



**University of Dundee**

## **Rhizosphere-scale quantification of hydraulic and mechanical properties of soil impacted by root and seed exudates**

Naveed, Muhammad; Brown, Lawrie K.; Raffan, Annette; George, Timothy; Bengough, Anthony; Roose, Tiina; Sinclair, Ian; Koebernick, Nicolai; Cooper, Laura ; Hallett, Paul D.

*Published in:*  
Vadose Zone Journal

*DOI:*  
[10.2136/vzj2017.04.0083](https://doi.org/10.2136/vzj2017.04.0083)

*Publication date:*  
2018

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

### *Citation for published version (APA):*

Naveed, M., Brown, L. K., Raffan, A., George, T., Bengough, A., Roose, T., ... Hallett, P. D. (2018). Rhizosphere-scale quantification of hydraulic and mechanical properties of soil impacted by root and seed exudates. *Vadose Zone Journal*, 17(1). DOI: 10.2136/vzj2017.04.0083

### **General rights**

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Rhizosphere-scale quantification of hydraulic and mechanical properties of**  
2 **soil impacted by root and seed exudates**

3 M. Naveed<sup>a</sup>, L.K. Brown<sup>b</sup>, A.C. Raffan<sup>a</sup>, T.S. George<sup>b</sup>, A.G. Bengough<sup>bc</sup>, T. Roose<sup>d</sup>, I.  
4 Sinclair<sup>d</sup>, N. Koebernick<sup>c</sup>, L. Cooper<sup>c</sup>, and P.D. Hallett<sup>a\*</sup>

5  
6  
7 <sup>a</sup>School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK

8 <sup>b</sup>The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

9 <sup>c</sup>School of Science and Engineering, University of Dundee, Dundee DD1 4HN, UK

10 <sup>d</sup>Faculty of Engineering and Environment, University of Southampton, Southampton, SO17

11 1BJ, UK

12  
13  
14 **\*Contact author**

15 Paul Hallett

16 [paul.hallett@abdn.ac.uk](mailto:paul.hallett@abdn.ac.uk)

17 +441224272264

## 1 **Abstract**

2 Using rhizosphere-scale physical measurements we test the hypothesis that plant exudates gel  
3 together soil particles and on drying they enhance soil water repellency. Barley and maize  
4 root exudates were compared with chia seed exudate, a commonly used root exudate  
5 analogue. Sandy loam and clay loam soils were treated with root exudates at 0.46 and 4.6 mg  
6 exudate g<sup>-1</sup> dry soil, and chia seed exudate at 0.046, 0.46, 0.92, 2.3 and 4.6 mg exudate g<sup>-1</sup>  
7 dry soil. Soil hardness and modulus of elasticity were measured at -10 kPa matric potential  
8 using a 3 mm diameter spherical indenter. Water sorptivity and repellency index of air-dry  
9 soil were measured using a miniaturized infiltrometer device with a 1 mm tip radius. Soil  
10 hardness increased by 28% for barley root exudate, 62% for maize root exudate, and 86% for  
11 chia seed exudate at 4.6 mg g<sup>-1</sup> concentration for sandy loam soil. For a clay loam soil, root  
12 exudates did not affect soil hardness, whereas chia seed exudate increased soil hardness by  
13 48% at 4.6 mg g<sup>-1</sup> concentration. Soil water repellency increased by 48% for chia seed  
14 exudate and 23% for maize root exudate, but not for barley root exudate at 4.6 mg g<sup>-1</sup>  
15 concentration for sandy loam soil. For clay loam soil, chia seed exudate increased water  
16 repellency by 45%, whereas root exudates did not affect water repellency at 4.6 mg g<sup>-1</sup>  
17 concentration. Water sorptivity and repellency were both correlated with hardness,  
18 presumably due to the combined influence of exudates on hydrological and mechanical  
19 properties of soils.

## 20 **Key words**

21 Root exudate, seed exudate, rhizosphere-scale indenter and infiltrometer, soil mechanical  
22 stability, soil water repellency

## 23 **Introduction**

24 Exudates produced by plant roots and microbes continually modify plant-soil interactions  
25 such as root penetration, soil aggregate formation, microbial dynamics, and water and

1 nutrients fluxes from soil to roots (Carminati et al., 2017; Oleghe et al., 2017; Hinsinger,  
2 2009). It has been well documented in a number of species such as sorghum, wheat, and rice  
3 that root exudation decreases with the age of plants and increases with soil stress such as  
4 compaction, drought, and limited nutrient supply (Neumann et al., 2014; Aulakh et al., 2001;  
5 Brady and Weil, 1999). Plant exudates are generally viscoelastic gels consisting of an array  
6 of compounds such as large molecular weight polysaccharides (with both free sugars and  
7 polymerised arabinose, fructose, glucose, maltose, xylose etc.), organic acids (acetic,  
8 gluconic, succinic, valeric acids etc.), amino acids (alanine, glycine, lysine, valine etc.), fatty  
9 acids, and sugar alcohols (Naveed et al., 2017; Aulakh et al., 2010).

10 Plant exudates can have a large influence on soil mechanical stability through  
11 resistance to disruption by mechanical and hydraulic stresses that depend on exudate  
12 chemical characteristics. The anions of organic acids present in root exudates may be  
13 adsorbed by soil mineral particles, thereby increasing the net negative charge of clays that  
14 would cause particles to disperse (Shanmuganathan and Oades 1983). Mucilages and other  
15 polysaccharides (sugars) present in root exudates, which can function as stabilizing materials,  
16 may offset this effect (Oades, 1984). Morel et al., (1991) showed that incorporation of maize  
17 root exudate in soils resulted in an immediate increase in soil aggregate stability, followed by  
18 a decrease over time due to microbial degradation. Traoré et al. (2000) also observed a  
19 significant increase in soil aggregate stability of soil by different substrates i.e.  
20 polgalacturonic acid, modelled soluble exudates, and maize root exudate. Czarnes et al.,  
21 (2000) found that adding polgalacturonic acid and xanthan to soil increased tensile strength  
22 and stability against disruptive effects of wetting and drying cycles. Peng et al. (2011) found  
23 improved aggregate stability for only certain biological exudates they studied, with cycles of  
24 wetting and drying decreasing stabilisation more rapidly in soils with swelling versus non-  
25 swelling clay minerals. Fracture tests on dry soil disks (Czarnes et al., 2000) or notched bars

1 (Zhang et al., 2008) have also quantified increased particle bond energy due to root exudate  
2 compounds. However, most of the above-mentioned studies have either used model root  
3 exudates, extreme test conditions such as air-dry soils, or test techniques such as soil  
4 aggregate stability that do not quantify mechanical processes directly (Hallett et al., 2013).

5         Just as exudates may influence mechanical behaviour, by coating soil particles and  
6 influencing soil water surface tension, they may also influence hydrological behaviour. The  
7 flow of water from soil to plant roots is controlled by the properties of soil in close contact  
8 with roots, known as the rhizosphere. Exudation is believed to strongly influence soil  
9 moisture dynamics in the rhizosphere (Bengough, 2012; Carminati et al., 2010 & 2016).  
10 Currently there are two concepts in the literature regarding hydraulic properties of the  
11 rhizosphere compared to bulk soil. The first is that polymeric gels present in root exudates  
12 increase the water holding capacity of soil on drying, but become significantly water repellent  
13 on re-wetting (Carminati et al., 2016; Ahmed et al., 2015; Moradi et al., 2011, Carminati et  
14 al., 2010). The second case is decreased water holding capacity of the rhizosphere on drying  
15 and more rapid re-wetting due to surfactants in root exudates (Dunbabin et al., 2006; Whalley  
16 et al., 2004; Read et al., 2003, Hallett et al., 2003). Soil water retention and degree of the  
17 hydrophobicity of the rhizosphere may therefore depend on the quantity and type of the root  
18 exudates and also on the drying history of the rhizosphere. Root scale quantification of  
19 hydraulic properties of soils either *in-situ* or using real root exudates of known physico-  
20 chemical characteristics from different plant species are needed to determine the net effect of  
21 the complete cocktail of exudates released by plant roots.

22         For rhizosphere-scale hydrological tests, Hallett et al. (2003) have already used a  
23 miniature infiltrometer to obtain measurements of sorptivity and water repellency. A suitable  
24 rhizosphere-scale mechanical approach could be a miniature spherical indenter, as used by  
25 Kanayama et al. (2012) to measure the micromechanical properties of clay. The approach

1 quantifies the mechanical properties Youngs Modulus of elasticity,  $E$  (stress vs. strain) and  
2 Hardness,  $H$  (related to strength) from the resistance to insertion and contact area of the  
3 sphere. Spherical indenters are not a new approach for soils and were first introduced to  
4 assess the mechanical behaviour of frozen soils in Russia in the 1940s (Zhang et al. 2016).  
5 Indenters come in a range of geometries, including cones similar to soil penetrometers used  
6 to measure mechanical resistance (Bengough 1992). We selected a spherical indenter over a  
7 cone to increase the contact surface area over the shallow depth to which an indenter can be  
8 inserted to accurately measure  $E$  and  $H$ . The sharp tip of a cone would result in considerable  
9 variability in measurements as it concentrates stress over a very small area (Zhang and Li  
10 2014), which in soil could be a few interacting particles.

11 We employed these rhizosphere-scale mechanical and hydrological tests to measure  
12 soil mechanical stability, soil water sorptivity, and water repellency index influenced by  
13 barley (*Hordeum vulgare L. cv. Optic*) and maize (*Zea mays L. cv. Freya*) root exudates. The  
14 impact of barley and maize root exudates were then compared with chia (*Salvia hispanica*)  
15 seed exudate, a commonly used root exudate analogue (with its own biological function and  
16 importance), at a range of concentrations. We advance the earlier research of Hallett et al.  
17 (2003), and other research exploring the water repellency of exudate amended soils (e.g. Peng  
18 et al., 2011), by amending soils with controlled amounts of real root exudates. Moreover,  
19 these data are combined with small-scale mechanical characteristics, providing a robust  
20 assessment of small-scale testing approaches for deployment in direct measurements of  
21 rhizosphere soil. The thrust of this research is to provide the first combined quantitative data  
22 on direct mechanical and hydrological shifts driven by natural plant exudates in soil. This  
23 combined data will improve our understanding of rhizosphere development and function, and  
24 allow for the inter-dependence of mechanical and hydraulic properties of the rhizosphere to  
25 be assessed.

## 1 **Materials and Methods**

### 2 *Collection of exudates*

#### 3 *Collection of barley and maize root exudates*

4 Barley and maize root exudates were collected using an aerated hydroponic method. The  
5 details regarding the method are available in Naveed et al., (2017). In short, barley (*Hordeum*  
6 *vulgare* L. cv. Optic) and maize (*Zea mays* L. cv. Freya) seeds were surface-sterilised in  
7 sodium hypochlorite solution (2%) for 10 min, then rinsed thoroughly in sterile deionised  
8 water. Sterilised seeds were pre-germinated on 0.5% distilled water (DW) agar until the  
9 radicals were approximately 1 cm long (2-3 days post germination). After discarding poorly  
10 germinated seeds, 180 individuals each of barley or maize plants were grown, successively,  
11 in a 60 litre aerated hydroponic tank. Nutrient solutions used in the aerated hydroponic tank  
12 were changed every 3 days beginning with  $\frac{1}{4}$  strength, followed by  $\frac{1}{2}$  strength, and  
13 continuing to full strength until harvest. Plants were harvested after 2 weeks of growth.  
14 Exudates were collected for 12 hours in 150 ml pots containing 75 ml DW with a set amount  
15 of plants per pot (barley = 5 or maize = 3). Plants were removed from the pots the following  
16 morning (12 hour collection period) and the remaining liquid in the collection pots was first  
17 frozen at  $-20^{\circ}\text{C}$  and then freeze-dried for the collection of the dry barley and maize root  
18 exudates. It was observed that the mucilage attached to the roots was dissolved in DW during  
19 the collection period. We have tested using chia seed exudate that freeze-drying followed by  
20 rehydration of the exudates did not influence their physical properties i.e. viscosity and  
21 surface tension (data not shown). The average dry weight of root exudates collected from  
22 individual barley and maize plants was  $4.1 \pm 0.9$  and  $6.4 \pm 1.7$  mg individual<sup>-1</sup>, respectively.

23         Freeze-drying was essential so that the exudates could be concentrated from the dilute  
24 growth solutions, and then rehydrated to local rhizosphere concentrations typical of exudates  
25 from roots in soil (Carminati et al., 2016). The amount of carbon and nitrogen present in

1 freeze-dried barley root exudate, maize root exudate, and chia seed exudate were measured  
2 using a CNS elemental analyser (CE Instruments). The surface tension of exudate solutions at  
3 4.6 mg exudate g<sup>-1</sup> water was measured using force tensiometers-K100 (Krüss) employing  
4 DuNüoy ring.

#### 5 *Extraction of chia (Salvia hispanica) seed exudate*

6 Chia seed exudate has been used in studies as a model root exudate (Ahmed et al., 2014;  
7 Kroener et al., 2014). It was extracted based on Ahmed et al. (2014), by mixing 100 g of  
8 distilled water with 10 g of Chia seeds using a magnetic stirrer for 2 min at 50°C, followed by  
9 cooling to room temperature (20°C) over four hours in sealed containers. The exudate was  
10 separated from the seeds by repeatedly pushing the mixture through a 500 µm sieve under  
11 pressure using a syringe that was cut at the end. This approach harvested the easily extracted  
12 seed exudate, with tightly bound exudate remaining on the seeds even after 5 repeated  
13 extraction attempts. The extracted chia seed exudates were freeze-dried so that its  
14 concentration was obtained i.e. 9.2 mg dry exudate g<sup>-1</sup> water. To obtain the total exudates the  
15 entire hydrated seed was freeze-dried, after which the exudate layer was easy to remove. Of  
16 0.13 ± 0.03 g g<sup>-1</sup> total exudate on seeds, only 0.10 ± 0.02 g g<sup>-1</sup> of seed exudate was harvested,  
17 so the extraction efficiency was 77 ± 8 %.

#### 18 *Selection and preparation of soils*

19 Soils were sampled from Bullion field, The James Hutton Institute, Dundee, UK (56° 27' 39"  
20 N & 3° 04' 11" W) at two locations denoted as "South Bullion" and "North Bullion". The  
21 south Bullion soil is a Dystric Cambisol and was under barley production. North Bullion soil  
22 is a Haplic Cambisol and was under fallow. The bulk soils were sampled from both locations  
23 at 0-100 mm depth. The soils were partially air-dried to 15 g water 100g<sup>-1</sup> dry soil, and then  
24 passed through a 2 mm sieve and stored at 4 °C before any measurements started. The soil  
25 texture was determined by the combination of wet sieving and hydrometer methods. The



1 amount of carbon and nitrogen present in soils were measured using CNS elemental analyser  
2 (CE Instruments). The pH<sub>CaCl<sub>2</sub></sub> of the soils was measured using a pH meter (Hanna  
3 Instruments, Bedfordshire, UK).

4 The sieved soils were mixed with either distilled water (control) or each of the  
5 exudates: barley root exudate, maize root exudate, and chia seed exudate, bringing all to 0.2 g  
6 g<sup>-1</sup> water content. As the soils were at 0.15 g g<sup>-1</sup> water content before amendments were  
7 applied, only 0.05 g g<sup>-1</sup> additional water or exudate solution was added, so potential ionic  
8 impacts of distilled water were assumed to be minimal. Barley and maize root exudates were  
9 amended with 0.46 and 4.6 mg exudate g<sup>-1</sup> dry soil. Chia seed exudate was amended at five  
10 concentrations i.e. 0.046, 0.46, 0.92, 2.3, and 4.6 mg exudate g<sup>-1</sup> dry soil. The amended soils  
11 were packed in triplicate in soil cores of 3 cm diameter and 1 cm height at 1.3 g cm<sup>-3</sup> bulk  
12 density at 0.2 g g<sup>-1</sup> water content. This provided homogeneous samples to test both the effects  
13 of different exudates on hydrological and mechanical properties of soil, as well as evaluate  
14 the direct measurement approaches deployed in this study.

#### 15 *Measurements of soil hardness and elasticity*

16 The packed soil cores were first saturated and then drained to -10 kPa matric potential on a  
17 suction plate (ecoTech, Umwelt-Bsysteme) at 4<sup>0</sup>C to suppress microbial decomposition of the  
18 exudates. One indentation measurement was carried out on each soil core using a 3 mm  
19 diameter spherical indenter. Figure 1 shows a typical load-displacement curve obtained from  
20 a soil indentation test. A corresponding schematic cross section of such an indentation is  
21 depicted in Figure 2. The soil hardness parameter (Oliver and Pharr, 1992) was obtained  
22 using Eq. 1:

$$23 \quad H = \frac{F_{max}}{A_c} \quad (1)$$

1 where  $F_{max}$  is the maximum force applied during an indentation as shown in Figure 1 and  $A_c$   
 2 is the projected contact area at the maximum applied force  $F_{max}$ . The projected contact area  
 3  $A_c$  for a spherical indenter was obtained using Eq. 2:

$$4 \quad A_c = \pi (2h_c R - h_c^2) \quad (2)$$

5 where  $R$  is the radius of a spherical indenter (i.e. 1.5 mm) and  $h_c$  is a contact depth as shown  
 6 in Figure 2. It was obtained using Eq. 3:

$$7 \quad h_c = h_{max} - h_s \quad (3)$$

8 where  $h_{max}$  is the indentation depth and  $h_s$  is the displacement of the surface at the perimeter  
 9 of the contact at the maximum applied force  $F_{max}$  before the unloading. The  $h_s$  was  
 10 determined by Eq. 4:

$$11 \quad h_s = \varepsilon \frac{F_{max}}{S} \quad (4)$$

12 where  $\varepsilon$  is a geometric constant, which for a spherical indenter is theoretically equal to 0.75  
 13 (Fisher and Cripps 2011).  $S$  is the stiffness i.e. the initial slope of the unloading curve as  
 14 shown in Figure 1. It was determined by taking the derivative of  $F$  with respect to  $h$  for the  
 15 initial linear part of the unloading curve. The reduced modulus of elasticity ( $E_r$ ) was obtained  
 16 following Oliver and Pharr (1992) using Eq. 5:

$$17 \quad E_r = \frac{S\sqrt{\pi}}{2\beta\sqrt{A_c}} \quad (5)$$

18 where  $\beta$  is the tip geometry correction factor that is equal to 1 for a spherical indenter. As  
 19 indenter's (steel) modulus of elasticity is much higher compared to soil, the indenter can be  
 20 treated as rigid body and Eq. 6 was used for determining modulus of elasticity.

$$21 \quad E = (1-\nu_s^2)E_r$$

22 where  $\nu_s$  is the Poisson ratio of soil, assumed as 0.30. The  $E$  was reported in addition to  
 23 hardness as soil at -10 kPa matric potential behaves as an elasto-plastic material, as evident  
 24 from the initial elastic followed by plastic unloading of the indenter shown in Figure 1.

25

**Figure 1**

1

**Figure 2**2 *Effect of indentation size on soil hardness and elasticity*

3 The effect of indentation depth on soil hardness and modulus of elasticity was estimated  
4 using a series of loading-unloading cycles with increasing indentation depth as shown in  
5 Figure 3a. The soil hardness and modulus of elasticity for each loading-unloading cycle was  
6 determined and plotted as a function of indentation depth as show in Figure 3b. Generally  
7 there was large variation in soil hardness and modulus of elasticity for an indentation depth  
8 shallower than 0.5 mm (Figure 3b) because of surface roughness of soil cores. In almost all  
9 soil cores soil hardness and modulus of elasticity data became stable for an indentation depth  
10 deeper than 0.5 mm. Therefore the hardness and modulus of elasticity data were averaged at  
11 0.6, 0.7, 0.8, 0.9, and 1.0 mm indentation depths to obtain an ultimate hardness ( $H_u$ ) and  
12 ultimate modulus of elasticity ( $E_u$ ) for each soil core as shown in Figure 3b. The  $H_u$  and  $E_u$   
13 data are reported in rest of the paper.

14

**Figure 3**15 *Measurements of soil hydraulic properties*

16 The soil cores used for indentation measurements were air-dried to measure soil hydraulic  
17 properties. Three different hydraulic properties for each soil core were obtained using a  
18 miniaturized infiltrometer of tip radius 1 mm: (1) water sorptivity, (2) ethanol sorptivity, and  
19 (3) water repellency. The complete details on the experimental setup are available in Hallett  
20 et al., (2003). Water sorptivity is the rate at which soil imbibes water, much like a wetting  
21 sponge. The soil matric potential, pore structure, and hydrophobicity all influence water  
22 sorptivity. Ethanol sorptivity was measured because the nonpolar nature of ethanol and its  
23 contact angle with hydrophobic surfaces provides a reference measurement not influenced by  
24 the hydrophobicity of soil. All measurements were conducted at -10 mm hydraulic head.  
25 Liquid uptake by the soil from the infiltrometer reservoir was logged from the balance at 1 s

1 intervals for 90 s. After about 20 s, the flow rate,  $Q$ , was steady and used to evaluate water  
 2 sorptivity ( $S_W$ ) and ethanol sorptivity ( $S_E$ ) using Eq. 6:

$$3 \quad S_W \text{ or } S_E = \sqrt{\frac{Qf}{4br}} \quad (6)$$

4 The parameter  $b$  depends on the soil-water diffusivity function and it can range from  $0.5 \leq b$   
 5  $\leq \pi/4$  with 0.55 being an average value used here,  $r$  is the radius of the infiltrometer tip of 1  
 6 mm used here, and  $f$  is the fillable air-porosity for the soil cores (Leeds-Harrison et al., 1994).  
 7 Equation 6 assumes infiltration as a function of square root of time, as confirmed from the  
 8 measured data shown in Figure 4.

9 The water repellency index ( $R$ ) was determined from the sorptivity measurements of water  
 10 ( $S_W$ ) and ethanol ( $S_E$ ) at -10 mm pressure head (Tilman et al., 1989). Accounting for  
 11 differences in surface tension and viscosity of the two liquids (water and ethanol), repellency  
 12 index ( $R$ ) was obtained using eq. 7:

$$13 \quad R = 1.95 \frac{S_E}{S_W} \quad (7)$$

14 The larger the value of  $R$  the more water repellent is the soil, with  $R = 1.0$  signifying a totally  
 15 non water repellent soil. The calculation of  $R$  assumes that ethanol wets the soil perfectly  
 16 (contact angle =  $0^\circ$ ) and that different effects of the wetting liquids on the hydrated behaviour  
 17 of the exudates are negligible. In reality, swelling of exudates by water and subsequent  
 18 mixing effects on viscosity, pore clogging and surface tension, may influence the result.  
 19 Ethanol may also dissolve organic compounds, so the interpretation of  $R$  needs to appreciate  
 20 these potential artefacts.

#### 21 **Figure 4**

##### 22 *Statistical Analysis*

23 All the statistical analyses were performed using SigmaPlot 13. To test the significant  
 24 differences in soil hardness, modulus of elasticity, water sorptivity, ethanol sorptivity and

1 repellency index among different exudate-treated soils as well as control soil, one-way  
2 analysis of variance (ANOVA) was carried out. To test the significant difference between the  
3 regressions, analysis of covariance (ANCOVA) was carried out using SigmaPlot 13 at  $p <$   
4 0.05 level. Pairwise comparison of means was carried out using Holm-Sidak method at 95%  
5 confidence level to test the significant difference. Linear, logarithmic or exponential models  
6 were fitted to the measured data to show trends depending upon the fitting efficiency i.e.  $R^2$   
7 value.

## 8 **Results**

9 The carbon and nitrogen contents of the plant exudates used are listed in Table 1. There were  
10 large differences depending on exudate origin, thereby providing a good range of compounds  
11 for further study. The physical properties of the studied soils are given in Table 2. The soil  
12 texture was confirmed as sandy loam for the soil sampled from South Bullion and clay loam  
13 for the soil sampled from North Bullion, with significant difference between soils in carbon  
14 contents ( $p < 0.05$ ).

### 15 **Table 1**

### 16 **Table 2**

17 Soil water content for sandy loam soil at -10 kPa matric potential decreased by 4.6%  
18 for barley root exudate, whereas it increased by 5.25% for maize root exudate and 7.29% for  
19 chia seed exudate at  $4.6 \text{ mg g}^{-1}$  concentration compared to unamended soil. Soil water content  
20 for clay loam soil at -10 kPa matric potential decreased by 2.09% for barley root exudate,  
21 whereas it increased by 2.33% for maize root exudate and 6.52% for chia seed exudate at  $4.6$   
22  $\text{mg g}^{-1}$  concentration (Table 3). Sandy loam soil hardness was not significantly impacted by  
23 barley root exudate at  $0.46 \text{ mg g}^{-1}$  concentration, whereas it at  $4.6 \text{ mg g}^{-1}$  concentration  
24 significantly increased ( $p < 0.05$ ) by 28% compared to control (Fig. 5). Soil hardness was  
25 increased by 20% for maize root exudate at  $0.46 \text{ mg g}^{-1}$  concentration ( $p < 0.05$ ) and 62% at

1 4.6 mg g<sup>-1</sup> concentration ( $p < 0.05$ ) for sandy loam soil (Fig. 5). Clay loam soil showed  
2 significantly greater hardness compared to sandy loam soil. However, this difference was  
3 overcome by the addition of barley root exudate at 4.6 mg g<sup>-1</sup> concentration and maize root  
4 exudate at both 0.46 and 4.6 mg g<sup>-1</sup> concentration. Both barley and maize root exudates at  
5 any of the studied concentrations did not improve soil hardness for clay loam soil (Figure 5).  
6 Soil hardness was sharply increased at lower chia seed exudate concentrations for sandy loam  
7 soil ( $p < 0.05$ ), whereas a more like a linear increase in soil hardness as a function of chia  
8 seed exudate concentration was observed for the clay loam soil ( $p < 0.05$ ). Soil hardness was  
9 significantly increased ( $p < 0.05$ ) by 33% at 0.46, 61% at 0.92, 69% at 2.3, and 86% at 4.6  
10 mg g<sup>-1</sup> chia seed exudate concentrations for sandy loam soil (Fig. 5). Soil hardness was only  
11 significantly increased ( $p < 0.05$ ) by 32% at 2.3 and 60% at 4.6 mg g<sup>-1</sup> chia seed exudate  
12 concentration for the clay loam soil. Similar to soil hardness, modulus of elasticity of sandy  
13 loam and clay loam soils were influenced by barley root exudate, maize root exudate and chia  
14 seed exudate at various concentrations (Figure 5).

15 **Table 3**

16 **Figure 5**

17 On air-dried samples, water sorptivity was significantly decreased ( $p < 0.05$ ) for  
18 sandy loam soil treated with barley root exudate at 0.46 and 4.6 mg g<sup>-1</sup> concentrations. Clay  
19 loam soil has significantly lower water sorptivity compared to sandy loam soil ( $p < 0.05$ ), and  
20 it is not influenced by the barley root exudate (Figure 6). Water sorptivity was significantly  
21 decreased by maize root exudate at 4.6 mg g<sup>-1</sup> concentration for both sandy loam and clay  
22 soils ( $p < 0.05$ ). Water sorptivity was exponentially decayed for both sandy loam and clay  
23 loam soils amended with increasing chia seed exudate concentrations ( $p < 0.05$ ). Water  
24 sorptivity was reduced by 30% for sandy loam soil and 37% for clay loam soil treated with  
25 chia seed exudate at 4.6 mg g<sup>-1</sup> concentration (Figure 6). Ethanol sorptivity was also

1 significantly less for sandy loam soil treated with both barley and maize root exudates at 0.46  
2 and 4.6 mg g<sup>-1</sup> concentrations. Ethanol sorptivity for sandy loam soil was unaffected with  
3 increasing chia seed exudate concentration. Ethanol sorptivity for clay loam soil was  
4 significantly lower compared to sandy loam soil ( $p < 0.05$ ), and it remains unaffected by  
5 treating soils with barley root exudate, maize root exudate and chia seed exudate (Figure 6).  
6 Water repellency for clay loam soil was significantly higher compared to sandy loam soil ( $p$   
7  $< 0.05$ ). Water repellency for both sandy loam and clay loam soils were not influenced by  
8 barley root exudate at 0.46 and 4.6 mg g<sup>-1</sup> concentrations ( $p < 0.05$ ). Water repellency was  
9 significantly greater by 23% for sandy loam soil treated with maize root exudate at 4.6 mg g<sup>-1</sup>  
10 concentration ( $p < 0.05$ ). Treating clay loam soil with maize root exudates did not impact its  
11 water repellency. Water repellency was linearly increased for sandy loam soil treated with  
12 increasing concentrations of chia seed exudate ( $p < 0.05$ ). Water repellency was increased by  
13 48% for sandy loam soil and 46% for clay loam soil treated with chia seed exudate at a  
14 concentration of 4.6 mg g<sup>-1</sup>. Water repellency was increased logarithmically with increasing  
15 chia seed exudate concentration for clay loam soil ( $p < 0.05$ ), and it was significantly higher  
16 compared to sandy loam soil (Figure 6).

### 17 **Figure 6**

## 18 **Discussion**

### 19 *Mechanical properties of soils influenced by plant exudates*

20 Soil mechanical properties, quantified by soil hardness and modulus of elasticity, were  
21 greatly enhanced by barley root exudate, maize root exudate, and chia seed exudate for the  
22 sandy loam soil. The smallest impact was observed for barley root exudate, moderate for  
23 maize root exudate, and greatest for chia seed exudate at a particular exudate concentration in  
24 soil. A possible explanation lies in the physico-chemical characteristics of the exudates  
25 (Naveed et al., 2017). The barley root exudate consists of the greatest amount of organic

1 acids, followed by maize root exudate and chia seed exudate (Naveed et al., 2017). The  
2 largest concentrations of (combined polysaccharide- and free-) sugars were observed in chia  
3 seed exudate, followed by maize and barley root exudate, respectively (Naveed et al., 2017).  
4 The anionic forms of organic acids present in root exudates may be increasing the net  
5 negative charge of clays that would cause particles to disperse, whereas the amount of  
6 polysaccharides (sugars) present in root exudates, which can function as stabilizing materials,  
7 may offset this effect by gelling of soil particles (Shanmuganathan and Oades 1983). This  
8 suggests that barley root exudates can have a net dispersing effect on soil whereas maize and  
9 chia seed exudates have net stabilizing effects.

10 Following this explanation, the enhanced mechanical stability of sandy loam soil upon  
11 the addition of barley root exudate at  $4.6 \text{ mg g}^{-1}$  concentration could be explained by two  
12 factors. The first is increased drainage of the soil at  $-10 \text{ kPa}$  (Table 3) driven by lowering of  
13 surface tension of soil solution by the exudate (Table 4). Secondly, although care was taken  
14 to minimise microbial decomposition of added exudates by storing and equilibrating samples  
15 at  $4^\circ\text{C}$ , some compounds may be metabolised to produce longer-chain compounds. We plan  
16 to study microbial decomposition impacts of plant exudates on soil physical properties in  
17 future research.

18 Viscosity of the exudates tended to be in the order barley root exudate < maize root  
19 exudate < chia seed exudate, reflecting their gelling potential with individual soil particles  
20 (Table 4). The greatest viscosity of the chia seed exudate thus resulted in the greatest  
21 mechanical stability of the soil, followed by maize root exudate and barley root exudate. Our  
22 results are in agreement with studies exploring impacts of maize root exudate on soil  
23 aggregate stability (Morel et al., 1991; Traoré et al. 2000). Similarly, most of the studies  
24 available in the literature have reported improved mechanical stability of soil amended with  
25 model root and microbial exudates such as polygalacturonic acid, xanthan, scleroglucan, and



1 *Capsella sp.* seed exudate (Traoré et al. 2000; Czarnes et al., 2000; Barré and Hallett, 2009;  
2 Peng et al., 2011; Deng et al., 2015). It should be noted that model root exudates, such as chia  
3 seed exudate, used in the present study, showed an exaggerated effect on soil mechanical  
4 properties compared to real root exudates from barley and maize roots. Further, it was also  
5 evident from our findings that the impact of exudation on soil mechanical stability depends  
6 on initial soil properties, such as texture. An insignificant effect of both barley and maize root  
7 exudation on soil hardness and modulus of elasticity for clay loam soil (Fig. 5) was most  
8 likely driven by the greater impact of clay minerals and possibly soil organic matter on  
9 interparticle bonding compared to the sandy loam soil. If soil was already in a stable  
10 condition, such as clay loam soil in the present study (Fig. 5), plant root exudation would be  
11 expected to have less of an impact on its mechanical stability.

#### 12 **Table 4**

##### 13 *Hydraulic properties of soils influenced by plant exudates*

14 Both water and ethanol sorptivities for sandy loam soil significantly decreased with  
15 increasing barley root exudate concentration (Fig. 6). Water repellency was not significantly  
16 different from the control (Fig. 6), suggesting a marked difference in pore structure drove  
17 decreased sorptivities. This difference in pore structure might originate from either pore  
18 clogging due to exudates swelling during measurements or different levels of shrinkage  
19 between control and exudate treated soil cores from air-drying. If it is assumed that all of the  
20 exudate remains in the pore water, then at 4.6 mg g<sup>-1</sup> barley exudate concentration, it has a  
21 surface tension of 45.59 mN m<sup>-1</sup> (Table 4). Sorption of exudates to soil surfaces may decrease  
22 exudate concentration in solution and therefore increase surface tension, but even at smaller  
23 concentrations, surface tension would be less than for water (Read and Gregory, 1997). This  
24 lower surface tension of soil solution as a result of barley root exudation might overcome soil  
25 water repellency by acting as a surfactant. Strong negative correlations were observed

1 between surface tension of barley root, maize root and chia seed exudates solutions and water  
2 sorptivity of exudates treated soils i.e. sandy loam ( $R^2 = 0.97$ ) and clay loam ( $R^2 = 0.99$ ).  
3 With maize root and chia seed exudates at  $4.6 \text{ mg g}^{-1}$  concentration the marked decrease in  
4 water sorptivity and greater repellency index (Fig. 6) for sandy loam soil was probably due to  
5 exudates creating a hydrophobic coating on soil particles. Moreover, the greater viscosity of  
6 these exudate solutions at  $4.6 \text{ mg g}^{-1}$  compared to barley root exudate, may affect water  
7 infiltration by retarding capillary flow and potentially clogging pores (Table 4). Similar to  
8 surface tension, strong negative correlations were observed between viscosity of the exudates  
9 solutions and water sorptivity of exudates treated soils i.e. sandy loam ( $R^2 = 0.97$ ) and clay  
10 loam ( $R^2 = 0.90$ ). Ethanol sorptivity was significantly lower for sandy loam soil treated with  
11 barley and maize root exudates but no effect of chia seed exudate was observed (Fig. 6). This  
12 suggests a different interaction of ethanol with different types of exudates, which could be  
13 explored further by quantifying time-dependent dissolution, swelling and viscous clogging by  
14 exudates as affected by either water or ethanol. As the hydraulic measurements were  
15 conducted on air-dried soils, greater sorption of exudates onto soil surfaces may have  
16 exacerbated the impacts of exudates compared to the mechanical tests that were done at  $-10$   
17 kPa water potential. It is feasible to use the same miniature infiltrometer setup at wetter water  
18 contents.

19 Our findings for barley root exudate treated soils agree with *in-situ* measurements of  
20 the barley rhizosphere by Hallett et al., (2003), who found only a slight impact. The maize  
21 root exudate hydraulic measurements follow trends reported by Ahmed et al. (2015), that the  
22 rhizosphere of maize stayed temporarily dry after irrigation. Both this study and Carminati et  
23 al.'s (2010) investigation of the lupin rhizosphere observed the development of water  
24 repellency when the rhizosphere dries beyond a critical threshold, which was attributed to  
25 root exudates. Whilst maize root exudates influenced the development of water repellency in

1 the sandy loam soils, for the clay loam soil the greater initial water repellency was likely  
2 driven by past soil management accumulating organic matter (Fig. 6). However, chia seed  
3 exudate, that is strongly hydrophobic in nature, significantly increased soil water repellency  
4 with increasing concentration for both sandy loam and clay loam soils.

#### 5 *Interaction between mechanical and hydraulic properties of soils*

6 Significant negative correlations were observed between water sorptivity and soil hardness for  
7 both sandy loam (Fig. 7a) and clay loam (Fig. 7b) soils treated with barley root, maize root  
8 and chia seed exudates. This suggests that the coating of soil particles by exudates increases  
9 interparticle adhesion and decrease water transport through either pore clogging or water  
10 repellency. Pore clogging was less likely as ethanol sorptivity was not correlated with soil  
11 hardness for either the sandy loam (Fig. 7c) or clay loam (Fig. 7d) soils. The non-polar nature  
12 of ethanol and its contact angle with hydrophobic surfaces provides a transport measurement  
13 not influenced by repellency. Biases due to differential swelling or dissolution of exudates by  
14 ethanol compared to water (Hallett et al., 2003) limit the reliability of this interpretation but it  
15 is supported by water repellency measurements. There was a significant positive correlation  
16 between the water repellency index and soil hardness for both sandy loam (Fig. 7e) and clay  
17 loam (Fig. 7f) soils treated with barley root, maize root and chia seed exudates. This revealed  
18 the dual effect of exudates; (1) coating of soil particles to form water repellent surfaces and  
19 (2) binding soil particles and thus making soil more stable.

20

#### **Figure 7**

#### 21 *Rhizosphere-scale mechanical and hydrological tests*

22 The rhizosphere-scale quantification techniques successfully measured the impact of plant  
23 exudates on soil mechanical properties and water transport. This could open up future  
24 research on direct *in-situ* measurements of mechanical and hydraulic properties in the  
25 rhizosphere of different plant species. Hallett et al. (2003) have already discussed

1 rhizosphere-scale hydrological measurements, including limitations to the approach caused  
2 by experimental artefacts such as soil contact and the development of erratic wetting bulbs  
3 over such a small area. Nevertheless, with adequate replication, both Hallett et al. (2003) and  
4 our current study, found realistic sorptivity values and elucidated the effects of plant  
5 exudates.

6         The spherical indenter also obtained realistic mechanical properties of the soil. In a  
7 different study (unpublished) we found from unconfined compression tests of the sandy loam  
8 soil tested at the same water potential that modulus of elasticity ranged from 1.1 to 1.9 MPa  
9 and failure stress ranged from 37 to 73 kPa depending on packing stress. . There is a scope to  
10 enhance the indentation approach to estimate fracture mechanics properties of the soil (Chen  
11 et al. 2016), which would be a useful technique given traditional approaches with notched  
12 bars require remoulded soil and have fragile samples that are difficult to handle (Yoshida and  
13 Hallett 2008).

#### 14 **Conclusions**

15 Using rhizosphere-scale tests, we provide strong evidence that exudates, depending upon  
16 their origin, have differing impacts on water transport and mechanical stability of the  
17 rhizosphere. Soil water repellency was measured at air-dry condition and soil mechanical  
18 stability was measured at -10 kPa matric potential. Barley root exudate did not significantly  
19 affect soil water repellency. The slight increase in water repellency by maize root exudates  
20 probably has little influence on the ability of plants to extract water from soil, however it  
21 depends on soil type and initial soil water content. A more important impact, observed for the  
22 sandy loam soil studied, is the capacity of barley, maize and chia exudates to increase  
23 rhizosphere mechanical stability. Our clay loam results showed less of an impact, which was  
24 possibly due to the greater inherent hardness and elasticity without added exudates.

1           Increased mechanical stability will drive the physical stabilisation and aggregation of  
2 rhizosphere soil. For the same amount of exudation, barley root exudate had less of an impact  
3 than maize root exudate or chia seed exudate, associated with differing chemical  
4 compositions. The variability of exudate chemistry and its impact on physical stabilisation  
5 between crop genotypes would be interesting to explore as a possible tool to select root traits  
6 that diminish the negative impacts of intensive farming on soils.

7           The measured data will assist in the development of models on rhizosphere physical  
8 formation and on water/nutrient transport from soil to plant roots. Model root exudates, such  
9 as chia seed exudate in the present study, can exaggerate the effects of real root exudates on  
10 soils, so care must be taken in extrapolating results from one exudate type or species to  
11 another. The use of root exudates collected using the aerated hydroponic method in the  
12 present study is a significant improvement on the use of model root exudate compounds. We  
13 appreciate that this approach may produce root exudates with different composition than  
14 would be produced in a soil environment. Root exudates from seedlings may also have  
15 characteristics that differ from older plants. The next step in our research is to apply the  
16 rhizosphere-scale hydrological and mechanical measurements in-situ along the length of plant  
17 roots in soil, to directly quantify the impacts of root age, root traits such as root hairs, and soil  
18 conditions.

## 1 **Acknowledgments**

2 We thank George Themistocleus who conducted preliminary research on the indentation  
3 technique as part of his MSc project. This work was funded by the Biotechnology and  
4 Biological Sciences Research Council (BBSRC) project ‘Rhizosphere by Design’  
5 (BB/L026058/1, BB/J000868/1 and BB/J011460/1) with support from a Royal Society  
6 University Research Fellowship, EPSRC EP/M020355/1 and ERC Consolidator grant DIMR  
7 646809. The James Hutton Institute receives funding from the Scottish Government.

8

## 1 **References**

- 2 Ahmed, M.A., E. Kroener, P. Benard, M. Zarebanadkouki, A. Kaestner, and A. Carminati.  
3 2015. Drying of mucilage causes water repellency in the rhizosphere of maize: measurements  
4 and modelling. *Plant Soil* 407:161-171. doi: 10.1007/s11104-015-2749-1.
- 5 Ahmed, M.A., E. Kroener, M. Holz, M. Zarebanadkouki, and A. Carminati. 2014. Mucilage  
6 exudation facilitates root water uptake in dry soil. *Functional Plant Biology* 41:1129-1137.  
7 <http://dx.doi.org/10.1071/FP13330>.
- 8 Aulakh, M.S., R. Wassmann, C. Bueno, J. Kreuzwieser, and H. Rennenberg. 2001.  
9 Characterisation of root exudate at different growth stages of ten rice (*Oryza sativa* L.)  
10 cultivars. *Plant biology* 3:139-148. doi: 10.1055/s-2001-12905.
- 11 Barré, P., and P.D. Hallett. 2009. Rheological stabilization of wet soils by model root and  
12 fungal exudates depends on clay mineralogy. *European Journal of Soil Science* 60:525-538.  
13 doi: 10.1111/j.1365-2389.2009.01151.x.
- 14 Bengough, A. G. 1992. Penetrometer resistance equation - its derivation and the effect of soil  
15 adhesion. *J. Agr. Engng. Res.* 53:163-68. [https://doi.org/10.1016/0021-8634\(92\)80080-C](https://doi.org/10.1016/0021-8634(92)80080-C).
- 16 Brady, N.C., and R.R. Weil. 1999. *The Nature and Properties of Soils*, 12th ed. Prentice Hall  
17 Inc., New Jersey.
- 18 Carminati, A., M. Zarebanadkouki, and E. Kroener et al. 2016. Biophysical rhizosphere  
19 processes affecting root water uptake. *Ann. Bot.* 118:561-571. doi: 10.1093/aob/mew113.
- 20 Carminati A., A.B. Moradi, and D. Vetterlein et al. 2010. Dynamics of soil water content in  
21 the rhizosphere. *Plant Soil* 332:163–176. doi:10.1007/s11104-010-0283-8.
- 22 Chen, Zhangwei, Xin Wang, Alan Atkinson, and Nigel Brandon. 2016. Spherical indentation  
23 of porous ceramics: Cracking and toughness. *Journal of the European Ceramic Society* 36:  
24 3473-80. doi:10.1016/j.jeurceramsoc.2016.05.010.

25

- 1 Czarnes, S., A.R. Dexter, and F. Bartoli. 2000. Wetting and drying cycles in the maize  
2 rhizosphere under controlled conditions. *Mechanics of the root-adhering soil. Plant and Soil*  
3 221:253-271. doi:10.1023/A:1004747323220.
- 4 Deng, W., P.D. Hallett, D.-S. Jeng, G.R. Squire, P.E. Toorop, and P.P.M. Iannetta. 2015. The  
5 effect of natural seed coatings of *Capsella bursa-pastoris* L. Medik. (shepherd's purse) on  
6 soil-water retention, stability and hydraulic conductivity. *Plant and Soil* 387:167-176. doi:  
7 10.1007/s11104-014-2281-8.
- 8 Dunbabin, V.M., S. McDermott, and A.G. Bengough. 2006. Upscaling from rhizosphere to  
9 whole root system: modelling the effects of phospholipid surfactants on water and nutrient  
10 uptake. *Plant and Soil* 283:57-72. doi:10.1007/s11104-005-0866-y.
- 11 Fischer-Cripps, A.C. 2011. *Nano indentation*, Springer, New York, NY, USA, 3rd edition.
- 12 Hallett, P.D., K.H. Karim, A.G. Bengough, and W. Otten. 2013. Biophysics of the vadose  
13 zone: From reality to Model systems and back again. *Vadose Zone Journal* 12:1-17. doi:  
14 10.2136/vzj2013.05.0090.
- 15 Hallett, P.D., D.C. Gordon, and A.G. Bengough. 2003. Plant influence on rhizosphere  
16 hydraulic properties: direct measurements using a miniaturized infiltrometer. *New*  
17 *Phytologist* 157:597-603. doi:10.1046/j.1469-8137.2003.00690.x.
- 18 Hinsinger, P., A.G. Bengough, D. Vetterlein, and I.M. Young. 2009. Rhizosphere:  
19 biophysics, biogeochemistry and ecological relevance. *Plant and Soil* 321:117-152. doi:  
20 10.1007/s11104-008-9885-9.
- 21 Kanayama, M., M. Ohtsubo, T. Higashi, M. Tanaka, and K. Soga. 2012. Evaluation of  
22 Microscopic Behaviour for Clay by Using Micro-indenter. *Journal of the Faculty of*  
23 *Agriculture Kyushu University*, 57: 189-94.



- 1 Kroener, E., M. Zarebanadkouki, A. Kaestner, and A. Carminati. 2014. Nonequilibrium water  
2 dynamics in the rhizosphere: How mucilage affects water flow in soils. *Water Resour. Res.*  
3 50:6479–6495. doi:10.1002/2013WR014756.
- 4 Leeds-Harrison P.B., E.G. Youngs, and B. Uddin. 1994. A device for determining the  
5 sorptivity of soil aggregates. *Eur. J. of Soil Sci.* 45:269–272. doi:10.1111/j.1365-  
6 2389.1994.tb00509.x.
- 7 Moradi AB, A. Carminati, and D. Vetterlein et al. 2011. Three-dimensional visualization and  
8 quantification of water content in the rhizosphere. *New Phytol.* 192:653–663. doi:609  
9 10.1111/j.1469-8137.2011.03826.x.
- 10 Morel, J.L., L. Habib, S. Plantureux, and A. Guckert. 1991. Influence of maize root mucilage  
11 on soil aggregate stability. *Plant and Soil* 136:111-119. doi:10.1007/BF02465226.
- 12 Naderi-Boldaji, Mojtaba, Reza Alimardani, Abbas Hemmat, Ahmad Sharifi, Alireza  
13 Keyhani, Mehari Z. Tekeste, and Thomas Keller. 2013. 3D finite element simulation of a  
14 single-tip horizontal penetrometer–soil interaction. Part I: Development of the model and  
15 evaluation of the model parameters. *Soil and Tillage Research*, 134:153-62.  
16 <http://doi.org/10.1016/j.still.2013.08.002>.
- 17 Naveed, M., L.K. Brown, A.C. Raffan, T.S. George, A.G. Bengough, T. Roose, I. Sinclair, N.  
18 Koebernick, L. Cooper, and P.D. Hallett. 2017. Plant exudates can either stabilise or weaken  
19 soils depending on species, origin and time. *Eur. J. Soil Sci.*, doi:10.1111/ejss.12487.
- 20 Neumann, G., S. Bott, M.A. Ohler, H.-P. Mock, R. Lippmann, R. Grosch, and K. Smalla  
21 2014. Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as  
22 affected by different soils. *Frontiers in Microbiology* 5:1-6. doi:10.3389/fmicb.2014.00002.
- 23 Oades, J.M. 1984. Soil organic matter and structural stability; mechanisms and implication  
24 for management. *Plant and Soil*, 76:319-337. doi:10.1007/BF02205590.

- 1 Oleghe, E. M. Naveed, E.M. Baggs, and P.D. Hallett. 2017. Plant exudates improve the  
2 mechanical conditions for root penetration through compacted soils. *Plant and Soil*,  
3 <https://doi.org/10.1007/s11104-017-3424-5>.
- 4 Oliver W.C. and G.M. Pharr. 1992. Improved technique for determining hardness and elastic  
5 modulus using load and displacement sensing indentation experiments. *Journal of Materials*  
6 *Research* 7:1564–1583.
- 7 Peng, X., P.D. Hallett, B. Zhang, and R. Horn. 2011. Physical response of rigid and non-rigid  
8 soils to analogues of biological exudates. *Eur. J. of Soil Sci.* 62:676-684. doi:10.1111/j.1365-  
9 2389.2011.01383.x.
- 10 Read, D.B., A.G. Bengough, and P.J. Gregory et al. 2003. Plant roots release phospholipid  
11 surfactants that modify the physical and chemical properties of soil. *New Phytologist*  
12 157:315–326. doi:10.1046/j.1469-8137.2003.00665.x.
- 13 Read, D. B. & Gregory, P. J. 1997. Surface tension and viscosity of axenic maize and lupin  
14 root mucilages. *New Phytologist*, 137, 623-628.
- 15 Shanmuganathan, R.T., and J.M. Oades. 1983. Modification of soil physical properties by  
16 addition of calcium compounds. *Aus. J. of Soil Res.* 21:285-300. doi:10.1071/SR9830285.
- 17 Traoré, O., V. Groleau-Renaud, S. Plantureux, A. Tubeileh, and V. Bceuf-Tremblay. 2000.  
18 Effect of root mucilage and modelled root exudates on soil structure. *Eur. J. of Soil Sci.*  
19 51:575–581. doi:10.1111/j.1365-2389.2000.00348.x.
- 20 Tilman R.W., D.R. Scotter, M.G. Wallis, and B.E. Clothier. 1989. Water-repellency and its  
21 measurement by using intrinsic sorptivity. *Aus. J. of Soil Res.* 27:637–644. doi:  
22 10.1071/SR9890637.
- 23 Whalley, W.R., B. Riseley, P.B. Leeds-Harrison, N.R.A. Bird, P.K. Leech, and W.P.  
24 Adderley 2005. Structural differences between bulk and rhizosphere soil. *Eur. J. of Soil Sci.*  
25 56:353–360. doi:10.1111/j.1365-2389.2004.00670.x.

- 1 Yoshida, S., and P. D. Hallett. 2008. Impact of hydraulic suction history on crack growth  
2 mechanics in soil. *Water Res. Res.* 44:1-9. doi:10.1029/2007WR006055.
- 3 Zhang, B., P.D. Hallett, and G. Zhang. 2008. Increase in the fracture toughness and bond  
4 energy of clay by a root exudate. *Eur. J. Soil Sci.* 55:855-862. doi:10.1111/j.1365-  
5 2389.2008.01045.x.
- 6 Zhang, L. L., and X. Y. Li. 2014. A Numerical study of indentation elastic-plastic behaviors  
7 based on indenters of different geometry. *Int. Conf. on Mech. and Mat. Engg. (Icmme 2014):*  
8 317-23.
- 9 Zhang, Z., H. Zhou, W. Feng, Z. Zhang, and D. Xiao. 2016. A spherical template indenter for  
10 a frozen soil long-term shear strength test. *Cold Regions Sci. and Tech.*, 131:10-15. doi:  
11 10.1016/j.coldregions.2016.07.011.
- 12

## 1 **Figures Captions**

2 **Figure 1:** Typical load-displacement curve obtained from an indentation test,  $F_{\max}$  =  
3 maximum measured force for the required indentation,  $h_e$  = elastic indentation i.e.  
4 deformation recovered on unloading,  $h_p$  = plastic indentation i.e. permanent deformation and  
5 not recovered on unloading and  $S$  = stiffness i.e. slope of the initial linear part of the  
6 unloading curve.

7 **Figure 2:** A schematic of an indentation test,  $h_e$  = elastic indentation i.e. deformation  
8 recovered on unloading,  $h_p$  = plastic indentation i.e. permanent deformation and not  
9 recovered on unloading,  $h_c$  = contact depth at maximum load,  $h_s$  = displacement of the  
10 surface at the perimeter of the contact.

11 **Figure 3:** (a) 10 loading-unloading cycles adopted during an indentation test and (b)  
12 corresponding soil hardness and modulus of elasticity were plotted as a function of  
13 indentation depth,  $H_u$  and  $E_u$  are average hardness and elasticity for 0.6, 0.7, 0.8, 0.9, and 1.0  
14 mm indentation depths.

15 **Figure 4:** An average water infiltration of three soil cores was plotted as a function of square  
16 root of time for unamended soil, barley root, maize root, and chia seed exudates treated soils  
17 at a concentration of 4.6 mg exudate  $\text{g}^{-1}$  dry soil.

18 **Figure 5:** Hardness and modulus of elasticity of sandy loam and clay loam soils treated with  
19 barley root, maize root, and chia seed exudates at different concentrations in mg exudate  $\text{g}^{-1}$   
20 dry soil measured at -10 kPa matric potential; different letters indicate significant difference  
21 between treatments at  $p < 0.05$ , regressions were significant at  $p < 0.05$ , and regressions  
22 between sandy loam and clay loam soils were not found significantly different.

23 **Figure 6:** Water sorptivity, ethanol sorptivity, and water repellency of sandy loam and clay  
24 loam soils treated with barley root, maize root, and chia seed exudates at different  
25 concentrations in mg exudate  $\text{g}^{-1}$  dry soil measured at air dry condition; different letters

1 indicate significant difference between treatments at  $p < 0.05$ , regressions were significant at  
2  $p < 0.05$ , and regressions between sandy loam and clay loam soils were found significantly  
3 different.

4 **Figure 7:** Water sorptivity, ethanol sorptivity, and repellency index were plotted as a  
5 function of soil hardness for sandy loam and clay loam soils treated with water (circle),  
6 barley root exudate (square), maize root exudate (triangle) and chia seed exudate (diamond).

7

1 Table 1: Carbon, nitrogen, and carbon nitrogen ratios for barley root, maize root, and chia  
 2 seed exudates.

Exudates	Carbon	Nitrogen	Carbon/Nitrogen
–	(g 100g <sup>-1</sup> )	–	–
chia seed	40.77 ± 0.14	1.09 ± 0.01	37.44 ± 0.52
barley root	14.89 ± 0.32	6.15 ± 0.08	2.42 ± 0.04
maize root	16.62 ± 0.79	3.25 ± 0.32	5.23 ± 0.63

3

4

5

6

7

8

9

10 Table 2: Physical properties of the studied soils.

Soils	Clay	Silt	Sand	Carbon	Nitrogen	Soil pH_CaCl <sub>2</sub>	Texture class
				(g. 100g <sup>-1</sup> )			
South Bullion	16	24	60	2.25 ± 0.14	0.16 ± 0.03	5.48 ± 0.07	Sandy loam
North Bullion	26	30	44	2.95 ± 0.12	0.23 ± 0.02	5.15 ± 0.04	Clay Loam

11

12

13

14

15

16

17

- 1 Table 3: Volumetric water contents at -10 kPa matric potential for soils treated with different  
 2 exudates (concentration is in mg exudate g<sup>-1</sup> dry soil).

Soil	Exudate treatment	Concentration (mg g <sup>-1</sup> )	Volumetric water content (m <sup>3</sup> m <sup>-3</sup> )
sandy loam	control	0	0.343 ± 0.010
		0.046	0.345 ± 0.014
	chia seed	0.46	0.351 ± 0.012
		0.92	0.361 ± 0.004
		2.3	0.364 ± 0.011
		4.6	0.368 ± 0.001
		0.46	0.327 ± 0.002
	barley root	4.6	0.323 ± 0.013
		0.46	0.341 ± 0.009
	maize root	4.6	0.361 ± 0.009
		control	0
	clay loam	control	0.046
0.46			0.451 ± 0.001
chia seed		0.92	0.452 ± 0.001
		2.3	0.461 ± 0.006
		4.6	0.457 ± 0.007
		0.46	0.420 ± 0.009
		barley root	4.6
0.46			0.443 ± 0.018
maize root		4.6	0.439 ± 0.027

3

4

5

- 6 Table 4: Surface tension and apparent viscosity at zero-shear rate of exudates solution at a  
 7 concentration of 4.6 mg exudate g<sup>-1</sup> water.

Exudates solution	Surface tension (mN m <sup>-1</sup> )	Zero shear viscosity (Pa.s)
chia seed	59.30 ± 0.89	95.1
maize root	49.90 ± 0.26	0.85
barley root	45.59 ± 0.73	0.50

8

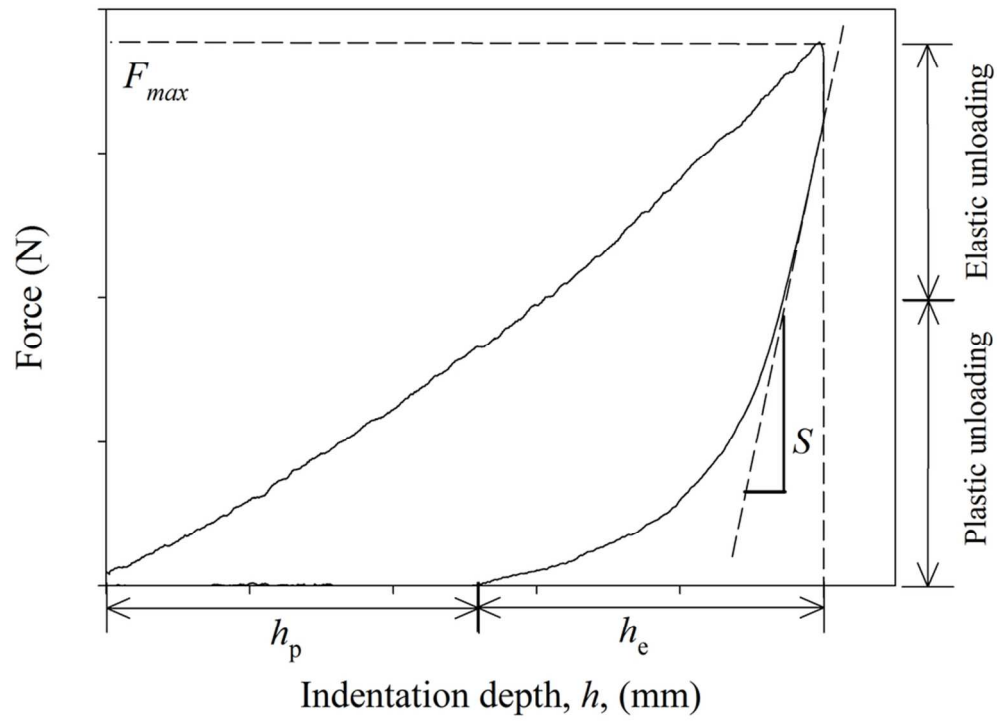
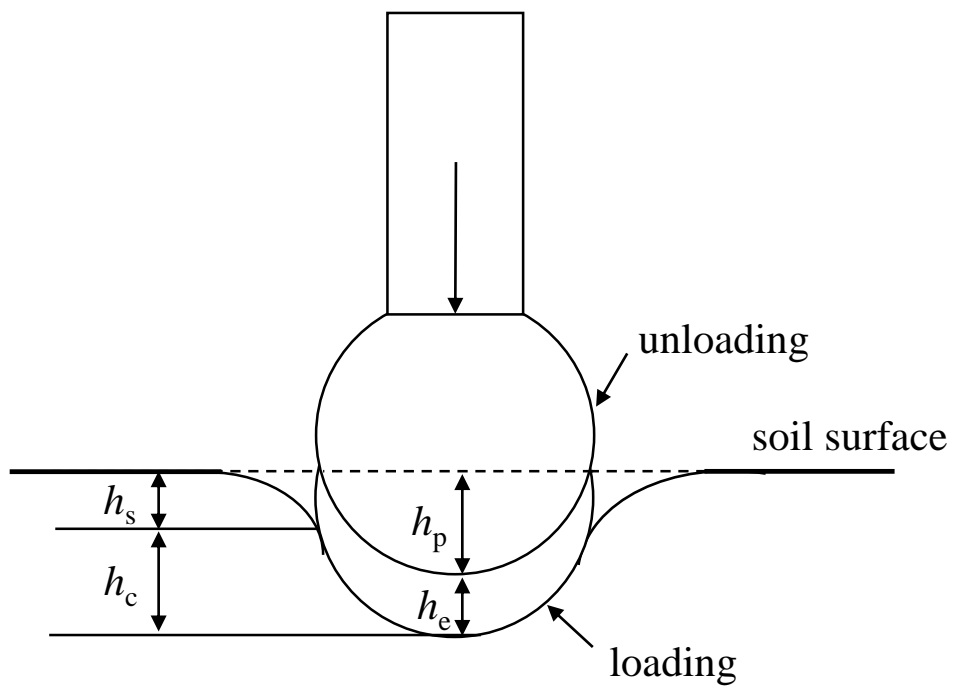


Figure 1: Typical load-displacement curve obtained from an indentation test,  $F_{max}$  = maximum measured force for the required indentation,  $h_e$  = elastic indentation i.e. deformation recovered on unloading,  $h_p$  = plastic indentation i.e. permanent deformation and not recovered on unloading and  $S$  = stiffness i.e. slope of the initial linear part of the unloading curve.

85x63mm (300 x 300 DPI)





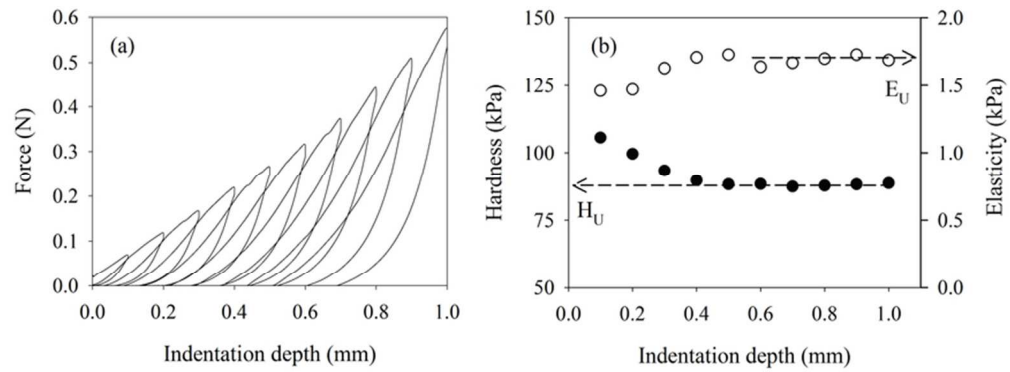


Figure 3: (a) 10 loading-unloading cycles adopted during an indentation test and (b) corresponding soil hardness and modulus of elasticity were plotted as a function of indentation depth,  $H_U$  and  $E_U$  are average hardness and elasticity for 0.6, 0.7, 0.8, 0.9, and 1.0 mm indentation depths.

73x28mm (300 x 300 DPI)

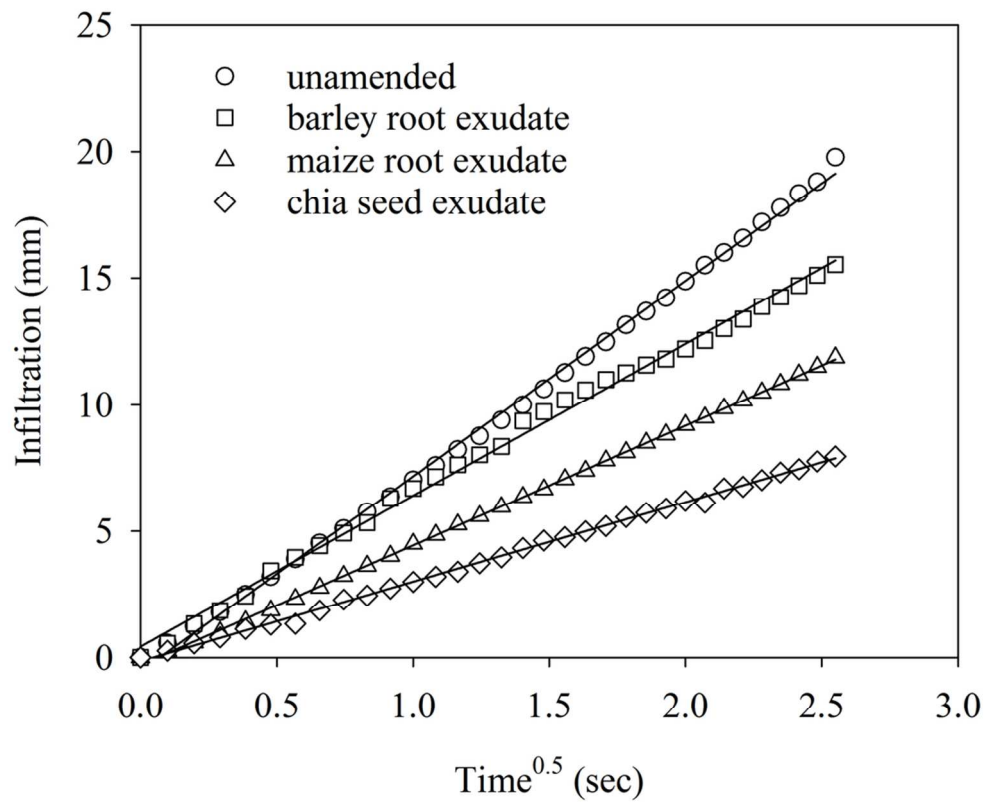


Figure 3: (a) 10 loading-unloading cycles adopted during an indentation test and (b) corresponding soil hardness and modulus of elasticity were plotted as a function of indentation depth,  $H_u$  and  $E_u$  are average hardness and elasticity for 0.6, 0.7, 0.8, 0.9, and 1.0 mm indentation depths.

93x76mm (300 x 300 DPI)

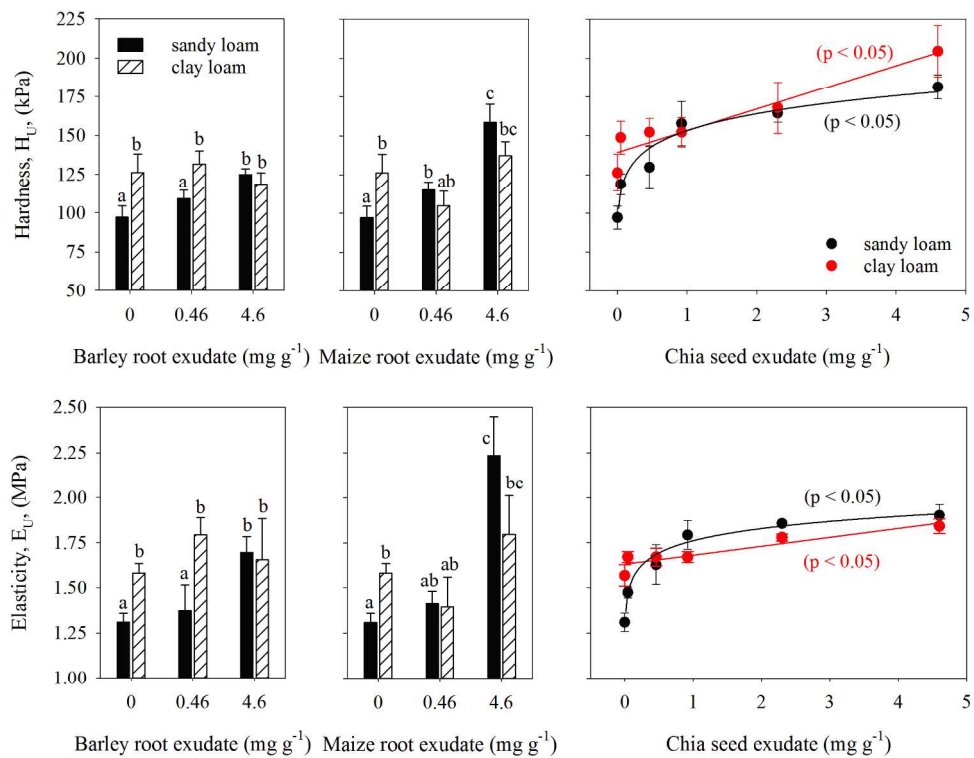


Figure 5: Hardness and modulus of elasticity of sandy loam and clay loam soils treated with barley root, maize root, and chia seed exudates at different concentrations in  $\text{mg exudate g}^{-1}$  dry soil measured at  $-10$  kPa matric potential; different letters indicate significant difference between treatments at  $p < 0.05$ , regressions were significant at  $p < 0.05$ , and regressions between sandy loam and clay loam soils were not found significantly different.

202x159mm (300 x 300 DPI)

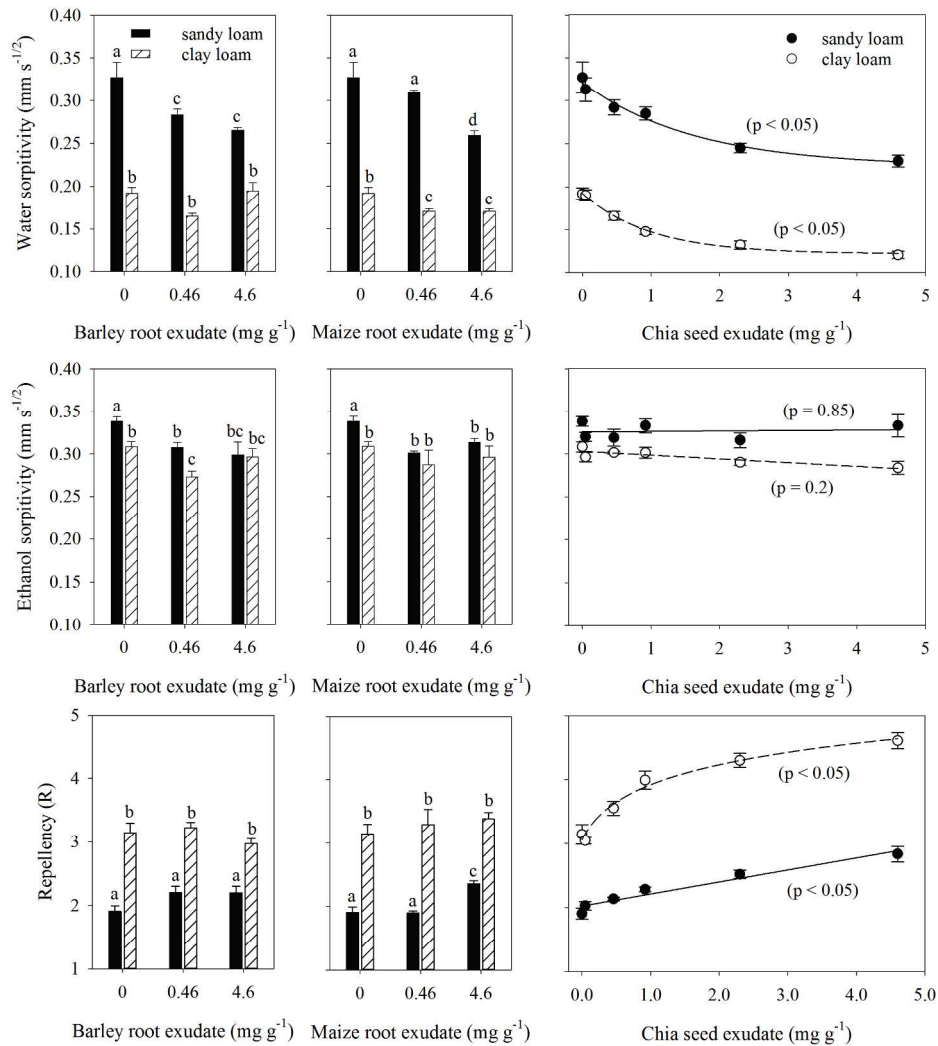


Figure 6: Water sorptivity, ethanol sorptivity, and water repellency of sandy loam and clay loam soils treated with barley root, maize root, and chia seed exudates at different concentrations in mg exudate g<sup>-1</sup> dry soil measured at air dry condition; different letters indicate significant difference between treatments at  $p < 0.05$ , regressions were significant at  $p < 0.05$ , and regressions between sandy loam and clay loam soils were found significantly different.

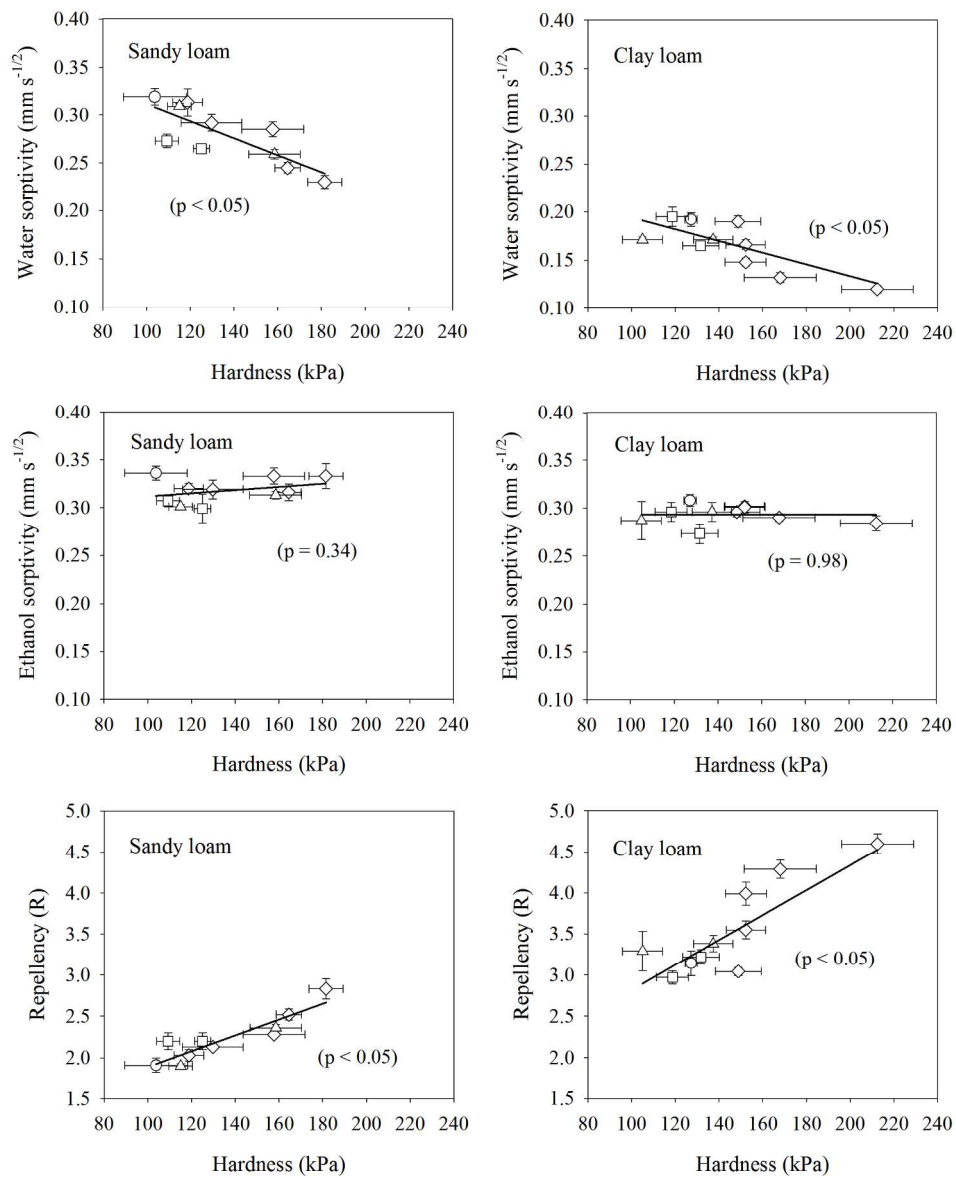


Figure 7: Water sorptivity, ethanol sorptivity, and repellency index were plotted as a function of soil hardness for sandy loam and clay loam soils treated with water (circle), barley root exudate (square), maize root exudate (triangle) and chia seed exudate (diamond).