

The Effects of Major Organic Compound Found in Roots and Biological Exudates Influencing Liquid Transport in Soil

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Abstract: *Soil organisms produce a range of exudates that may affect water uptake and transport by inducing hydrophobicity, clogging of pores or acting as a surfactant. Using the Capillary Rise Method, the rate of liquid uptake of a range of biological exudates were measured in model sands of varying texture and a range of soils with differing water repellency. The hypothesis was that root exudate compounds will counteract water repellency. The compounds examined were malic acid, malonic acid and oxalic acid, which are common components of root exudates, in addition to xanthan produced by bacteria. The results demonstrate that agricultural soil, Culbin forest, is extremely hydrophobic. Both fine sand and Glass Ballotini Contact Angle ($\theta = 0^\circ$) are completely wettable. Coarse sand texture is hydrophobic and silica flour is moderately. Sorptivity of Ethanol is large in Culbin forest; bullion field and Inch soil are less. Sorptivity of malonic in coarse sand is much higher than in fine sand, silica flour and Glass Ballotini. have little impact of sorptivity. High organic matter content dried unto the agricultural soil, is water repellent, but is overcome in solution of the root exudate compound; malonic acid has the greatest impact in relative wettability of soil with hydrophobic coating. The surface tension of xanthan at a concentration of 5 mg/g is greater than that at 1 mg/g.*

Keywords: Wettability, sorptivity, exudate, biological plant exudate contact angle

1. Introduction

The common assumptions in soil physics that soils are completely wettable (contact angle = 0°) and pore water has the same surface tension as water (73 mN m^{-1}) rarely hold true in natural soils (Hallett et al., 2013). Most soils have decreased wettability due to water repellency induced by physical, chemical and biological process taking place in the soil (woche et al, 2017). The organic matter content and the texture of soil are major drivers of wettability capacity of soil (Saulick et al, 2016). The discharge of carbon based compounds (rhizodeposit) by plant roots, such as organic acid and amino acid, into the soil can significantly decrease wettability of soil particles (Jones et al, 2009, Carlos and Bachmann (2013). The exudates have different viscosity and surface tension, which influences liquid transport. In wettable soils this may overcome water repellency and speed up water flow. Exudate from the root of plant play a diversity of role in nutrient uptake, nutrient solubilisation, and influence of soil microbes. when these compound dried unto the soil particles they become hydrophobic which causes hindrance not only to the wetting of soils, but how water move in the rhizosphere during the phase of rewetting (Carminati et al 2013, Ahmed et al, 2015) and the degree of water repellency of soil depend on the amount and types of organic substance found in the soil (Hallett et al, 2002)

Reduction in the rate of wetting and storage of water is influenced by the existence of hydrophobic deposits on soil particles are referred to as soil water repellency. In extreme conditions, water repellency can lead to a high water stress resulting in a decrease in yield of agricultural produce or grass (Hallett, 2008). Studies revealed that plant exudate compound and microbial activities impact the occurrence of water repellency in soil (Hallett et al. 2003; Feeney et al. 2006 ;).The influence of root exudate compounds on aggregation and stabilization is the function of the nature of

exudate, soil pore structure and soil water matric potential (Peng et al, 2011). The phenomenon of water repellency is influenced by the surface of non- polar organic compounds adsorbed on soil particles (Woche et al, 2017) The movement of water in soil is determined by the distribution of matric potential within the soil profile, caused by gradients in water content or gravity (Hallett, 2008). Sorptivity is the ability of the soil to absorb or take up water or liquid, which is generally greater in dry soils than moist soil. Normally, water tends to move from a region of higher potential to one of lower potential brought about by hydraulic conductivity and matric potential controlled by the shape, volume and distorted shape of pores in the soil. In general, soils with large pores have higher hydraulic conductivity with low sorptivity compared to soil with smaller pore sizes (Hallett, 2008). Organic compounds in soil may alter this wetting of porous material particularly root exudates. The water holding capacity curve of zone of soil next to the root, the rhizosphere, can enhanced largely due to root mucilage being a gel with the ability to absorb large volumes of water (Carminati, 2012; McCully and Boyer, 1997). A small quantity of root exudate may decrease surface tension of water (Read and Gregory, 1997) driven by their lipid content (Read et al, 2003). If the lipids become adsorbed to soil particles they may decrease the wettability of the rhizosphere (Hallett et al, 2003) through an increase in soil water contact angle (Urbanek et al, 2007). However, the drop in surface tension could also possibly increase water flow in hydrophobic soils (Read et al., 2003). Generally the smaller the pore size of soil the higher the capillary rise (Hallett, 2008), but in exceedingly water repellent soil, capillary rise and sorptivity is equal to zero (0) and their contact angle is $> 90^\circ$. Different type of soils have specific limits of water repellency in which water infiltration decreases as contact angle increases between 0 and 90° (Tilman et al, 1989).

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The production of biofilm by bacterial cell have influence on soil texture as this would cover the pore wall thereby preventing the flow of liquid in the soil (Philippe et al, 1992) this occur when the deposit from the suspended solid dominates the pore diameter, for example coarse texture soil have the least effect while fine texture are largely affected and decreases permeability of liquid as more of these organic compound are being deposited (Rice (1974), Vandevivere et al 1995). High activities of microorganism in soil give rise to biological clogging, when this phenomenon occurs it decreases the flow of liquid through the surface of the soil. (Seki et al, 2013). The multiplication of bacterial within the rhizosphere is a function of the root exudate in which the microorganism initiate changes to modifies the root exudate and released carbon containing substances and by-product of microorganism that cause water repellency of soil. (Dennis et al, 2010) Soil water repellency is commonly tackled in agriculture and amenity turf by applying surfactants that interact directly with soil particles to decrease contact angle or decrease surface tension to improve wettability. Organic compounds produced by biology in soil may serve a similar role. (Read & Gregory (1997) Xanthan gum is produced by the bacterial *Xanthomonas campestris* (Sigma –Aldrich). It is known to affect a range of soil mechanical properties and induced water repellency when dry (Czarnes et al., 1999) but the flow of xanthan in soil is not known. The wetting of soil particles can be influenced positively when the surface tension of mucilage becomes low which allow the liquid to move through the porous material (Read and Gregory 1997 Reduction in wettability of soil is influenced by the increase of root exudate compound of plant and microbial cells resulting in higher contact angle (Hallett and Young, 1999) which is driven by the individual species of the organism (White et al, 2000).

A decrease in surface tension caused by root mucilage can leading to changes in moisture characteristic curve of soil to decrease water content at a specific tension by 10 – 50% depending on pore size distribution (read et al, 2003). The changes in physical properties imply that if root is able to retain appreciable concentration of exudate compound within the rhizosphere, allowing water and nutrient to flow through the small pore size soil particles which becomes more accessible as compared to large pores (Gregory, 2006). The hypothesis recoded in (Walker et al., 2003) that when soil dries and the hydraulic conductivity reduces, exudate losses water to the bulk soil occurs. When this condition takes place, the exudate surface tension becomes less and its viscosity becomes much greater (Walker et al, 2003). The capacity of the exudates compound to wet soil particle tend to be higher when the surface tension of the wetting liquid become lower, an increase in viscosity counteract the movement of soil particle in contact with the exudate becomes higher and greater stability in the rhizosphere is visible (Walker et al., 2003). Multiple of different factors are influenced as a result of changes in the wettability of soil, such factors include infiltration and shear strength of soil (Saulick et al., 2016) the polymeric material of the mucilage greatly influence water holding capacity of soil with large amount of water content in the rhizosphere.(Carminati al.,2012) The differences that exist between rhizosphere and bulk soil in terms of water retention dynamic especially during wetting and drying conditions can

be influence by root exudate compound (Carminati et al, 2016) in which mucilage increase the water retention capacity of soil. However, majority of tests done on the impact of root exudates on water repellency were measured on the impacts on subsequent soil wetting using water. In this laboratory study, plant root exudate compound including malic acid, malonic acid, oxalic acid and xanthan were use as the wetting liquid. I hypothesized that root exudate compounds counter-act water repellency that develops in soil from the deposition of the organic compounds. The objectives of this study are: (i) to assess the impact of a range of soil biological compounds primarily found in root exudates, on soil wetting characteristics using the capillary rise method with the exudate as the wetting liquid. (ii) To assess whether water repellency in dried soil is overcome by subsequent wetting by a plant exudate compound instead of water.

2. Material and Methods

Soil

The soil samples were graded sands and natural soils. The natural or agricultural soil was sampled in Scotland from Culbin forest in Moray shire, Bullion field at the James Hutton Institute, Dundee, and Inch soil in Aberdeen shire. The soil samples were air dried at 25° C for 48 hours after which sieving of the soil sample was carried out to classify the soil according to their particle size distribution. USDA system of classification was employed with the sieve ranging from 1 mm, 500 µm, 250 µm and 100 µm. The sieving is done manually by shaking the sieve to allow passage of the soil particles through a given size as indicated above, soil samples within < 500 µm – 1 mm are medium coarse sand and < 100 µm– 250 µm are the fine sand, other properties of the soil sample such as texture, carbon content and bulk density are indicated in Table 1 below.

Table 1: General property of the soils

Soil samples	Textural size	Carbon content (g)	pH	weight after	class distribution
Culbin forest	Sandy	100	3.9	3.47 cm	
Bullion field	Sandy Loam	2.29	4.9	3.86 cm	
Insch soil	Sandy loam	2.00	3.57	4.13 cm	
Silica flour	Clay< 50 µm	-	4.7	3.37 cm	
Glass bollotini	Coarse 1 mm	-	6.2	3.39 cm	
Coarse sand	Medium coarse 500µm – 1 mm- 5.5	-	5.4	3.41 cm	
Fine sand	< 100 – 200 µm			3.39 cm	

Root Exudate Compound and Capillary Rise Measurements

A range of exudate compounds consisting of organic acids or polysaccharides were used as the treatment. Xanthan gum obtained from the bacteria *Xanthomonas campestris* by (sigma – Aldrich Lot MKBQ9467V) served as an analogue to bacterial exudate. Major components of real root exudates were also studied: Malic acid (Alfa Easer Lot 10202834, C₄H₆O₅, Molecular weight 134.09 g/mol, 98%) Oxalic acid (Fisher Scientific UK, Lot 1401325, C₂H₂O₄ with

molecular weight 90.03 g/mol) malonic acid (Alfa - Aesar, Lot 10194472, C₃H₄O₅, 104.06 g/mol, 99%) which are the major components of a real root exudate. (Rohrabacher and St-Arnaud (2016); Hyvärinen, et al, 2006, Luo, et al 2014) Exudates were prepared at 1 mg/g and 5 mg/g. Preliminary investigations were carried out to optimise the configuration of the capillary rise method. The tests were done in an Attension 701 Process Tensiometer, using a capillary rise attachment that included a spring loaded plunger. When too much soil is added, the spring causes greater compaction of the soil so affects pore sizes. If too little soil is added the time to complete wetting is very quick so it is difficult to obtain a reliable measurement. A range of soil weights 2g, 3g, 4g, 5g, 6g, and 7g, were tested for capillary rise of either double deionized water or Ethanol. Soils or sands were poured into the metal capillary rise attachment. There was whatman GF filter paper lining the base and the soil was packed with the spring loaded plunger. After inserting the attachment the process tensiometer, it was lowered at a rate of 10 mm/ minute until Attension Sigma Software detected that it had touched the surface of a liquid reservoir. The rate of the liquid uptake was recorded. The experimental procedure for packing and measurement is in line with the existing one by (Bachmann et al, 2003, Ramirez et al, 2008 and 2010). Hang on the slope and material C values of the wetted sample were recorded, contact angle were calculated using Lucas- Washburn equation Repeat the same steps for ethanol 4 replicates each of the find sand and course for different weight of the soil sample as in the above. A total of 48 replicates were obtained for water and ethanol sorptivity/wettability of the soil samples and 5g weight, 0.5 mm insertion depth and 10 mm / min insertion speed were used. The height before and after packing were measured using vainer caliper, this procedures applies to all the seven soil samples so as to determine the bulk density of each soil. About 3 ml of the prepared solution of malic were collected in the small vessel as the wetting liquid and placed on the probe with the canister hanged on it containing the sample

3. Statistical Analysis

Data were subjected for Normality and homogeneity of variance test on IBM SPSS. Using Two Way Analysis of Variance (ANOVA) to test the impact of biological exudates

compound, sorptivity of water the contact angle relative, wetting rates and water repellency of the soil samples material of natural and artificial of different particles size distribution. To determine the mean differences in relative wettability and sorptivity of the materials, least significant difference was used for the analysis of 112 replicates and (LSD) is significant at $(p < 0.05)$

4. Results

Table 2 the surface tension of exudates at the concentration of 1 mg/g and 5 mg/g

Concentration	Liquid	Mean	Std. Deviation	N
1	Ethanol	21.9997	.02822	10
	Malic	69.2269	3.27897	10
	Malonic	67.4440	2.41190	10
	Oxalic	69.6694	4.00417	10
	Water	71.2796	1.02886	10
	Xanthan	68.0645	3.93199	10
	Total	61.2807	17.96750	60
5	Ethanol	21.9997	.02822	10
	Malic	64.9167	3.36654	10
	Malonic	66.8329	1.36917	10
	Oxalic	65.9713	5.44083	10
	Water	71.2796	1.02886	10
	Xanthan	75.2393	1.84511	10
	Total	61.0399	18.16154	60

Changes in the concentration of biological exudates compound from 1 mg/g to 5 mg/g decreases the surface tension of root exudate compound and increase that of the biological leading to high viscosity as indicated above in (Table.2)

Contact Angle of the Sands and Soils

The Contact Angle of the agricultural soils and the sands varied considerably (Figure 1a and 1b). In agricultural soil, out of the three investigated, Culbin forest was most water repellent whereas, Inch soil was more wetttable. Regardless, the contact angle was always $> 50^{\circ}$ despite some of the soils being sampled from intensively managed agricultural fields. For the sand, both fine sand and Glass Ballotini had a contact angle of zero degrees (CA = 0°). The coarse sand was very water repellent and the silica flour moderately water repellent based on the contact angle (Figure 2b)

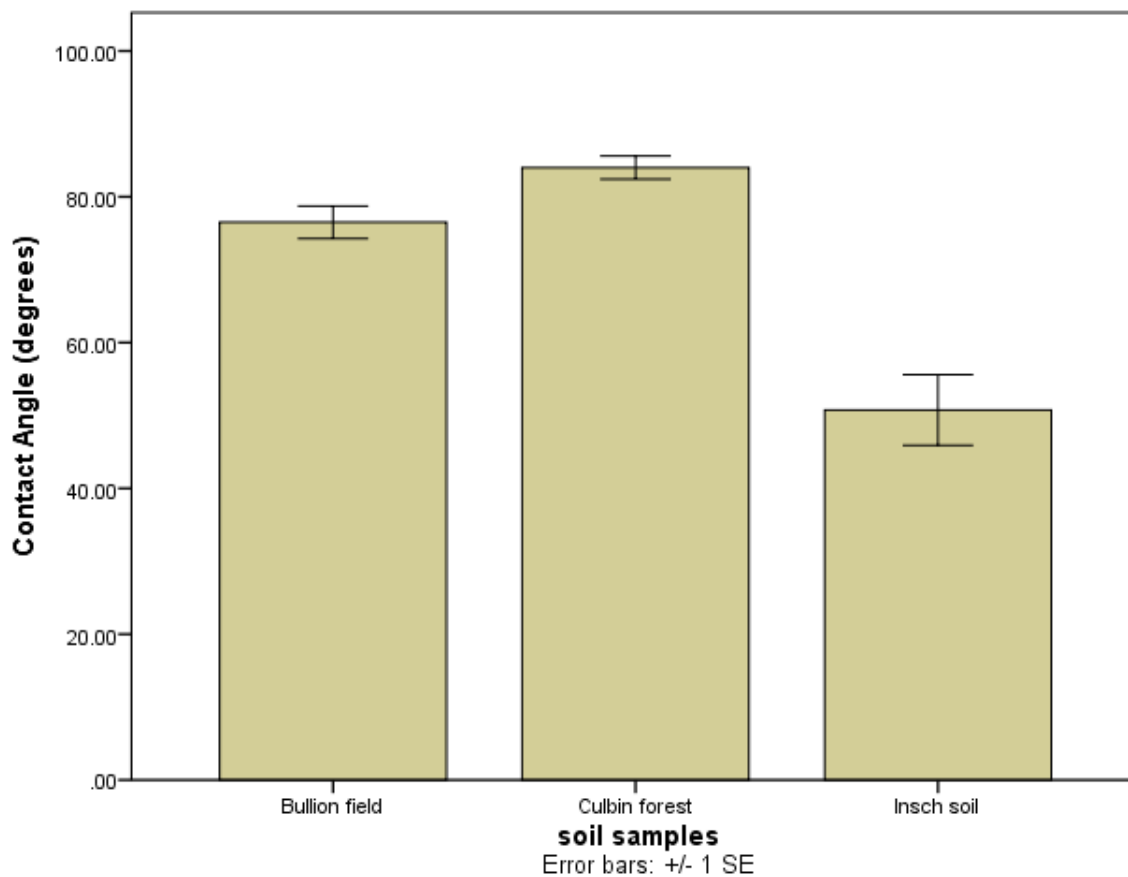


Figure 1 (a): Graphical representation of contact angle of agricultural soil, Culbin forest, Bullion field and Insch soil in water, standard errors in bars.

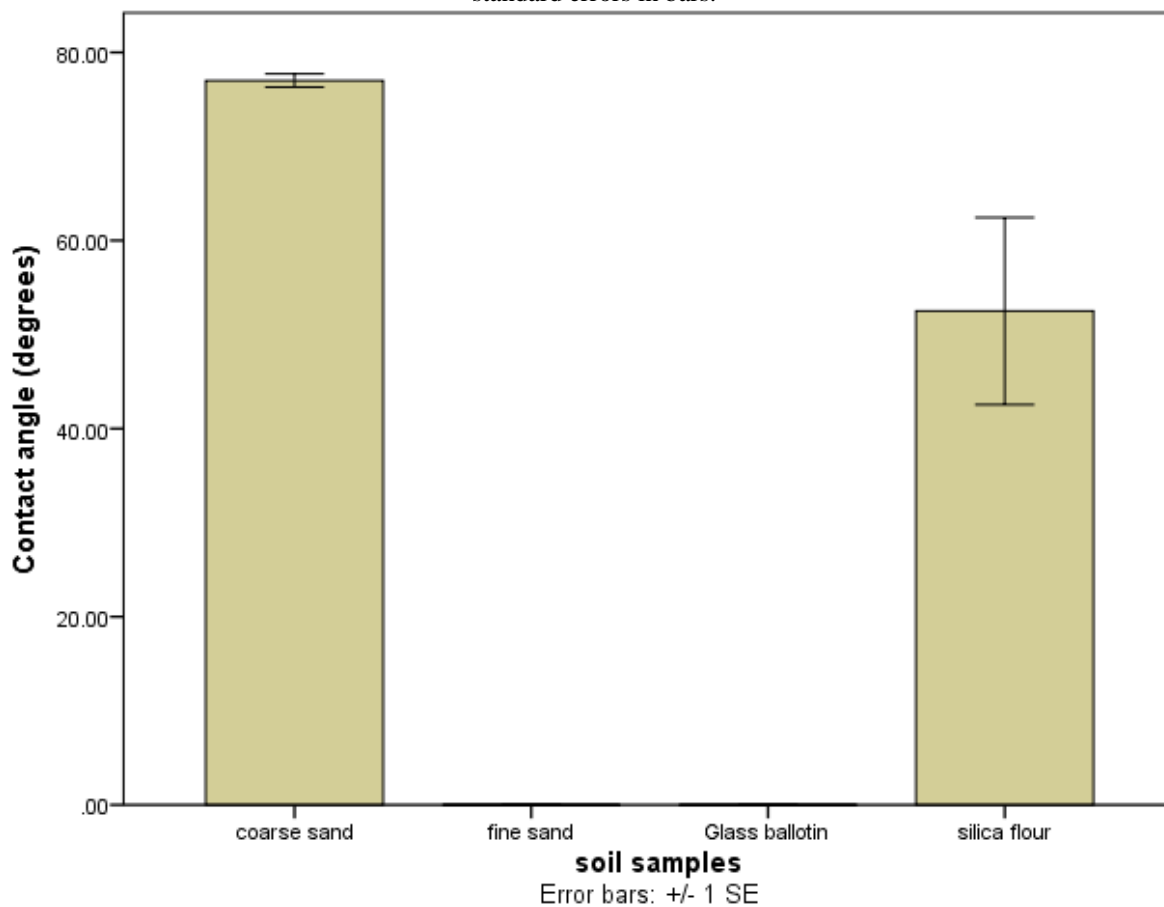


Figure 1(b): Graphical representation of contact angle sand, medium coarse, fine sand, Glass Ballotini and Silica flour in water, bars are the standard errors.

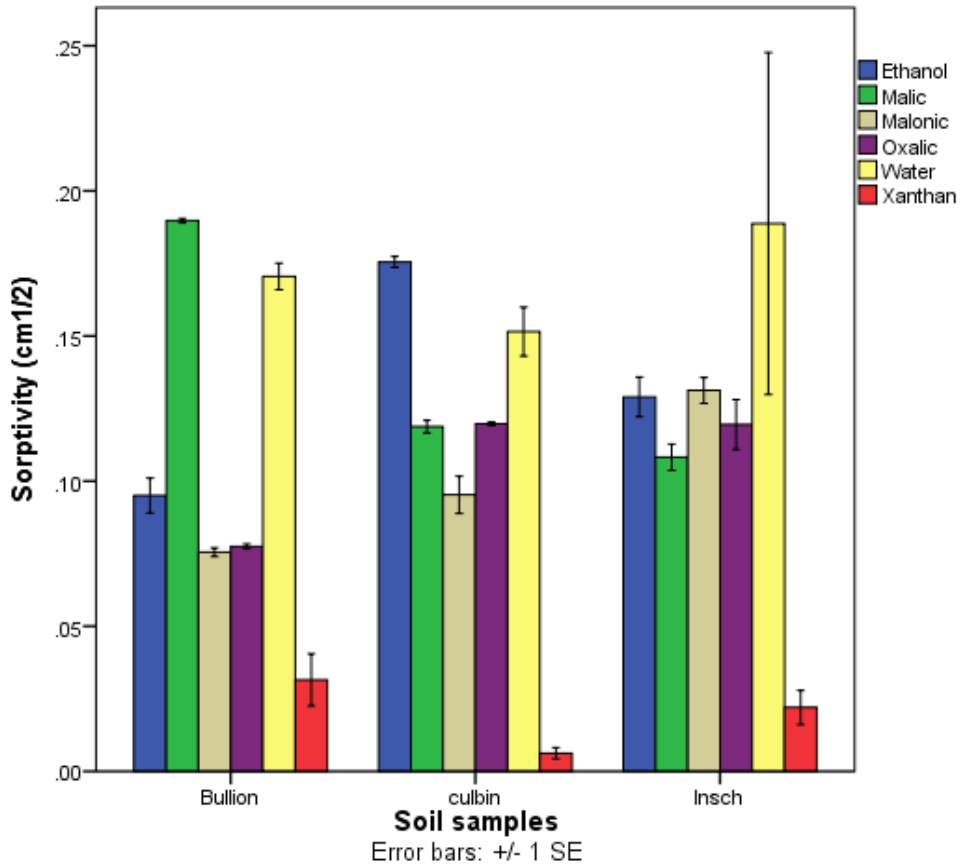


Figure 2 (a): Sorptivity (cm^{-1/2}) of malic, malonic, oxalic and xanthan by Bullion field, Culbin forest and Insch soil

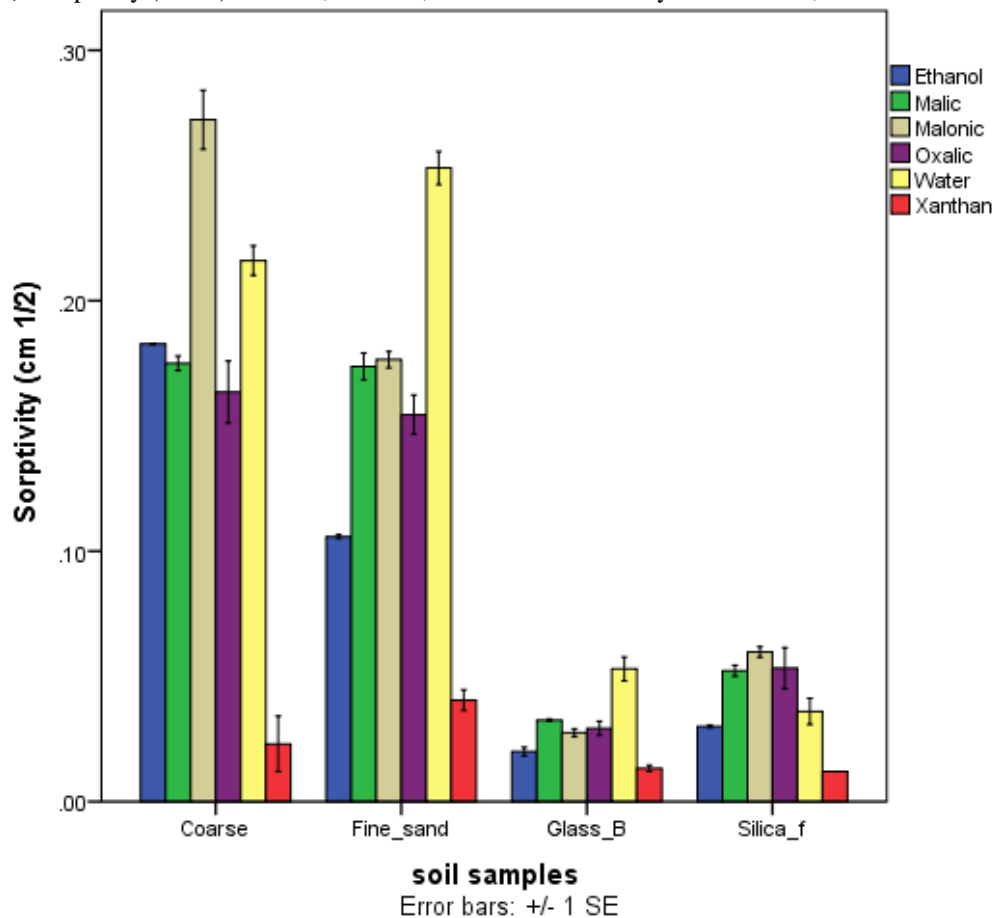


Figure 2(b): sorptivity of malic, malonic, oxalic and xanthan by artificial sand of Coarse, Fine sand, Glass Ballotini and Silica flour

The three agricultural soils studied, Culbin forest which is extremely hydrophobic (figure 1a) decreased sorptivity in water ($P < 0.05$) (figure 2a) except in Inch soil with sandy loam texture similar to Bullion field and Culbin forest, has the largest tendency of sorptivity in water ($P > 0.05$).with ethanol, the evidence of sorptivity was large in Culbin forest while Bullion field and Inch soil has the least impact. Out of the four biological exudate compound used in this experiment, malic acid caused the highest sorptivity in Bullion and Culbin soils. Of the four sands, the moderately water repellent silica flour causes a greater decrease of water sorptivity ($P < 0.05$). Coarse sand with hydrophobic coating also lower the sorptivity in water (Figure 2b), whereas fine sand increased the rate that the soil to absorbed water than the remaining three of the sands. The transport of malonic acid in coarse sand increased drastically as compared to other sand particles with the mean and standard deviation (0.27 and 0.02). There was a large impact in the transport of ethanol in coarse sand compare to other sand particles. The sorptivity of the four biological exudate and the soil types was statistically significant at ($P < 0.05$).

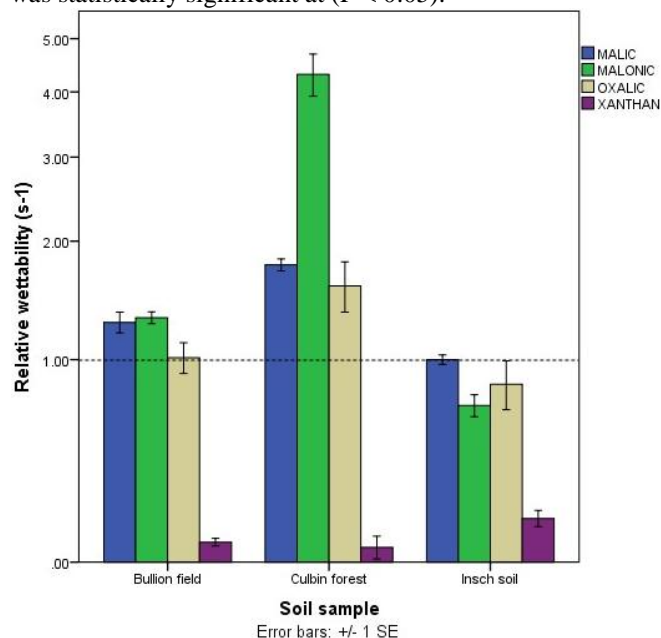


Figure 3 (a): Relative wettability of Soil in 1 mg/g concentration of malic, malonic, oxalic and xanthan

Data were transformed to relative wettability by dividing the wetting rate of the exudates by the wetting rate of water. For the agricultural soils (Figure 3a), the greatest increase in wettability caused by exudates was observed for Culbin forest, which is the most water repellents (extremely hydrophobic). However, Inch soil with the least water repellency has a smallest impact of relative wettability with the exudate. Malonic acid had the greatest impact on the relative wetting of the soils as compared to malic and oxalic acid. Xanthan has the lowest impact for both soils. In Figure 3b the exudates had the largest impact on the initially hydrophobic coarse sand and silica flour. As with the natural soils, malonic acid had the greatest impact. The relative wettability of both the soil types and the biological exudate ($P < 0.05$) is indicated in table below.

Table 4: Analysis of Variances of relative wettability of soil types and biological exudate

Source	df	Mean Square	F	Sig.
Soil_type	6	19.547	15.024	$P < 0.05$
Root	1	61.795	47.496	$P < 0.05$
Soil_type * Root	6	4.368	3.357	0.005
Error	98	1.301		

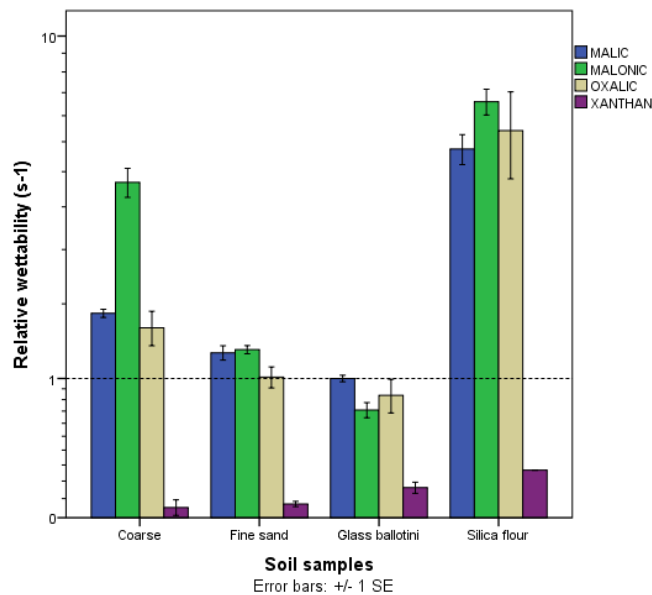


Figure 3b: Graphical representation of the relative wettability (s^{-1}) of the organic acids 1 mg/g concentration in artificial sand of Coarse, Fine, Glass Ballotini and silica flour, the standard errors in bars

5. Discussion

It was found that biological exudate compounds can greatly alter water transport into soils, with the effects being the greatest for the most hydrophobic sands and soils. Liquid movement in porous media can be described using the Washburn equation. This allows for the theoretical impact of surface tension, density and viscosity of infiltrating liquid, and contact angle of the soil or porous media to be calculated. The Washburn equation is:

$$\frac{m^2}{t} = \frac{c \cdot \rho^2 \cdot \sigma \cdot \cos\theta}{\eta}$$

Where m is the Mass of infiltrated liquid; t is the time; σ is the Surface tension of the liquid; c is a Capillary constant describing the structure of the porous media; ρ is the Density of the liquid; θ is the Contact angle; and η is the Viscosity of the liquid. Many of these data were available from our study as listed in Tables 5 and 6.

Table 5: Liquid properties used to parameterize the Washburn Equation. Concentration used was 1 mg/g.

Liquid	Malic	Malonic	Oxalic	Xanthan
σ – surface tension	69.2	67.4	69.7	69.8
ρ – density	1	1	1	0.932*
θ – contact angle	Soils	Soils	Soils	Soils
η – viscosity, mPa s ⁻¹	1	1	?	4.5*

Table 6: Contact Angle of the soils used in solution of the exudate compound

Liquid	Malic	Malonic	Oxalic	Xanthan	Water
C= 1	69.2	67.4	69.7	13.47	72
Coarse Sand	57.054	55.57	57.466	11.108	59.3631
Fine Sand	166.08	161.76	167.28	32.33	172.8
Glass Ballotini	8.3	8.08	8.36	1.62	8.64
Silica Flour	10.83	10.55	10.91	2.11	11.27
Bullion field	5.18	5.04	5.22	1.01	5.39
Culbin forest	13.09	12.76	13.19	2.55	13.62
Insch soil	69.44	67.63	69.94	13.52	72.25

From the Washburn equation, the relative wetting rates (s) of the liquid as compare to water are calculated as follows: 0.96, 0.94, 0.97 and 0.19 for malic, malonic, oxalic and xanthan respectively.

In (Figure 3b) Glass Ballotini with a smooth pore walls and measured contact Angle of zero ($\theta = 0$), the wetting rate is not far off what was measured. As indicated, the calculated wetting rate of Glass Ballotini in solution of the exudates were approximately 0.968 in oxalic, 0.961 in malic and 0.936, 0.187 in malonic and xanthan respectively. This may be as a result of low surface tension of the liquids as compare to water which is in agreement with the study of (Read and Gregory 1997) as stated above. It could also be the function of pores as a large size particle has low sorptivity (Hallett, 2007). while other sands does not follow the same trend due to their large impacts.

For Agricultural soils (Figure 3a), Insch soil relative wetting rate in water was 1 and the calculated wetting rate of Insch in solution of all exudate were 0.961, 0.936 s^{-1} 0.968 s^{-1} and 0.187 s^{-1} for malic, malonic, oxalic and xanthan respectively which are not far off as compare with water infiltration level whereas, Culbin forest and Bullion field are far above water level. This is due to contact angle alteration by the exudate compounds; they act like a surfactant, clogging of the pores by xanthan, the decrease in surface tension of the liquid and as a result of changing the contact angle as the soils wet. (Read and Gregory (1997); Seki. K 2013,

Root exudates have a positive impact of liquid uptake in soils, particularly if they are initially water repellent. As a consequence, this physiological cost to the plant will improve the transport of exudates to soil mineral surfaces where nutrients may be accessed. Moreover, the exudates could ease water uptake. (Carminati et al, 2013) study revealed that exudates cause the rhizosphere to be water repellent, but they can also overcome it.

Differences between soils and exudates

The wettability of both agricultural soils and sands used in this experiment by the root exudate was significant (Figure 3a and b). The extremely water repellent Culbin forest is completely wettable in the solution of the exudate compound. This finding is opposite to the recent study by Ahmed et al, 2016, and Carminati et al 2013, that root exudates causes' water repellency within the rhizosphere especially when they are dried to the soil particle.

Wettability is higher in silica flour as compare to coarse sand (Figure 3b), which is the function of pore size. This study

agrees strongly with Hallett 2008 in the literature review smaller size particle absorbed more liquid than large particle.

Texture and hydrophobicity of the soil

The development of hydrophobicity is influenced by texture of soil, organic matter content and pH (Figure 1b and Table 1). Coarse sand 500 μm – 1 mm is water repellent as compare with silica flour with particle size less than fifty μm , the higher quantity of carbon (100 g) and the low pH (3.9) in Culbin forest is the cause of the high water repellency (Figure 1a). The result of this experiment confirm with the study done by (Lozano et al, 2013) stated that organic matter has impact in decreasing the pH that give rise to hydrophobicity

In figure 1b and 2b show that one of the soils is completely wettable as it assume the contact angle of ($CA = 0$) and the extremely water repellent soil has become hydrophilic when in contact with root exudate. The wettability, is a function of the existence of organic compound in solution of exudates reacting with the substances coating in the dry soil

Viscosity and Surface tension of the liquids

The exudates act as surfactant, organic compound that decreases the surface tension of water (Table 2.) wettability of water repellent soil is enhance by the surfactant when it come in contact with another surfactant in dry soil that resulted in wetting of the soil. (Figure 3a). Wetting in Culbin forest soil is greater with malonic exudate compound. This is so, because of the low surface tension (Table 2) the effect of oxalic in wetting of Bullion field is lower, this might also be the function of surface tension which is higher compare to malonic, and malic. The. Decreased wettability of both agricultural soils and sand in xanthan is associated with higher viscosity (Figure 3a and b) this result agreed with the previous finding of (Walker et al., 2003, Dennis et al, 2010)

The activity of microorganism in soil influence the clogging of pore sand, micro-organism like bacterial, produces substance like xanthan with high viscosity capable of cause water repellency in soil. The decrease in wetting rate of xanthan is an evident of high viscosity (Figure 3a and b) that clog on to the surface of soil leading low wetting. The result is in line to the study of Seki (2013) and the sorptivity of xanthan in all the soils decreases (figure 2a and b) this is as a result of high surface tension of the compound Table 2. The result of this study is in agreement with the previous research of (Carnes et al., 1999) which state that the mechanical properties

6. Conclusions

Using the capillary rise method, this study demonstrates that the root and biological exudate compounds can greatly influence the wetting of soils particularly when the compounds are dried unto the soil. The result of this experiment demonstrated that the solution of the root exudate overcome the water repellency of soil and facilitate transport of liquid. This is also in line to my earlier hypothesis which state that root exudate compounds will counteract water repellency that developed from the deposition of organic compounds.

The hydrological nature of soil is a major drive to many major processes taken place in the system, starting from the flow of nutrient to the microbial functioning which provides nourishment to plant for a better yield. If these are tempered as a result of the existence of organic substances coating the soil particle resulting in water repellency which causes poor infiltration of water leading to a decreased in agricultural produces and amenity turf. Exploration of spatial impacts of exudate, more direct measurement of contact angle of exudates with sessile drop method Tested over a range of water content and Use of real root exudates with and greater study with real plants.

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