstration of fungal effects on SWR and infiltration as a function of the wetting properties of the fungal strain added. The ability of fungi to enmesh soil particles and fill pore spaces allows for the alteration of SWR and infiltration. The measured surface properties of fungi determined the level of SWR in the soil and affected the infiltration rate. Under these conditions, the use of fungal strains to decrease or to change the repellency in soil can be proposed.

8.2 Future Research

Soil water availability for plants is one of the key issues facing reclaiming disturbed landscapes in the Athabasca Oil Sands Region of Canada due to the dominance of coarse textured soils, organic soils and hydrocarbons. A major limiting factor to soil water availability is the presence of soil water repellency. The work presented in this dissertation will aid in improving reclamation practices to maximize soil water availability to plants by mitigating and understanding SWR in these soils. We proposed effective characterization of the severity and persistence of water repellency and CWC must be done to accurately determine the influence on soil water availability. To better understand water flow in repellent soils, determination of what pores sizes are influenced by SWR and how SWR influences the conducting porosity is also important. Since soil water repellency is dependent on many inter-related and dynamic factors including soil organic matter content, hydrocarbon concentration, fungi and plant exudates, fire, and water content (Doerr et al., 2000), examination of the causes of SWR is important to understand how they relate to degree and persistence of SWR and provides us with more information on factors to consider when determining the implications of SWR. As well, research into the causes of SWR will also aid in management and remediation practices of severely repellent sites.

The hydrological implications of SWR are closely related to the soil hydraulic properties; hydraulic conductivity and soil water retention characteristics. In order to assess the hydrological implications of SWR, it is important to gain more insights about the relationship between SWR and hydraulic properties. Yet, investigations of a direct, causal link between SWR and hydraulic properties are mainly restricted to the laboratory scale or single methods to evaluate SWR (Bauters et al., 2000). However, in recent studies of SWR, it has become increasingly apparent, that the use of only one method to assess the SWR is not sufficient (Chapter 3) (Bachmann et al., 2003). A few studies exist which examine the effect of SWR on the soil hydraulic properties. Bauters et al. (1998) conducted infiltration experiments on artificially hydrophobized materials and reported that the water entry pressures increased according to the degree of SWR reaching a positive value for the extremely water repellent materials. In contrast drainage curves showed that only the non-repellent sand was different compared with the artificial hydrophobic mixtures. Czachor (2006) determined drainage and infiltration water saturation curves of various soil types with different degrees of SWR. It was shown that in the case of drainage the effect of SWR is less pronounced than in case of infiltration. Lamparter et al. (2010) examined the effect of SWR on the water saturation curves for initially dry materials using artificially hydrophobized sand. For the same pressure head value, less water was taken up by the dry materials for a higher degree of SWR. Although there are a few studies which examine the effect of SWR on soil hydraulic properties, they are limited to classical hydraulic properties measurements.

Measurement of soil hydraulic properties using water can be difficult and may lead to erroneous results because of different water contents present at an equal capillary pressure in water-repellent soils compared with completely wettable soils (Bauters et al., 2000; Beatty and Smith, 2013). Additionally, the water phase in water repellent soils might be discontinuous, leading to decreased water infiltration rates (Chapter 4). SWR is not a static soil property. It changes with water content and the time the soil has been in contact with water (Chapter 3) (DeJonge et al., 1999). The changing wettability with time complicates the measurement of soil hydraulic properties (Clothier et al., 2000).

9. LITERATURE CITED

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10. APPENDIX A

Table A.1. P values from t-tests comparing the severity of soil water repellency between sites.

	SV 27	CPA	SV10	SV 30	SV26	ALFH	SS	AE1	AE2	SCB	ATS	Melfort	Goodale
SV 27	1.0000	0.1004	0.0009	0.0001	0.0001	0.0001	0.0001	0.0001	0.0034	0.0001	0.0001	0.0138	0.0252
CPA		1.0000	0.0005	0.0001	0.0001	0.0001	0.0001	0.0001	0.0047	0.0001	0.0001	0.0278	0.1114
SV 10			1.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.1950	0.0008	0.0055	0.0022	0.0010
SW 30				1.0000	0.0720	0.0284	0.0001	0.0001	0.0001	0.0140	0.0133	0.0001	0.0001
SV 26					1.0000	0.5212	0.0022	0.0001	0.0001	0.0007	0.0011	0.0001	0.0001
ALFH						1.0000	0.0316	0.0135	0.0001	0.0038	0.0030	0.0001	0.0001
SS Trial							1.0000	1.0000	0.0001	0.0001	0.0001	0.0001	0.0001
AE1								1.0000	0.0001	0.0001	0.0001	0.0001	0.0001
AE2									1.0000	0.0001	0.0008	0.0353	0.0133
SCB										1.0000	0.4295	0.0001	0.0001
ATS											1.0000	0.0001	0.0001
Melfort												1.0000	0.1950
Goodale													1.0000

Table A.2. P values from t-tests comparing the persistence of soil water repellency between sites.

	SV 27	CPA	SV 10	SW 30	SV 26	ALFH	SS Trial	AE1
SV 27	1	0.0358	0.4806	0.0001	0.0086	0.0015	0.9889	0.0111
CPA		1	0.0073	0.0001	0.0004	0.0001	0.0029	0.0001
SV 10			1	0.0001	0.0015	0.0001	0.1114	0.0001
SW 30				1	0.0001	0.0001	0.0001	0.0001
SV 26					1	0.6311	0.0031	0.0985
ALFH						1	0.0001	0.0066
SS Trial							1	0.0001
AE1								1

11. APPENDIX B

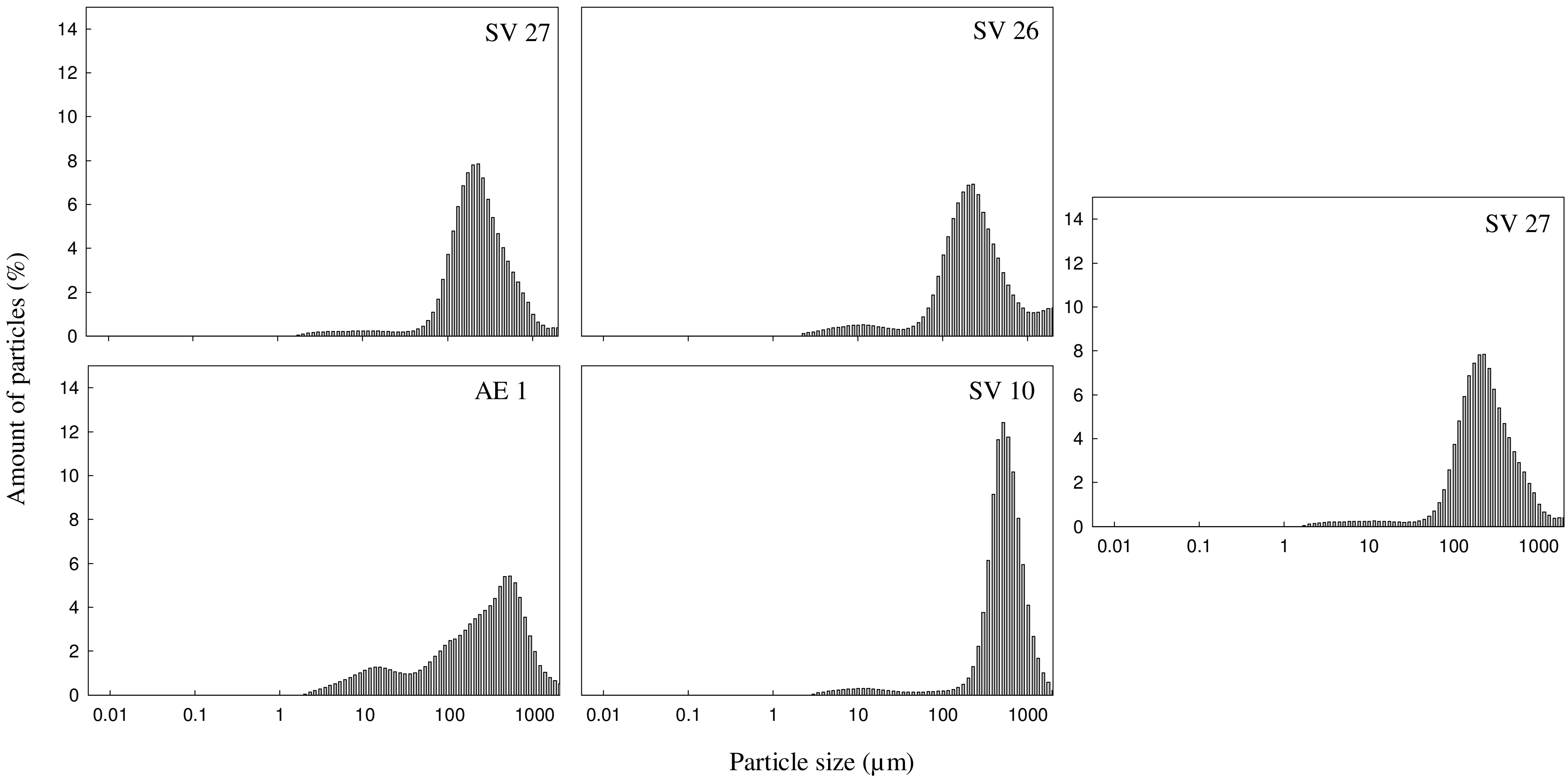

Fig. B.1. Soil particle size distribution for five natural jack pine ecosite.

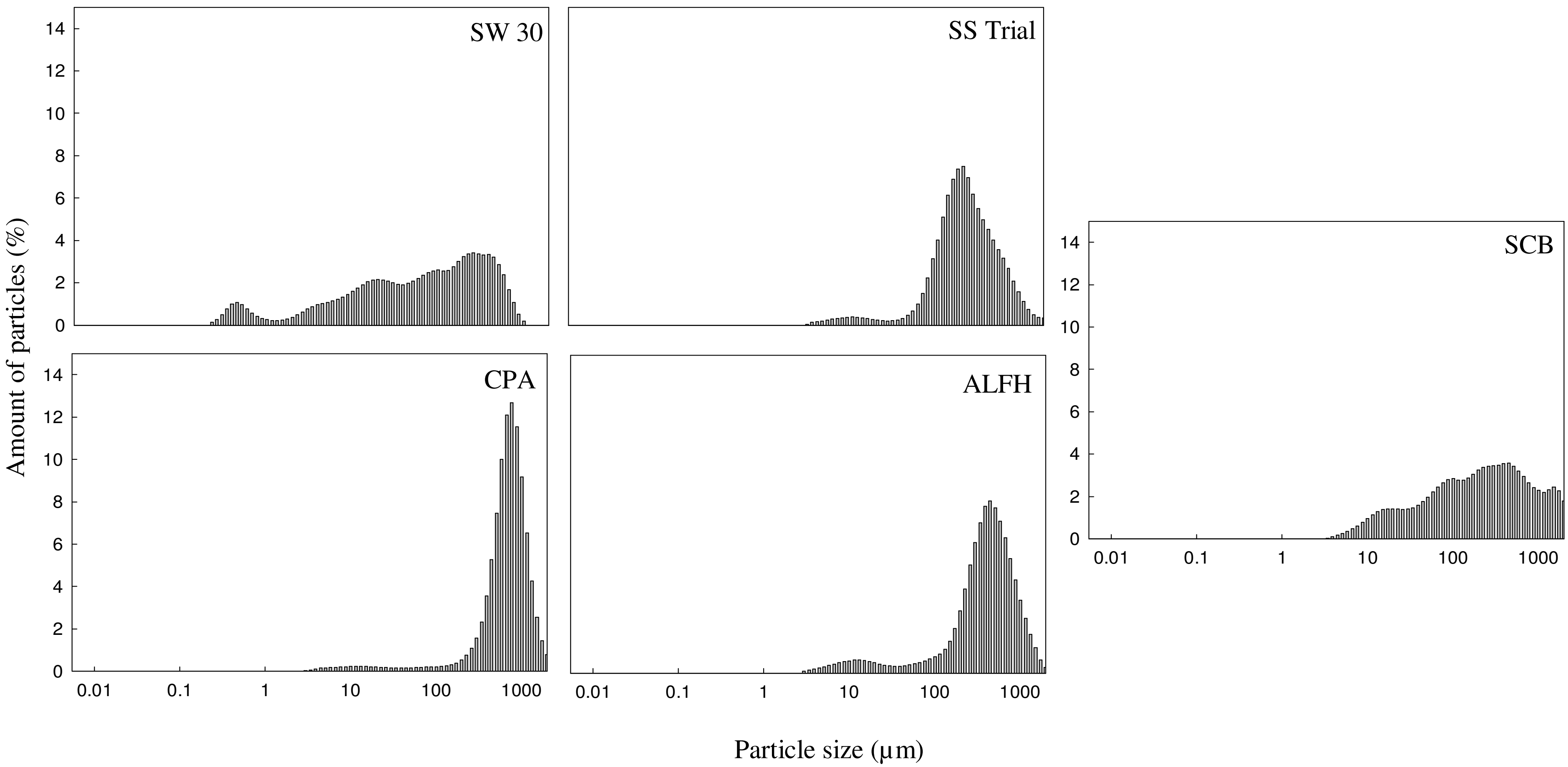

Fig. B.2. Soil particle size distribution for five disturbed/reclaimed sites.