# Preliminary Evidence that the Microbial Community may Produce Aliphatic Constituents and Induce Soil Water Repellency as a Response to Desiccation

By
Emma Chilcote

A THESIS

Submitted to

Oregon State University

Honors College

In partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in BioResource Research
(Honors Scholar)

Presented June 27, 2018
Commencement June 2019

AN ABSTRACT OF THE THESIS OF

Emma Chilcote for the degree of Honors Baccalaureate of Science in BioResource Research presented on June 27, 2018. Title: Preliminary Evidence that the Microbial Community may Produce Aliphatic Constituents and Induce Soil Water Repellency as a Response to Desiccation.

Abstract Approval: _____
Markus Kleber

Soil water repellency is one cause of rill soil erosion and overland flow that results in the loss of fertile topsoil. Previous research suggests that since microbial growth depends on temperature/moisture and follows diurnal and seasonal cycles, a biological mechanism would be able to explain the observed seasonality and climate dependence of soil wettability. Here we test whether soil water repellency is induced by the microbial community through the production of aliphatic constituents in their extracellular polymeric substances (EPS) as a response to desiccation stress. In a laboratory setting Quincy soil was subjected to wetting/drying cycles in order to induce desiccation stress. Analyses conducted were water drop penetration time (WDPT), contact angle, chloroform fumigation extraction for microbial biomass, and a hexane extraction to quantify aliphatic constituents using gas chromatography mass spectroscopy (GCMS). Results suggest there may be a threshold moisture content around 1.8% moisture content for repellency. There is also evidence to suggest that the critical surface tension of water penetration of a repellent sand is around a contact angle of 50°- 60°. There was a trend of an increasing aliphatic content as repellency increased. Differences were observed to suggest that the presence of aliphatic constituents induces soil water repellency, however there was no conclusive evidence that this is the mechanism microbes use to induce water repellency.

Key words: soil water repellency, extracellular polymeric substances, aliphatic constituents, desiccation stress

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Honors Baccalaureate of Science in BioResource Research project of Emma Chilcote presented on June 27, 2018.

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Emma Chilcote, Author

## 1. Introduction

### *1.1. General Problem*

Soil degradation is one of the top global threats to the suitability of the biosphere as a human habitat (World Wildlife Fund). Types of soil degradation include erosion, acidification, a reduction in soil fertility, and declining organic matter (Chen et al., 2002). Soil water repellency is one cause of water erosion that results in the loss of fertile topsoil, rill erosion, and overland flow (Woolverton, 2013).

### *1.2. Specific Research Question*

Can soil water repellency be induced by the soil microbial community through the production of aliphatic constituents in their extracellular polymeric substances (EPS) as a response to desiccation stress?

It has been observed that, depending on soil moisture content, the wettability of a particular soil surface can change with the seasons, and even within days (Bisdom et al., 1993). The appearance and intensity of water repellency has also been shown to be a function of organic matter content as well as a function of the chemical composition of soil organic matter. Bisdom et al., in 1993 found that water repellency in sandy soils increased as the quantity of organic matter increased. The intensity of water repellency was reduced by the decomposition of organic matter, a process mediated by soil biota, and likely affecting both organic matter quantity and organic matter chemistry. Micro-aggregates isolated from sandy Quincy soils were also found to exhibit high water drop penetration times (WDPT); a metric used to quantify water repellency (Bisdom et al., 1993). Considering the apparent dependence of the intensity of water repellency

on variables such as soil moisture, microbiological activity, organic matter quantity and organic matter chemistry, a mechanism with the potential to explain the interactions between these variables and soil water repellency warrants further investigation.

Most soil microbes live in micro-colonies held together by excreted extracellular polymeric substances (EPS) that provide various supportive functions for the micro-colony. EPS are substances exuded by the microbial community. The majority of EPS consist of polysaccharides, and proteins (Fish et al., 2015; Hu et al., 2012; Kakumanu et al., 2013). Secondary components consist of lipids, and DNA (Fish et al., 2015; Hu et al., 2012; Kakumanu et al., 2013). The functions of EPS include (i) the attachment of cells to solid surfaces, (ii) facilitation of cell-to-cell adhesion, (iii) protection of cells from desiccation, lysis, and toxic metals, and (iv) assistance with the gathering of nutrients from the surrounding soil solution (Kleber et al., 2015). EPS is also known to form contiguous films on mineral surfaces, such as soil (Kleber et al, 2015). The lipid/aliphatic constituents of EPS have not been as thoroughly investigated as the polysaccharide and protein content, and there is evidence that suggest the role of lipids would warrant increased attention.

(1) Some microorganisms respond to desiccation stress with an increased production of aliphatics/lipids. Eusterhues et al. in 2016 conducted adsorption experiments using goethite and EPS that demonstrated a preferential adsorption of lipids. The entire biofilm grown in the presence of goethite had an EPS composition with a greater proportion of lipids than of proteins (Eusterhues et al., 2016).

(2) Lipids are non-wetting (hydrophobic) materials. Hence, this may be a mechanism to reduce loss of moisture by the formation of a hydrophobic skin surrounding the microbial micro-colony. In early work on the topic, Bisdom et al. in 1993 found that water-repellent organic

coatings on sand grains consisted predominately of amorphous substances. Later, Mainwaring et al. in 2013 found that a combination of long chain acid and alkane compounds were the most effective at inducing water repellency compared to amides, esters, and cholesterol. These long chain alkane compounds may be what Bisdom et al. in 1993 labeled as amorphous substances.

(3) When lipids come in contact with surrounding soil particle or aggregate surfaces, they will render these surfaces water repellent. Mainwaring et al. in 2013 speculated that there is a van der Waals interaction between hydrophobic alkyl chains adsorbed to the mineral surface and experimentally-added alkanes that resulted in a hydrocarbon coating that is able to prevent water molecules from adsorbing to the silicate surfaces.

(4) Since microbial growth depends on temperature/moisture and follows diurnal and seasonal cycles, such a mechanism would be able to explain the observed seasonality and climate dependence of soil wettability.

### *1.3. The Scientific Unknown*

It is inferred that soil water repellency is induced by the microbial community through the production of aliphatic constituents in their extracellular polymeric substances (EPS) as a response to desiccation stress. When a soil is allowed to dry out, one of several possible physiologic responses of the microbiota is an increase in the production of hydrophobic aliphatic constituents as part of their EPS production. This response may significantly add to and/or enhance other, known processes capable of creating *temporarily* repellent surfaces (such as moisture dependent rearrangement of amphiphilic compounds).

To either corroborate or refute this assumption the following details need to be resolved: Soil water repellency is observed during dry conditions, and is influenced by organic matter content. In turn, organic matter content is influenced by the soil microbial community, which is

known to produce extracellular polymeric substances that protect the cells from desiccation. It is not known whether the microbial community actively influences water repellency, the mechanism the community utilizes, and under what conditions this occurs.

### *1.4. Conceptual Approach*

In a laboratory setting Quincy soil was subjected to wetting/drying cycles in order to induce desiccation stress, which is thought to be the trigger for water repellency. Sandy soils like the Quincy often exhibit severe water repellency (Ma'shum and Farmer, 1985). Water drop penetration time (WDPT) and contact angle were measured in order to determine the degree of water repellency. The total microbial biomass was of interest, because it was hypothesized that the microbial community induces soil water repellency. The quantity of aliphatic constituents were measured, because the hypothesized mechanism by which the microbial community induces soil water repellency, and it is commonly thought to be one of the causes of water repellency in sandy soils (Ma'shum and Farmer, 1985).

### *1.5. Objectives*

(1) Determine the moisture contents for the control, low, moderate, and high desiccation treatments. (2) Determine the quantity of carbon and nitrogen to add to the hydrating solution. (3) Determine the degree of water repellency for each soil sample. (4) Determine the quantity of microbial biomass for each soil sample. (5) Determine the quantity of aliphatic constituents in each soil sample.

*1.6. Hypotheses*

**(1) The degree of water repellency increases as the moisture content decreases.**

If accepted this means water repellency is a function of the moisture content. If refuted this means water repellency is not a function of the moisture content. This was tested by measuring the water repellency metrics of water drop penetration time, and contact angle at varying levels of moisture content.

$$Repellency_{water\ drop\ penetration\ time\ (seconds)} = f(Moisture\ content)$$

$$Repellency_{contact\ angle\ (degrees)} = f(Moisture\ content)$$

**(2) The degree of water repellency is greater for the subsoil (0.2-1cm) than the crust (0-0.2cm).**

It has been observed in-situ that the soil crust readily accepts water, while the subsoil layer exhibits extreme water repellency. If the hypothesis is accepted this means the water repellency of the subsoil is greater than the water repellency displayed on the crust. If refuted this means the water repellency of the subsoil is less than the water repellency displayed on the crust. This was tested by measuring the water repellency metrics of water drop penetration time, and contact angle for the curst and the subsoil at varying levels of moisture content.

$$Repellency_{water\ drop\ penetration\ time\ (subsoil,\ 0.2-1cm)} > Repellency_{\ water\ drop\ penetration\ time\ (crust,\ 0-0.2cm)}$$

$$Repellency_{contact\ angle\ (subsoil,\ 0.2-1cm)} > Repellency_{\ contact\ angle\ (crust,\ 0-0.2cm)}$$

**(3) The quantity of microbial biomass decreases as the moisture content decreases.**

If accepted this means the microbial biomass is a function of the moisture content. If refuted this means the microbial biomass is not a function of the moisture content. This was tested by measuring the quantity of microbial biomass at varying levels of moisture content.

$$Microbial\ biomass_{\mu g\ carbon/g\ dry\ soil} = f(Moisture\ content)$$

**(4) The quantity of aliphatic constituents of soil increases as the moisture content decreases.**

If accepted this means the quantity of aliphatic constituents of soil is a function of the moisture content. If refuted this means the quantity of aliphatic constituents of soil is not a function of the moisture content. This was tested by determining the relative percentage of aliphatic constituents at varying levels of moisture content.

$$Quantity\ of\ aliphatic\ constituents_{relative\ \%} = f(Moisture\ content)$$

**(5) The quantity of aliphatic constituents is greater for the subsoil (0.2-1cm) than the crust (0-0.2cm).**

If accepted this means the quantity of aliphatic constituents in the subsoil is greater than the aliphatic constituents in the crust. If refuted this hypothesis the aliphatic constituents in the subsoil is less than the aliphatic constituents in the crust. This was tested by determining the relative percentage of aliphatic constituents in the subsoil and the crust.

$$Quantity\ of\ aliphatic\ constituents_{relative\ \%\ (subsoil, 0.2-1cm)} > Quantity\ of\ aliphatic\ constituents_{relative\ \%\ (crust,\ \ 0-0.2cm)}$$

**(6) The degree of water repellency increases the longer the soil is subjected to wetting/drying cycles that induce desiccation stress.**

If accepted this means the degree of water repellency is a function of the duration of the wetting/drying cycles. If refuted this means the degree of water repellency is a function of the duration of the wetting/drying cycles. This was tested by measuring the degree of water repellency after one week, and after two weeks of stressful wetting/drying cycles.

$$Repellency_{water\ drop\ penetration\ time} = f\ (duration\ of\ wetting/drying\ cycles)$$

$$Repellency_{contact\ angle} = f\ (duration\ of\ wetting/drying\ cycles)$$

**(7) The microbial biomass decreases the longer the soil is subjected to wetting/drying cycles that induce desiccation stress.**

If accepted this means the microbial biomass is a function of the duration of the wetting/drying cycles. If refuted this means the microbial biomass is not a function of the duration of stressful wetting/drying cycles. This was tested by quantifying the microbial biomass after one week, and two weeks of wetting/drying cycles.

$$Microbial\ biomass_{\mu g\ carbon/g\ soil} = f\ (duration\ of\ wetting/drying\ cycles)$$

**(8) The quantity of aliphatic constituents increases the longer the soil is subjected to wetting/drying cycles that induce desiccation stress.**

If accepted this means the quantity of aliphatic constituents is a function of the duration of the wetting/drying cycles. If refuted this means the quantity of aliphatic constituents is not a function of the duration of the wetting/drying cycles. This was tested by measuring the quantity of aliphatic constituents after one week, and after two weeks of wetting/drying cycles.

$$Quantity\ of\ aliphatic\ constituents_{relative\ \%} = f\ (duration\ of\ wetting/drying\ cycles)$$

## 2. Methods

### *2.1. Soil*

Soil from Boardman, OR, in the Quincy soil series, a Mixed, mesic Xeric Torripsamment, was used for this experiment. The high content of fine-sand has made this soil a favorite for those producing high-value, below-ground crops such as onions, and potatoes (Lovell, 1980). The Quincy soil series has also demonstrated in-situ water repellency (Ma'shum and Farmer, 1985). The soil was collected from Boardman, frozen for two years and then kept in an air convection cabinet at 80ºC in Ziploc bags for 51 days; until the start of the experiment.

### *2.2. Preliminary Calculations*

The typical application rate of a fertigation system for potatoes in Boardman, OR is 39.17 kg Nitrogen/ha/week (Light, 2016). The fertigation rate for a 24-hour irrigation cycle was calculated to be 5.59 kg Nitrogen/ha, and used to determine the concentration of and nitrogen of the hydration solution. The carbon was calculated considering a 6:1 ratio at the above specific nitrogen rate to encourage the growth of the microbial community. Glucose and urea were used

as the carbon and nitrogen sources respectively. The final molarity of the solution was 0.033M glucose, and 0.014M urea.

The soil for all treatments was wetted to an initial gravimetric moisture content of 6%. This allowed for the 8-hour treatment to stay above permanent wilting point of 3%, the 24-hour treatment to stay above 2%, and the 48-hour treatment to stay above 1.8%. It was assumed that the moisture content at permanent wilting point causes desiccation stress to the microbial community (Sinegani and Maghsoudi, 2011).

### *2.3. Treatments*

Four repetitions of each soil sample were subjected to one of four different wetting/drying cycles, where the hours after hydration were used to target specific moisture contents. The 8-hour wetting/drying cycle reached a moisture content of 3% gravimetric moisture on average by the end of each 8-hour period and was then rewet to the original moisture content of 6%. The 24-hour cycle treatment reached a moisture content of 2%, and was rewet to 6% every 24 hours. The 48-hour cycle treatment reached a moisture content of 1.8%, and was rewet to 6% every 48 hours. The dry control treatment had a moisture content of 0.65%, and received no water for 39 days. The soil used for the dry control group was not subject to any wetting/drying cycles. The soil used for the dry control treatment remained in the 28ºC air convection cabinet for 39 days longer than the other treatments.

### *2.4. Experimental Design*

A 154ml cup was filled with soil, and leveled off. The soil was thoroughly mixed with 14.15ml of the carbon-nitrogen solution, and repacked into the sample cup, so that all treatments

started at 6% gravimetric moisture content. The initial total weight of the soil and cup was recorded for each sample. The treatments were rewet to the initial total weight at the 8, 24, or 48 hours (see table 1 and figures 1-3). Bringing the sample back up to the initial weight ensured that the samples reached the same initial moisture content. A 5ml disposable pipette was used to administer the solution. The samples were also subject to a diurnal temperature flux in order to replicate the day and nighttime temperature conditions in the Boardman area. Each day, all the samples spent 16hrs in an air convection chamber at 28ºC, and 8hrs in an incubator at 8ºC.

**Table. 1. Summarization of the treatments, hydration cycles, and moisture contents.**

| **Gravimetric Moisture Content** | **Week** | **Rewetting Intervals** |
|---|---|---|
| Percentage | | Hours |
| 3 | 1 | 8 |
| 3 | 2 | 8 |
| 2 | 1 | 24 |
| 2 | 2 | 24 |
| 1.8 | 1 | 48 |
| 1.8 | 2 | 48 |
| 0.65 | n/a | n/a |

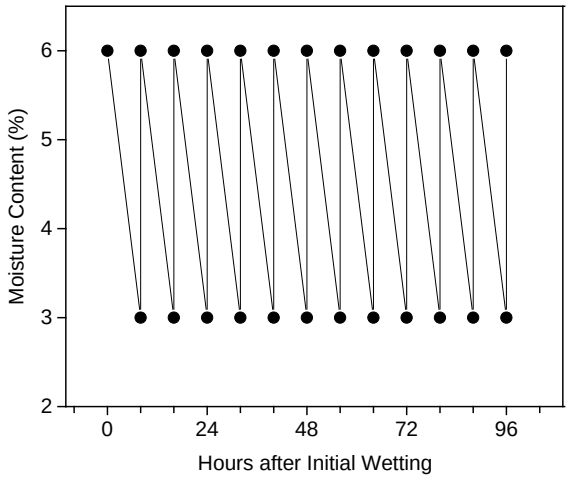


**Fig. 1.** A sample of the wetting interval and resulting moisture content for the 8-hour wetting/drying cycle.

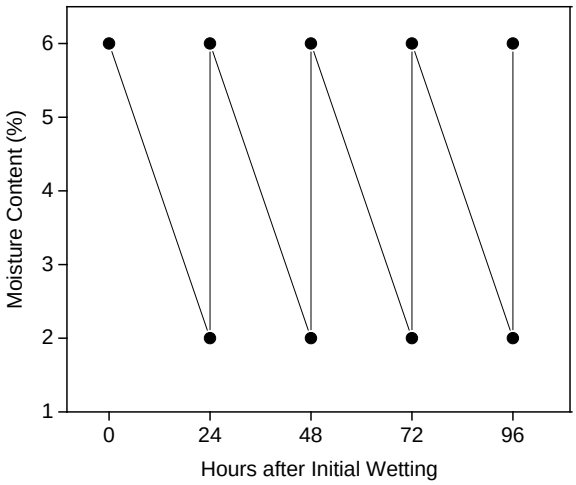


**Fig. 2.** A sample of the wetting interval and resulting moisture content for the 24-hour wetting/drying cycle.

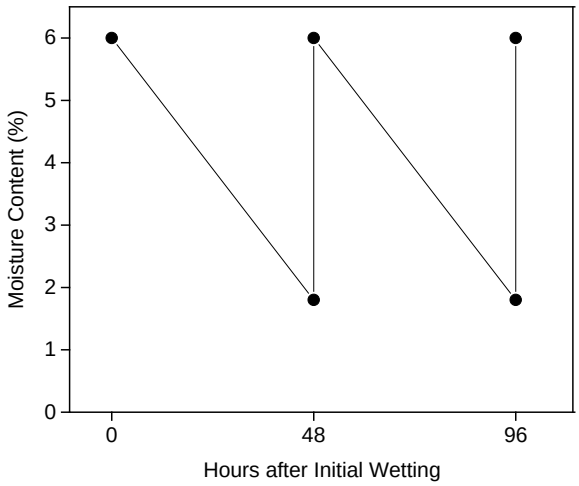


**Fig. 3.** A sample of the wetting interval and resulting moisture content for the 48-hour wetting/drying cycle.

*2.5. Sampling*

Each treatment contained four replications. The moisture content treatments of 3%, 2%, and 1.8% were destructively sampled at the end of week one and week two. The dry control treatment was sampled after 39 days without rewetting. At the end of each week water drop penetration time (WDPT), and contact angle (CA), were measured immediately at both the crust and subsoil layer. These samples were stored in the freezer in individual falcon tubes prior to the quantification of aliphatic constituents. The rest of the treatment was sampled immediately for gravimetric moisture content, and the remainder was stored in a Ziploc bag in the freezer.

*2.6. Measurements*

*2.7. Gravimetric Moisture Content*

Gravimetric moisture content is the water content of a soil reported on a mass basis. At the end of each week, the samples were mixed thoroughly and approximately 10g of soil was

weighed out and placed in a 105ºC oven for 24hrs. The tin weight, the wet weight, and the dry weight were recorded. The gravimetric moisture content was calculated using the follow equation:

$$\%\ gravimetric\ moisture = \left(\frac{wet\ soil\ wtih\ tin - dry\ soil\ with\ tin}{dry\ with\ tin - tin\ weight}\right) 100\% \quad (1)$$

*2.7.1. Water Drop Penetration Time*

Water drop penetration time (WDPT) measures the time it takes a single drop of water to infiltrate the soil. It is used to determine the degree of water repellency of a permeable surface (Doerr, 2000). By quantifying the degree of water repellency for each treatment, the results can be compared to the other quantitative results of microbial biomass, and aliphatic content. The WDPT analysis answers the first set of hypotheses concerning whether desiccation stress will induce water repellency.

The crust (soil depth of 0-0.2cm), and the subsoil (soil depth of 0.2cm-1cm) of each sample was measured by WDPT. A high-speed camera was used to record the time it took for the drop of water to infiltrate the surface. Three replications (drops) were applied for the crust and subsoil layers. The surface measurements were taken from the left side of the sample cup, and the subsurface measurements were taken from the right side to minimize moisture impacts on the subsoil layer. The sample cups were oriented with the label facing the person measuring, for a consistent division of the left and right sides.

A 20µl syringe connected to tubing and a small dropper was used to administer the water, and was placed 2cm above the soil. The height from the soil was determined by the camera's

frame. The syringe was arranged so that only its tip was visible in the frame. The camera was placed 7.7cm from the sample cup. A bright studio light was used to illuminate the scene.

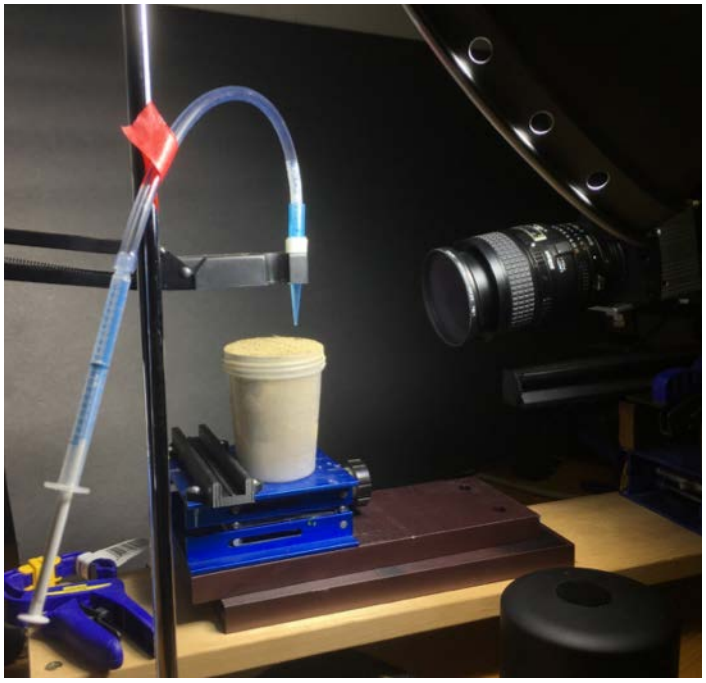

**Fig. 4.** WDPT, and contact angle set up with the high-speed camera.

The WDPT was determined immediately after each drop. The video was played back, and the viewer determined the frames for the point when the water drop first hit the soil, and when the water drop had completely infiltrated. HiSpec Control Software (Fastec Imaging Corp, San Diego, California, USA) was used to control the camera and record the WDPT.

### *2.7.2. Contact Angle*

Contact angle (CA) describes the angle of incidence between a solution and a surface. It is a method used to infer the degree of water repellency of a material (Wessel, 1988). If a contact angle is greater than 90°, the surface is considered water repellent. The greater the contact angle the more water repellent the surface.

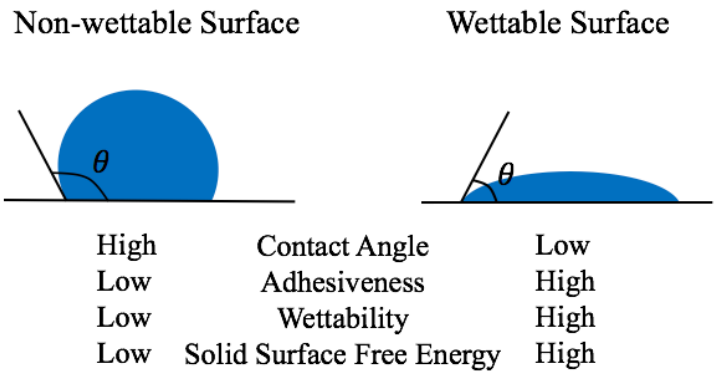


**Fig. 5.** The contact angle, denoted as theta, and the implications the degree has on the characteristics of the surface.

For each WDPT measured, a contact angle was also measured. The surface (0-0.2cm), and the subsurface (0.2cm-1cm) of each sample was measured for contact angle. Three replications (drops) were done for the surface, and three replications were done for the subsurface. The surface measurements were taken from the left side of the sample cup, and the subsurface measurements were taken from the right side. The sample cups were oriented with the label facing the person measuring, for a consistent division of the left and right sides.

Screenshots of the water drops were taken from the WDPT videos, immediately after WDPT was recorded. The screenshot was taken when the water drop stopped oscillating from the impact with the soil. The time it took from impact for the water drop to stabilize varied.

A screenshot of each droplet was saved to the SCA20_U (DataPhysics Instruments GmbH, Filderstadt, Germany) to calculate the contact angles. The lighting cast a shadow on the right side of the drop so the left side of the drop was used whenever possible to determine the contact angle. The angle was calculated using the ‘define ellipse’ function.


**Fig. 6.** Screenshot of the SCA20_U program (DataPhysics Instruments GmbH, Filderstadt, Germany) used for contact angle measurements.

### *2.7.3. Microbial Biomass*

The chloroform fumigation extraction is a common method for quantifying microbial biomass (Brookes et al., 1985; Vance et al., 1987). Microoganisms that are exposed to chloroform fumes are killed though the lysing of their cells. This releases their soluble organic carbon, which is proportional to the microbial biomass in the soil.

The procedures by Brookes et al., in 1985, and Vance et al., in 1987 were used to determine the microbial biomass in the soil. Two sets of 50mL falcon tubes with 10g of soil each were weighed out. One set was labeled ‘fumigated’, and the other was labeled ‘non-fumigated’. Paper with penciled sample IDs were placed in each falcon tube labeled for fumigation. This was done, because the chloroform removes permanent marker labels from the falcon tubes. All of the samples were placed in a glass vacuum desiccator inside of a fume hood. A 150mL beaker with desiccation chips, and 100mL of chloroform was placed in the middle of the samples. Silicon

grease was used to lubricate the rim of the desiccation chamber. The lid was additionally sealed with parafilm to prevent gas loss. The desiccation chamber was then vacuumed pumped until the chloroform started to boil. Once the chloroform had boiled, the nob sleeve was turned ¼ of the way around, and parafilm was wrapped around the vent to prevent gas loss. A dark bag was placed over the desiccator, and allowed to incubate for 24 hours.

After incubation, the parafilm was removed and the nob sleeve was realigned to allow for air flow. If no air flow is heard after realigning the nob sleeve, the seal was not successful, and the extraction must be redone.

The fumigated and non-fumigated samples were then extracted with 40mL of 0.5M $K_2SO_4$, and shaken (160 rpm) for one hour. The samples were filtered through Whatman #1 filter paper. The filtrate was put into new falcon tubes, and stored in the fridge until analyzed.

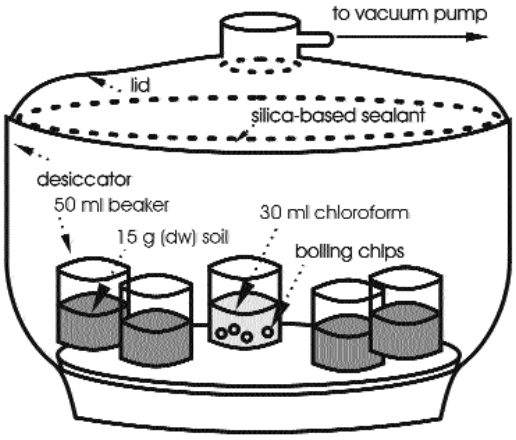


**Fig. 7.** A general set up for the chloroform fumigation extraction (Okalebo et al., 1993).

Both non-fumigated and fumigated extracts were analyzed for solution organic carbon (C) using a Shimadzu TOC/TN Analyzer (Shimadzu Corporation, Kyoto, Japan). The difference between the two extractions is assumed to be the carbon from inside of the microbial cells that

were lysed through the extraction process. The difference in the solution organic C between the fumigated and non-fumigated samples represents the C in the microbial biomass.

$$[CFs] - [CNFs] = [C]\ in\ the\ microbial\ biomass \quad (2)$$

Where [CFs] represents concentration of carbon in fumigated sample, and
[CNFs] represents concentration of carbon in non-fumigated sample.

This is converted into µg C by multiplying the corresponding values obtained from the Shimadzu TOC/TN Analyzer by the volume of the extract (40 mL plus the mL of water in the soil that was extracted) and dividing by the dry weight of the soil that was extracted. Division of the C flush values by a correction factor ($K_c$) of 0.45 for C will yield the microbial biomass C. The correction factor is defined as the fraction of biomass C mineralized by carbon dioxide (Horwath and Paul, 1994). Literature values are often used, because it is difficult to develop the $K_c$ for each soil (Horwath and Paul, 1994).

$$\frac{\left(\frac{[C](40mL+soil\ water)}{dry\ soil}\right)}{0.45} = \mu g\ C\ in\ microbial\ biomass\ g^{-1}\ dry\ soil \quad (3)$$

*2.7.4. Hexane Extraction*

The hexane extraction was used to extract the aliphatic constituents from the soil samples. Lipids are soluble in organic solvents, such as hexane. Hexane was chosen as the solvent, because it targets nonpolar lipids, like aliphatic constituents.

Five grams of soil was weighed out into a cellulose thimble and placed in the extraction chamber. Three hundred mL of hexane was placed in a 500mL boiling flask and attached to the Soxhlet apparatus. The heat source was placed under the boiling flask and powered at 40 volts. Aluminum foil was placed over the extraction chamber to maintain the high temperature as the vapor traveled to the extraction chamber. The extraction was run for five hours. After extraction, the extract allowed to evaporate from the boiling flask for 10 minutes, and then stored in a 250ml dark glass bottle in the freezer. One day prior delivering samples for analysis the extracts were condensed to approximately 15ml using a beaker in a water bath, and stored in glass vials. The cellulose tubes were cleaned by extracting with hexane for three hours.

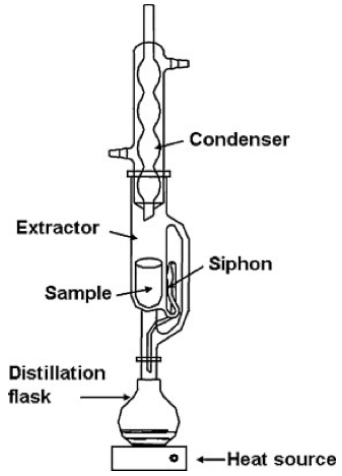


**Fig. 8.** Schematic diagram of a Soxhlet extractor (Arisa and Morada, 2006).

### *2.7.5. Gas Chromatography Mass Spectroscopy*

Gas chromatography mass spectroscopy (GCMS) ionizes liquid extracts, and each compound is separated by mass where heavier compounds have a longer retention time. Based on the retention time of the compounds, peaks were created using the compound's mass to charge ratio (Sahil et al., 2011). The peaks generated were identified by comparing them to

known compounds in the NIST (National Institute of Standards and Technology) database. The peak areas were quantified by their relative percentage of the total sample. Only the compounds that reported a peak area of 1% or greater were analyzed, because compounds that reported a peak area of less than 1% were assumed to not have a high enough quantity to significantly influence the degree of water repellency.

The nominal oxidation state of each compound was used to determine which compounds were aliphatic constituents. Naturally occurring environmental compounds inhabit a specific range of oxidation states. The oxidation state of lipids ranges from -2 to around -1 (Masiello et al., 2008). Due to the similarity in structure and function of aliphatic constituents and lipids, the oxidation range of lipids was used as a benchmark for determining the threshold for aliphatic components. Compounds that reported a nominal oxidation state number of -2 or less were considered aliphatic constituents.

The nominal oxidation state was calculated from the equation given in Masiello et al. in 2008. For molecules denoted as $C_xH_yO_zN_w$ the equation is:

$$C_{ox} = \frac{2z-y+3w}{x} \quad (4)$$

*2.8. Statistics*

Welch's t-test was used to compare the mean WDPT, contact angle, microbial biomass, and quantity of aliphatic constituents of one treatment to another. This test was chosen over other t-tests (such as the student t-test) due to unequal sample sizes, and the heterogeneity of variance of some of the data. The Games-Howell post-hoc test was used to correct for a type I error from repeated t-tests. This post-hoc test was used due to the unequal sample sizes, and heterogeneity

of variance of some of the data. For each analysis, a two-way ANOVA was used to determine if there was any interaction between moisture content and the duration of wetting/drying cycles, as well as between the moisture content and soil depth.

## 3. Results

*3.1. Hypothesis 1: The degree of water repellency increases as the moisture content decreases.*

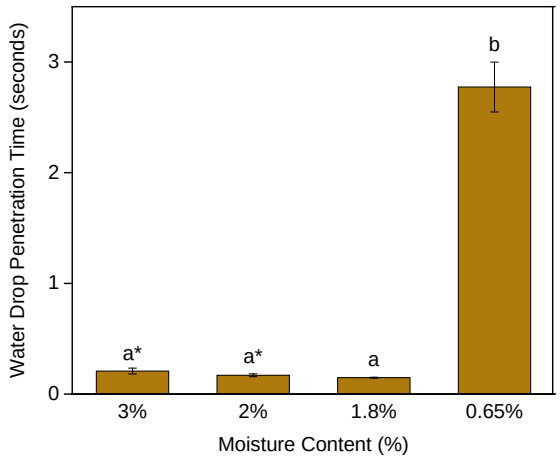


**Fig. 9.** The impact of moisture content on water drop penetration time. Different letters indicate a significant difference between treatments ($p < .05$). The error bars were calculated using the standard deviation. Asterisks denote that the treatment displayed a difference in average water drop penetration time between week one and week two.

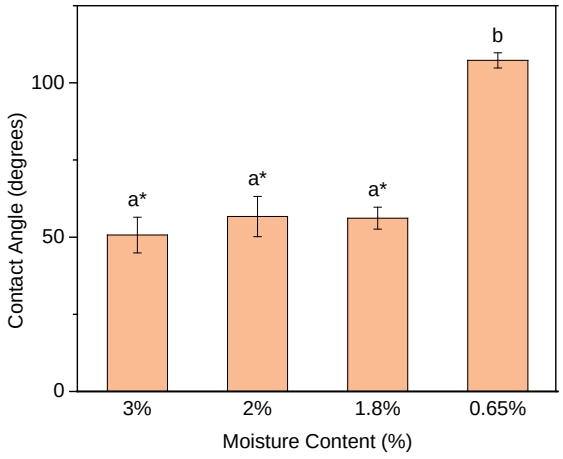


**Fig. 10.** The impact of moisture content on contact angle. Different letters indicate a significant difference between treatments ($p < .05$). The error bars were calculated using the standard deviation. Asterisks denote that the treatment displayed a difference in average contact angle between week one and week two.

The WDPT decreases from 3% to 1.8% moisture content treatments, however there is no statistical difference. The 0.65% moisture content treatment did have a statistically significant longer WDPT. While there was no difference in the re-wetting treatments, this data shows that soil that has been dry for a prolonged period of time displays water repellency. The WDPT increases significantly at a threshold value somewhere between 1.8% and 0.65%. Therefore, the hypothesis is not refuted.

The results for contact angle follow the same pattern as the WDPT. The contact angle for the 3%, 2%, and 1.8% moisture content treatments are not statically different from one another. However, the 0.65% moisture content did have a statistically different contact angle than the re-wetting treatments. Again, we see a threshold between 1.8% and 0.65%. The combination of these results with the WDPT results show that a contact angle around 50° does delay water infiltration. Further investigation showed a linear relationship between contact angle and WDPT for values ranging the threshold region from 40° to 100° of $y = 0.328x + 4.14$ with an $R^2$ of 0.52. Where y is the contact angle and x is the WDPT in milliseconds. The WDPT increases at a threshold value between 1.8 and 0.65% moisture content treatments. While our treatments did not show a stepwise relationship, the hypothesis is not refuted.

*3.2. Hypothesis 2: The degree of water repellency is greater for the subsoil (0.2-1cm) than the crust (0-0.2cm)*

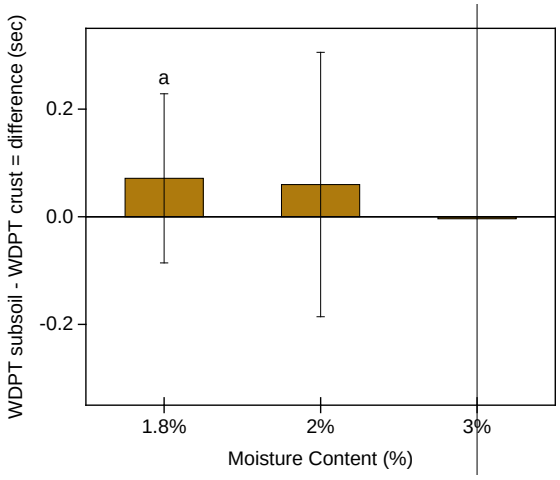


**Fig. 11.** The impact of moisture content on the difference in contact angle between the subsoil and the crust, where the difference in WDPT = $WDPT_{subsoil} - WDPT_{crust}$. Positive values indicate a greater contact angle for the subsoil. Negative values indicate a lower contact angle for the subsoil. Error bars were calculated using the standard deviation. Letters indicate that the difference in water drop penetration time is statistically different from zero.

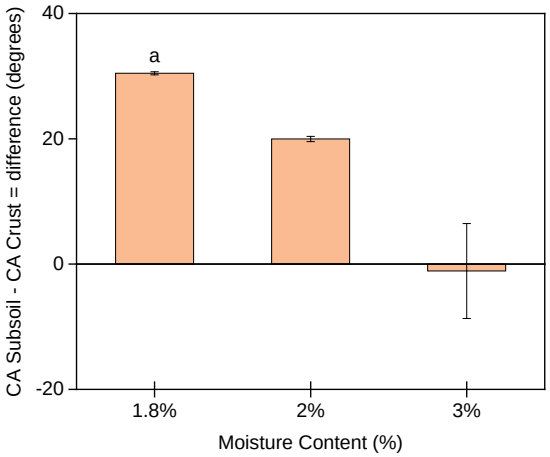


**Fig. 12.** The impact of moisture content on the difference in contact angle between the subsoil and the crust, where the difference in contact angle (CA) = $CA_{subsoil} - CA_{crust}$. Positive values indicate a greater contact angle for the subsoil, and values indicate a lower contact angle. Error bars were calculated using the standard deviation. Letters indicate that the difference in contact angle is statistically different from zero.

On average, the 1.8% and 2% moisture content treatments showed a slightly longer WDPT in the subsoil than the surface crust but some replicates did demonstrate a negative difference. The average difference of the WDPT of the 1.8% treatment was greater than the 2% treatment, but there was no statistical difference in these values. The 3% moisture content treatment showed a slightly shorter WDPT in the subsoil than the crust, but the value was very near zero showing no difference in WDPT between the two depths. This data shows that WDPT is not inherently different between the surface crust and subsoil, but may be different under drier moisture conditions. Therefore, we do not have enough evidence to accept or refute the hypothesis.

The results for contact angle follow the same pattern as the WDPT. The 1.8%, and 2% moisture content treatments displayed a positive difference in contact angle. On average, the 1.8%, and 2% moisture content treatments showed a slightly greater contact angles in the subsoil than the crust. The average difference of contact angle of the 1.8% moisture content treatment was greater than the 2% moisture content treatment. The 3% moisture content treatment showed a slightly lower contact angle for the subsoil than the crust, but the value was very near zero showing no difference in contact angle between the two depths. This data shows that contact angle is not inherently different between the subsoil and the crust, but may be different under drier moisture conditions. Therefore, we do not have enough evidence to accept or refute the hypothesis.

*3.3. Hypothesis 3: The microbial biomass decreases as the moisture content decreases*

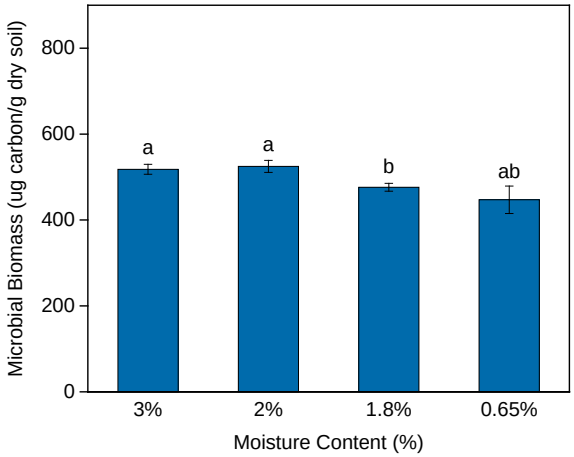


**Fig. 13.** The impact of moisture content on the microbial biomass. Different letters indicate a significant difference between treatments ($p < .05$). Error bars were calculated using standard deviation.

While there was not a statistical difference between moisture content treatments, the microbial biomass decreased as the moisture content decreased. Therefore, the hypothesis cannot be refuted.

*3.4. Hypothesis 4: The quantity of aliphatic constituents increases as the moisture content decreases*

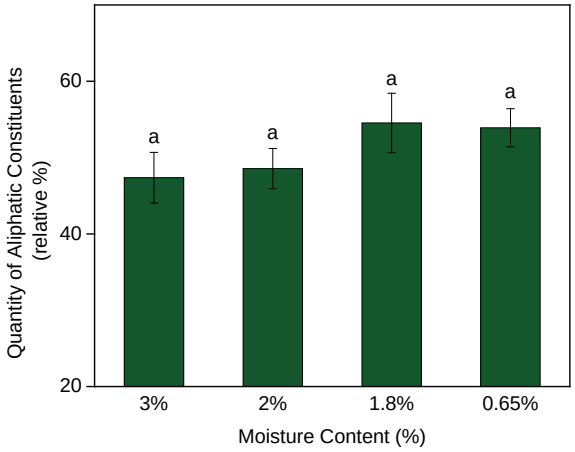


**Fig. 14.** The impact of moisture content on the quantity of aliphatic constituents. Different letters indicate a significant difference between treatments ($p > .05$). Error bars were calculated using the standard deviation.

The data was not statistically significant, but there is an apparent increase in the quantity of aliphatic constituents as the moisture content decreased. Therefore, the hypothesis cannot be refuted.

*3.5. Hypothesis 5: The quantity of aliphatic constituents is greater for the subsoil (0.2-1cm) than the crust (0-0.2cm)*

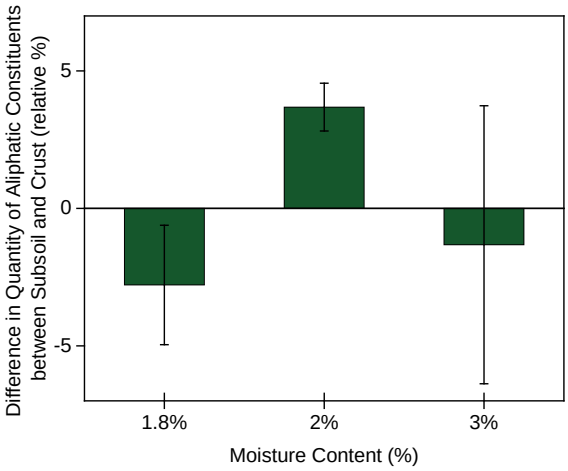


**Fig. 15.** The impact of soil depth and moisture content on the quantity of aliphatic constituents. The difference in the quantity of aliphatic constituents between the subsoil and the crust is plotted on the y-axis. Positive values indicated a greater quantity of aliphatic constituents for the subsoil. Negative values indicated a greater quantity of aliphatic constituents for the crust. Error bars were calculated using the standard deviation.

The 2% moisture content treatment displayed a positive difference in the quantity of aliphatic constituents between the subsoil and the crust. The 1.8%, and 3% moisture content treatments exhibited a negative difference in the quantity of aliphatic constituents between the subsoil and the crust. There was no discernable pattern between soil depth and the quantity of aliphatic constituents, therefore the hypothesis is refuted.

*3.6. Hypothesis 6: The degree of water repellency increases the longer the soil is subjected to wetting/drying cycles that induce desiccation stress*

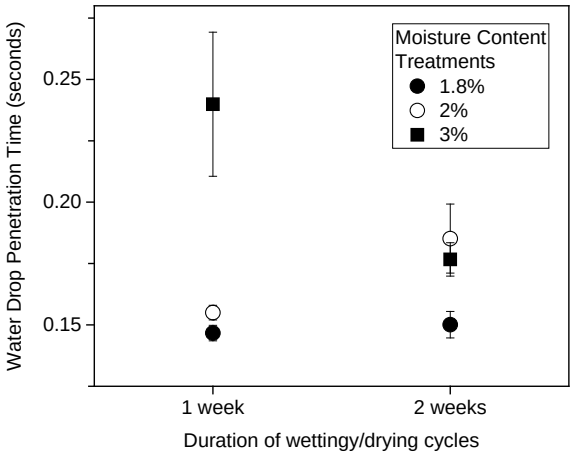


**Fig. 16.** The impact of the duration of wetting/drying cycles on water drop penetration time. Error bars were calculated using the standard deviation.

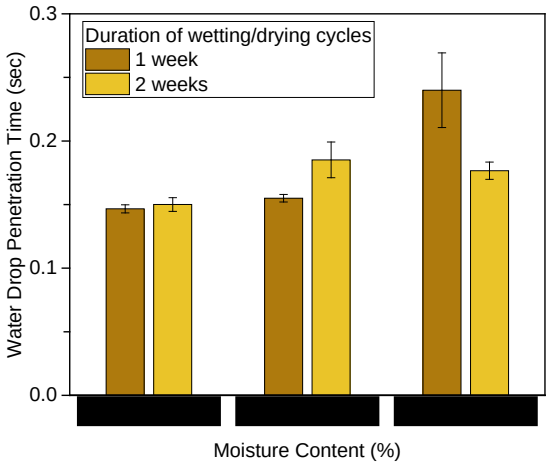


**Fig. 17.** The impact of the duration of wetting/drying cycles on water drop penetration time. Error bars were calculated using the standard deviation. There was a significant interaction between moisture content and the duration of wetting/drying cycles ($p < .05$).

This data shows that some treatments did change over time. The 2% moisture content treatment increased in WDPT between week one and week two. There was no difference in WDPT in the 1.8% moisture treatment. The 3% moisture content treatment decreased WDPT between week one and week two. The 3% moisture content treatment that was re-wetted every 8 hours was designed not to induce repellency and showed that over time the WDPT was reduced. The 2% moisture content treatment that was re-wet at the current management practice interval of 24 hours did show an increase in WDPT over time. The WDPT from week one to week two

was enhanced when the soil was subjected to wetting/drying cycles that induced desiccation stress, and suppressed when the wetting/drying cycles did not induce stress. This hypothesis is not refuted.

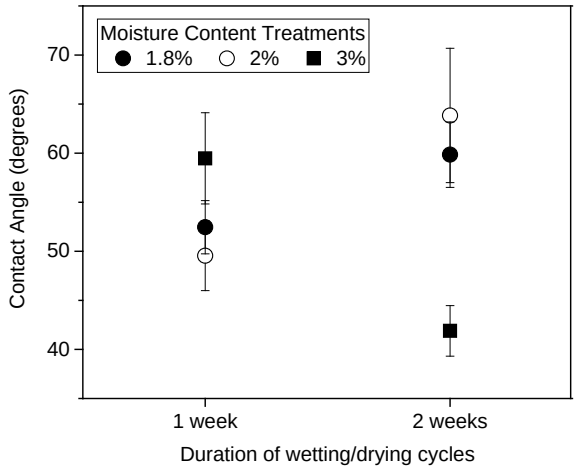


**Fig. 18.** The impact of the duration of wetting/drying cycles and moisture content on contact angle. Error bars were calculated using the standard deviation.

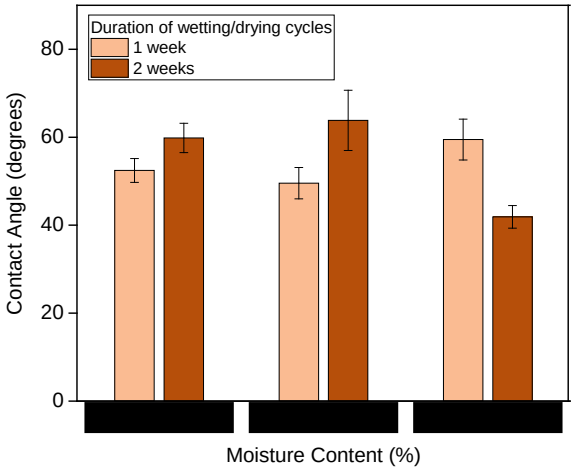


**Fig. 19.** The impact of moisture content and duration of wetting/drying cycles on contact angle. Error bars were calculated using the standard deviation. Overall, there was a significant interaction between moisture content and duration of wetting/drying cycles ($p < .05$).

The results for contact angle follow the same pattern as the WDPT. The 1.8%, and 2% moisture content treatments increased in contact angle between week one and week two, while the 3% moisture content treatment decreased. The treatments designed to induce desiccation

stress did show a larger contact angle from week one to week two, while the treatment that was designed to limit desiccation stress decreased in contact angle. This hypothesis is not refuted.

### *3.7. Hypothesis 7: The microbial biomass decreases the longer the soil is subjected to wetting/drying cycles that induce desiccation stress*

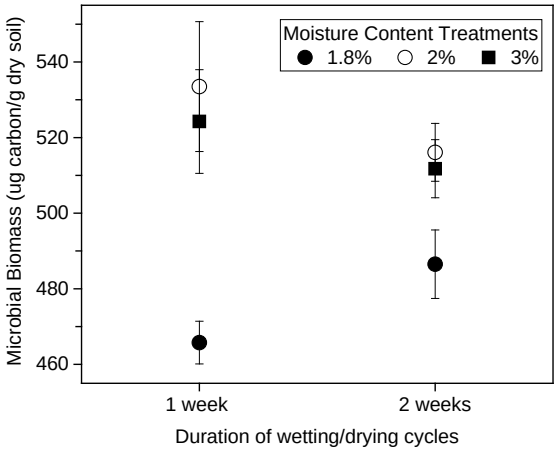


**Fig. 20.** The impact of the duration of wetting/drying cycles on the microbial biomass. Error bars were calculated using the standard deviation.

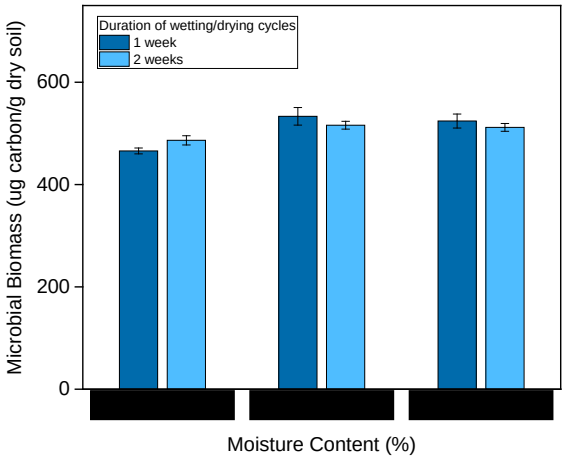


**Fig. 21.** The impact of moisture content and duration of wetting/drying cycles on the microbial biomass. Error bars were calculated using the standard deviation.

Overall, there was not a significant interaction between moisture content and the duration of wetting/drying cycles ($p > .05$).

The 1.8% moisture content treatment increased in microbial biomass between week one and week two. However, the microbial biomass for the 2% and 3% moisture content treatments were not different between weeks. The 1.8% moisture content treatment which was designed to induce desiccation stress resulted in an increase in microbial biomass. This hypothesis is refuted.

*3.8. Hypothesis 8: The quantity of aliphatic constituents increases the longer the soil is subjected to wetting/drying cycles that induce desiccation stress*

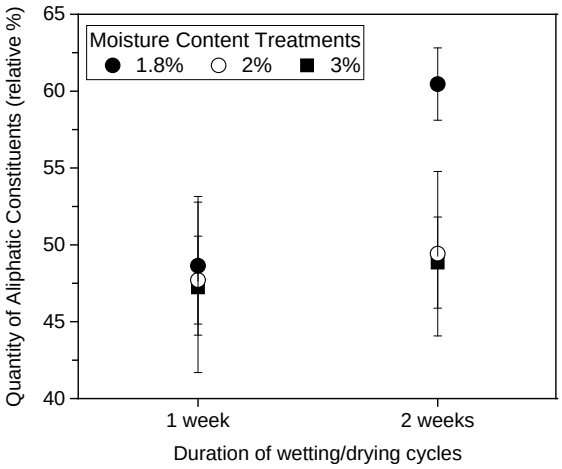


**Fig. 22.** The impact of the duration of wetting/drying cycles on the quantity of aliphatic constituents. Error bars were calculated using the standard deviation.

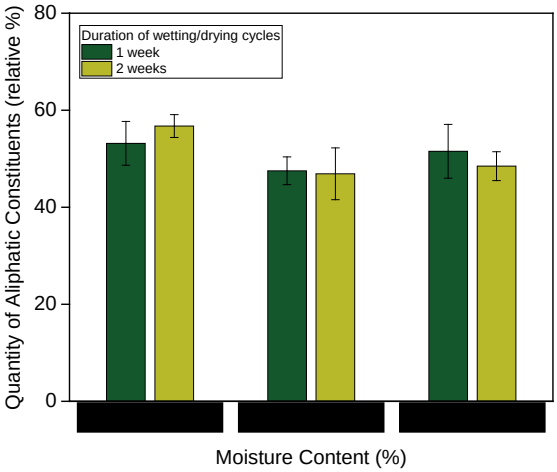


**Fig. 23.** The impact of moisture content and the duration of wetting/drying cycles on the quantity of aliphatic constituents. Error bars were calculated using the standard deviation. Overall, there was not a significant interaction between moisture content and the duration of wetting/drying cycles ($p > .05$).

The 2% and 3% moisture content treatments did not exhibit a difference in the aliphatic content between weeks. The treatment experiencing the greatest desiccation stress, 1.8%, did show a difference in the aliphatic content from week one to week two. Therefore, the hypothesis cannot be refuted.

*3.9. Interactions between variables*

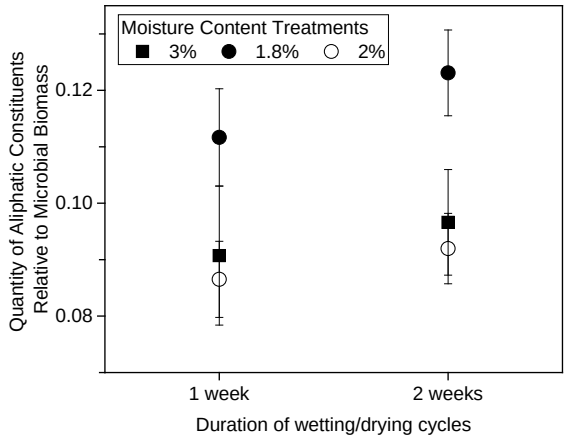


**Fig. 24.** The impact of the duration of wetting/drying cycles on the quantity of aliphatic constituents in relation to the microbial biomass. Error bars were calculated using the standard deviation.

On average, all of the moisture content treatments displayed a slight increase in aliphatic content relative to the microbial biomass between week one and week two. However, some of the replicates decreased, resulting in no significant difference between weeks.

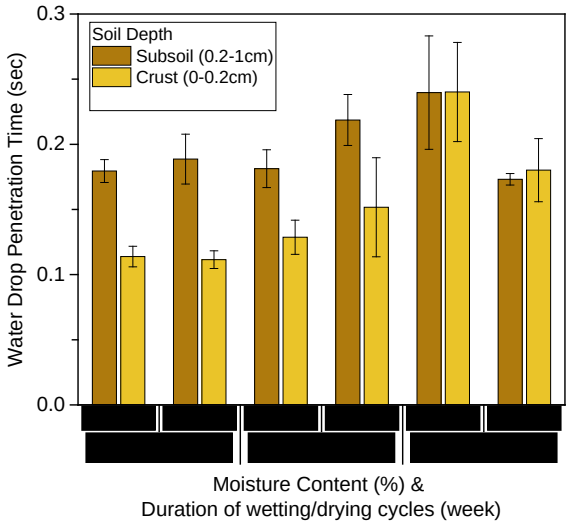


**Fig. 25.** The effect of water drop penetration time as a response to moisture content, duration of wetting/drying cycles, and soil depth. Error bars were calculated using the standard deviation.

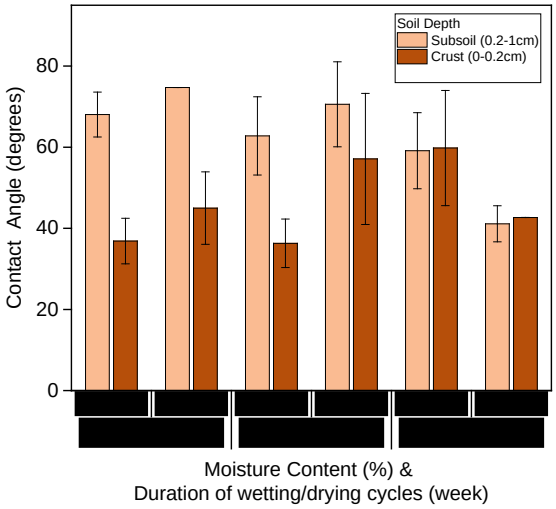


**Fig. 26.** The effect of contact angle as a response to moisture content, duration of wetting/drying cycles, and soil depth. Error bars were calculated using the standard deviation.

The 3% moisture content treatment did not display any difference in water repellency between soil depths, or between weeks. The 2% treatment shows a significant difference in soil depth for WDPT, and only in the first week for contact angle. The subsoil for the 2% treatment also shows a difference between weeks. The 1.8% treatment exhibited repellency difference by soil depth but not across weeks. While not all of the interactions between variables are significant, notice that both WDPT and contact angle follow the same trend throughout moisture content treatments, duration of wetting/drying cycles and soil depth.

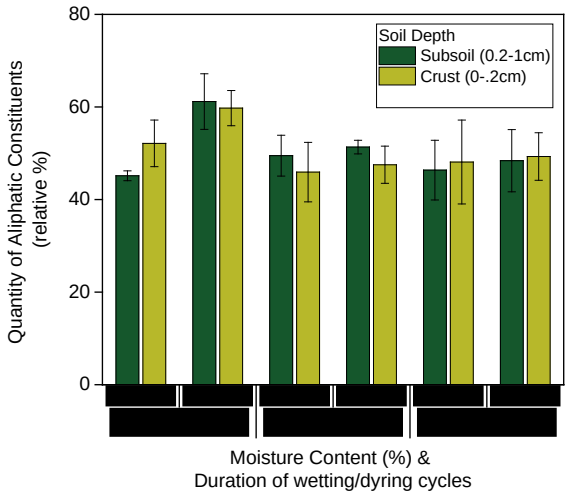


**Fig. 27.** The effect of the quantity of aliphatic constituents as a response to moisture content, the duration of wetting/drying cycles, and soil depth. Error bars were calculated using the standard deviation.

Neither the 2%, nor 3% moisture treatments displayed any differences in the aliphatic content by soil depth or between weeks. While there were few statistical differences there was a weak trend of greater aliphatic content in the subsoil than the crust as moisture content decreased, and the 1.8% moisture content treatment did exhibit an increase in aliphatic content from week one to week two.

## 4. Discussion

The overarching hypothesis of this project is that the soil microbial community actively controls water repellency. When the individually tested hypotheses are taken as a whole we find evidence that the microbial production of aliphatic constituents may induce water repellency. This comes from three primary results that will be discussed below. 1) The degree of repellency changed by moisture content treatment over time. 2) The lower moisture content treatments were associated with a higher degree of water repellency. 3) The low moisture and high repellency treatments were found in samples that had a higher relative percentage of aliphatic constituents.

### *4.1. The degree of repellency changed by moisture content treatment over time*

In the unstressed treatment (3% moisture content) that was rewet every eight hours the WDPT and contact angle decreased significantly, while the microbial biomass and the relative percent of aliphatic constituents were not significantly different. Under an eight-hour wetting interval treatment the number of weeks the soil was subjected to the wetting/drying cycles did not impact the microbial biomass. The 3% moisture content treatment was designed to limit desiccation stress. Without desiccation stress, there was no need for the microbial community to adjust its biomass or aliphatic content. The soil had been dry for months prior to the treatments, under non-stressed conditions the repellency decreased over time.

In the 2% moisture content treatment WDPT and contact angle increased significantly from week one to week two, while the microbial biomass and the quantity of aliphatic constituents were not significantly different. At a 24-hour rewetting interval it appears that the microbial community has not been significantly stressed enough to increase its desiccation response. The change in WDPT while significant may not be meaningful. The difference in an

infiltration time of 0.155 and 0.185 seconds will likely not have an impact on overland flow and erosion. However, these results may indicate that this treatment was right near a threshold that causes meaningful repellency. The contact angle and WDPT indicate repellency and were made on a large number of samples. This might lend statistical significance to these variables while the aliphatic content is a complex metric and was measured with limited repetitions.

In the 1.8% moisture content treatment contact angle, the quantity of aliphatic constituents, and the microbial biomass increased over time, while the WDPT did not change when taken as a whole. However, when examining the crust versus the subsurface WDPT, we do see that it follows the same trend as the contact angle. The contact angle changes from 52° to 59°, and there was also a trend suggesting a slight increase in WDPT at the subsurface depth. This lends evidence to the idea that the contact angle is indicative of repellency somewhere around 60°. Hamlett et al., in 2011 found that a contact angle around 61°- 65° could describe the critical surface tension of water penetration of a repellent sand. The change in contact angle also shows that there is a change in the surface interaction. The change in both aliphatic content and contact angle over time indicated there could be a greater increase in water repellency if this trial had been extended. The increase in microbial biomass could have been an artifact of the chloroform fumigation extraction procedure. There is evidence that one hour of exposure to the 0.5M potassium sulfate extraction solution may not be enough to extract the EPS from the surface of soil particles (Redmile-Gorden et al., 2014). However, using chloroform to lyse the microbial cells may have also released the EPS from the soil surface (Sang and Steinberger, 1993) thereby increasing the total dissolved carbon in the fumigated samples and displaying and artificial increase in microbial biomass.

Previous research shows that it takes time for the microbial community to change (Sang and Steinberger, 1993). Lauber et al., in 2013 found that microbial diversity within field plots were variable over time, and showed change on a month-to-month basis. Other research has shown the microbial community it is also capable of changing within a week. Kakumanu et al., in 2013 subjected the microbial community to varying levels of desiccation stress for three consecutive days and found that the microbial biomass carbon had already started to decrease at the lowest water potential. Our experiment showed that the microbial community can start to change over the course of a few weeks. Further work should extend these trials to see if a more pronounced change occurs over time.

### *4.2. The lower moisture content treatments were associated with a higher degree of water repellency*

Water drop penetration time and contact angle are both indicators of water repellency. By using both metrics the repellency phenomenon can be verified through theoretical surface tension as measured by contact angle and by practical measure of water actually moving into the soil with WDPT. The WDPT and contact angle results followed the same trends when taking into account the interactions between variables of moisture content, soil depth, and duration of the wetting/drying cycles, and gave us confidence in each measurement method (see figure 25 and 26).

Given the agreement between WDPT and contact angle, the marked difference in repellency between the 0.65% treatment and the other moisture content treatments may be an indicator of a threshold moisture content for repellency around 1.8%. There was also a difference in the degree of water repellency after two weeks of wetting/drying cycles for the 1.8% moisture

content treatment. Around this 1.8% moisture content threshold WDPT exhibits a high correlation with repellency, and contact angle display a moderate correlation. Literature previously defined a contact angle of 90° or greater as repellency (Shirtcliffe et al., 2006). New research suggests that the threshold contact angle is less than 90° (Shirtcliffe et al., 2006**)**. The results of this experiment suggest the threshold is near 50°- 60°.

### *4.3. The low moisture and high repellency treatments (after two weeks) were found in samples with a higher relative percentage of aliphatic constituents*

Aliphatic constituents were identified by the nominal oxidation state of carbon (NOSC) of the compound where a NOSC of -2 or less was considered an aliphatic constituent. Naturally occurring environmental compounds occupy a typical range of oxidation states. The NOSC for lipids ranges from -2 to -1 (Maisello et al., 2008). The long, non-polar carbon chains of a lipid is structurally similar to aliphatic constituents, and both are non-wetting materials. Thus, the NOSC for lipids was used as the benchmark to determine which compounds were labeled as aliphatic constituents.

Previous research has shown that microbes change the structure of their EPS in response to environmental stressors. Under desiccation stress EPS undergoes morphological changes that decreases the distance between its cross-linked strands resulting in a dense structure that surrounds the microcolony (Orr, 2007). EPS production has also been shown to be a major factor of microbial survival during desiccation. Chang et al., in 2007 found that bacteria that had a diminished production of EPS had a lower survival than bacteria with fully functioning EPS production. The results of this experiment also give evidence that under desiccation stress microbes increase their production of aliphatic constituents. Aliphatic constituents may form a

protective skin around microbes to shield them from the detrimental effects of desiccation. Its function being theorized as similar to a phospholipid bilayer, where a cell's membrane encompasses the organelles as the aliphatic constituents and EPS encompass a microbial community.

After one week of rewetting there were no differences in the average quantity of aliphatic constituents between moisture treatments. After two weeks, the sample under the highest desiccation stress (1.8% moisture content treatment) exhibited a significant increase in the average production of aliphatic constituents. When the aliphatic content was further analyzed by soil depth these results were confirmed. In the 1.8% moisture content treatment only the subsoil exhibited a significant increase in the quantity of aliphatic constituents over time. However, between week one and week two there was a weak trend of an increasing quantity of aliphatic content that surpassed the content in the crust (see figure 27). These results also lend evidence to the belief that the desiccation stress threshold is somewhere around a moisture content of 1.8%.

The difference in repellency by soil depth in the 1.8% treatment is similar to what is found in the field sites where this phenomenon has been observed. While, we do not have enough evidence to explain the mechanisms of this occurrence, it is worth noting that under stress, there seems to be a significant difference in the reaction of the microbial community at the surface crust versus the subsoil just 2mm deeper. The controlled laboratory conditions eliminated the explanation that this is caused by wind-blown particles that have settled on the surface causing roughness that induces repellency (Yoshimitsu et al., 2002; McHale et al., 2005**)**. If the soil just below the surface is the zone of greatest repellency, it would cause an infiltration barrier leading to a saturated crust that leads to the movement of surface particles with overland flow thus increasing the severity of rill erosion.

## 5. Conclusion

This project provided preliminary evidence to suggest that the presence of aliphatic constituents induces soil water repellency when the microbial community is subjected to desiccation stress. While we cannot conclusively say this is the mechanism microbes use to induce water repellency, we did demonstrate the ability to see differences. More research is needed to determine if this trend is significant.

## 6. Acknowledgements

I would first like to thank Markus Kleber for his mentorship. I would also like to thank Shannon Andrews for her guidance, patience, and numerous revisions. Thank you to Maria Dragila, and Matt Konkler for instrumentation help. Thank you to Chris Burgess for Shimazu instrumentation and statistics help. Lastly, thank you to my friends and family for your support.

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