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

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## Special Section: The Root Zone: Soil Physics and Beyond



## Core Ideas

- We hypothesized that plant exudates gel soil particles and on drying enhance water repellency.
- This has been carried out using rhizosphere-scale mechanical and hydraulic measurements.
- Plant exudates enhanced soil hardness and modulus of elasticity as chia seed > maize root > barley root.
- Plant exudates caused measurable decreases in soil wetting rates through water repellency.

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# Rhizosphere-Scale Quantification of Hydraulic and Mechanical Properties of Soil Impacted by Root and Seed Exudates

M. Naveed, L.K. Brown, A.C. Raffan, T.S. George, A.G. Bengough, T. Roose, I. Sinclair, N. Koebernick, L. Cooper, and P.D. Hallett\*

Using rhizosphere-scale physical measurements, we tested the hypothesis that plant exudates gel together soil particles and, on drying, enhance soil water repellency. Barley (*Hordeum vulgare* L. cv. Optic) and maize (*Zea mays* L. cv. Freya) root exudates were compared with chia (*Salvia hispanica* L.) seed exudate, a commonly used root exudate analog. Sandy loam and clay loam soils were treated with root exudates at 0.46 and 4.6 mg 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> dry soil. Soil hardness and modulus of elasticity were measured at -10 kPa matric potential using a 3-mm-diameter spherical indenter. The water sorptivity and repellency index of air-dry soil were measured using a miniaturized infiltrometer device with a 1-mm tip radius. Soil hardness increased by 28% for barley root exudate, 62% for maize root exudate, and 86% for chia seed exudate at 4.6 mg g<sup>-1</sup> concentration in the sandy loam soil. For the clay loam soil, root exudates did not affect soil hardness, whereas chia seed exudate increased soil hardness by 48% at 4.6 mg g<sup>-1</sup> concentration. Soil water repellency increased by 48% for chia seed exudate and 23% for maize root exudate but not for barley root exudate at 4.6 mg g<sup>-1</sup> concentration in the sandy loam soil. For the clay loam soil, chia seed exudate increased water repellency by 45%, whereas root exudates did not affect water repellency at 4.6 mg g<sup>-1</sup> concentration. Water sorptivity and repellency were both correlated with hardness, presumably due to the combined influence of exudates on the hydrological and mechanical properties of the soils.

Abbreviations: DW, distilled water.

**Exudates** produced by plant roots and microbes continually modify plant–soil interactions such as root penetration, soil aggregate formation, microbial dynamics, and water and nutrient fluxes from soil to roots (Carminati et al., 2016; Oleghe et al., 2017; Hinsinger et al., 2009). It has been well documented in a number of species such as sorghum [*Sorghum bicolor* (L.) Moench], wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.) that root exudation decreases with the age of the plant and increases with soil stress such as compaction, drought, and limited nutrient supply (Neumann et al., 2014; Aulakh et al., 2001; Brady and Weil, 1999). Plant exudates are generally viscoelastic gels consisting of an array of compounds such as large molecular weight polysaccharides (with both free sugars and polymerized arabinose, fructose, glucose, maltose, xylose, etc.), organic acids (acetic, gluconic, succinic, valeric acids, etc.), amino acids (alanine, glycine, lysine, valine, etc.), fatty acids, and sugar alcohols (Naveed et al., 2017; Aulakh et al., 2001).

Plant exudates can have a large influence on soil mechanical stability through resistance to disruption by mechanical and hydraulic stresses that depend on exudate chemical characteristics. The anions of organic acids present in root exudates may be adsorbed by soil mineral particles, thereby increasing the net negative charge of clays that would cause particles to disperse (Shanmuganathan and Oades, 1983). Mucilages and other polysaccharides (sugars) present in root exudates, which can function as stabilizing materials, may

offset this effect (Oades, 1984). Morel et al. (1991) showed that incorporation of maize root exudate in soils resulted in an immediate increase in soil aggregate stability, followed by a decrease with time due to microbial degradation. Traoré et al. (2000) also observed a significant increase in aggregate stability of soil by different substrates, i.e., polygalacturonic acid, modeled soluble exudates, and maize root exudate. Czarnes et al. (2000) found that adding polygalacturonic acid and xanthan to soil increased tensile strength and stability against the disruptive effects of wetting and drying cycles. Peng et al. (2011) found improved aggregate stability for only certain biological exudates they studied, with cycles of wetting and drying decreasing stabilization more rapidly in soils with swelling vs. non-swelling clay minerals. Fracture tests on dry soil disks (Czarnes et al., 2000) or notched bars (Zhang et al., 2008) have also quantified increased particle bond energy due to root exudate compounds. However, most of these studies have used model root exudates, extreme test conditions such as air-dry soils, or test techniques such as soil aggregate stability that do not quantify mechanical processes directly (Hallett et al., 2013).

Just as exudates may influence mechanical behavior, by coating soil particles and influencing soil water surface tension, they may also influence hydrological behavior. The flow of water from soil to plant roots is controlled by properties of the soil in close contact with roots, known as the rhizosphere. Exudation is believed to strongly influence soil moisture dynamics in the rhizosphere (Carminati et al., 2010, 2016). Currently there are two concepts in the literature regarding hydraulic properties of the rhizosphere compared with the bulk soil. The first is that polymeric gels present in root exudates increase the water holding capacity of the soil on drying but become significantly water repellent on rewetting (Carminati et al., 2010, 2016; Ahmed et al., 2015; Moradi et al., 2011). The second case is decreased water holding capacity of the rhizosphere on drying and more rapid rewetting due to surfactants in root exudates (Dunbabin et al., 2006; Whalley et al., 2005; Read et al., 2003; Hallett et al., 2003). Soil water retention and the degree of the hydrophobicity of the rhizosphere may therefore depend on the quantity and type of the root exudates and also on the drying history of the rhizosphere. Root-scale quantification of hydraulic properties of soils either in situ or using real root exudates with known physicochemical characteristics from different plant species are needed to determine the net effect of the complete cocktail of exudates released by plant roots.

For rhizosphere-scale hydrological tests, Hallett et al. (2003) have already used a miniature infiltrometer to obtain measurements of sorptivity and water repellency. A suitable rhizosphere-scale mechanical approach could be a miniature spherical indenter, as used by Kanayama et al. (2012) to measure the micromechanical properties of clay. The approach quantifies the mechanical properties of Young's modulus of elasticity,  $E$ , (stress vs. strain) and hardness,  $H$ , (related to strength) from the resistance to insertion and contact area of the sphere. Spherical indenters are not a new

approach for soils and were first introduced to assess the mechanical behavior of frozen soils in Russia in the 1940s (Zhang et al., 2016). Indenters come in a range of geometries, including cones similar to soil penetrometers used to measure mechanical resistance (Bengough, 1992). We selected a spherical indenter over a cone to increase the contact surface area over the shallow depth to which an indenter can be inserted to accurately measure  $E$  and  $H$ . The sharp tip of a cone would result in considerable variability in measurements because it concentrates stress in a very small area (Zhang and Li, 2014), which in soil could be a few interacting particles.

We used these rhizosphere-scale mechanical and hydrological tests to measure soil mechanical stability, soil water sorptivity, and water repellency index influenced by barley and maize root exudates. The impact of barley and maize root exudates were then compared with chia seed exudate, a commonly used root exudate analog (with its own biological function and importance), at a range of concentrations. We advance the earlier research of Hallett et al. (2003), and other research exploring the water repellency of exudate-amended soils (e.g., Peng et al., 2011), by amending soils with controlled amounts of real root exudates. Moreover, these data are combined with small-scale mechanical characteristics, providing a robust assessment of small-scale testing approaches for deployment in direct measurements of rhizosphere soil. The thrust of this research is to provide the first combined quantitative data on direct mechanical and hydrological shifts driven by natural plant exudates in soil. These combined data will improve our understanding of rhizosphere development and function and allow the interdependence of mechanical and hydraulic properties of the rhizosphere to be assessed.

## Materials and Methods

### Collection of Exudates

**Collection of Barley and Maize Root Exudates**  
Barley and maize root exudates were collected using an aerated hydroponic method. The details regarding the method are available in Naveed et al. (2017). In short, barley and maize seeds were surface sterilized in NaClO solution (2%) for 10 min, then rinsed thoroughly in sterile, deionized water. Sterilized seeds were pre-germinated on 0.5% distilled water (DW) agar until the radicals were approximately 1 cm long (2–3 d post-germination). After discarding poorly germinated seeds, 180 individuals each of barley and maize plants were grown, successively, in a 60-L aerated hydroponic tank. Nutrient solutions used in the aerated hydroponic tank were changed every 3 d beginning with one-quarter strength, followed by half strength, and continuing to full strength until harvest. Plants were harvested after 2 wk of growth. Exudates were collected for 12 h in 150-mL pots containing 75 mL of DW with a set amount of plants per pot (barley = 5 or maize = 3). Plants were removed from the pots the following morning (12-h collection period) and the remaining liquid in the collection pots was

first frozen at  $-20^{\circ}\text{C}$  and then freeze-dried for the collection of the dry barley and maize root exudates. It was observed that the mucilage attached to the roots was dissolved in DW during the collection period. We have determined, using chia seed exudate, that freeze-drying followed by rehydration of the exudates did not influence their physical properties, i.e., viscosity and surface tension (data not shown). The average dry weight of root exudates collected from individual barley and maize plants was  $4.1 \pm 0.9$  and  $6.4 \pm 1.7$  mg per individual, respectively.

Freeze-drying was essential so that the exudates could be concentrated from the dilute growth solutions and then rehydrated to local rhizosphere concentrations typical of exudates from roots in soil (Carminati et al., 2016). The amounts of C and N present in freeze-dried barley root exudate, maize root exudate, and chia seed exudate were measured using a CNS elemental analyzer (CE Instruments). The surface tension of exudate solutions at  $4.6$  mg exudate  $\text{g}^{-1}$  water was measured using a Sigma 700 force tensiometer (Attension) and a DuNüoy ring.

### Extraction of Chia Seed Exudate

Chia seed exudate has been used in studies as a model root exudate (Ahmed et al., 2014; Kroener et al., 2014). It was extracted based on the method of Ahmed et al. (2014) by mixing 100 g of DW with 10 g of chia seeds using a magnetic stirrer for 2 min at  $50^{\circ}\text{C}$ , followed by cooling to room temperature ( $20^{\circ}\text{C}$ ) for 4 h in sealed containers. The exudate was separated from the seeds by repeatedly pushing the mixture through a  $500\text{-}\mu\text{m}$  sieve under pressure using a syringe that was cut at the end. This approach harvested the easily extracted seed exudate, with tightly bound exudate remaining on the seeds even after five repeated extraction attempts. The extracted chia seed exudate was freeze-dried so that its concentration was obtained, i.e.,  $9.2$  mg dry exudate  $\text{g}^{-1}$  water. To obtain the total exudates, the entire hydrated seed was freeze-dried, after which the exudate layer was easy to remove. Of  $0.13 \pm 0.03$  g  $\text{g}^{-1}$  total exudate on seeds, only  $0.10 \pm 0.02$  g  $\text{g}^{-1}$  of seed exudate was harvested, so the extraction efficiency was  $77 \pm 8\%$ .

### Selection and Preparation of Soils

Soils were sampled from Bullion field at The James Hutton Institute, Dundee, UK ( $56^{\circ}27'39''$  N,  $3^{\circ}4'11''$  W) at two locations denoted as South Bullion and North Bullion. The South Bullion soil is a Dystric Cambisol and was under barley production. The North Bullion soil is a Haplic Cambisol and was under fallow. The bulk soils were sampled from both locations at the 0- to 100-mm depth. The soils were partially air dried to  $150$  g water  $\text{kg}^{-1}$  dry soil and then passed through a 2-mm sieve and stored at  $4^{\circ}\text{C}$  before any measurements started. The soil texture was determined by the combination of wet sieving and hydrometer methods. The amounts of C and N present in the soils were measured using a CNS elemental analyzer (CE Instruments). The pH in  $\text{CaCl}_2$  of the soils was measured using a pH meter (Hanna Instruments).

The sieved soils were mixed with either DW (control) or one of the exudates: barley root exudate, maize root exudate, and chia seed exudate, bringing all to  $0.2$  g  $\text{g}^{-1}$  water content. Because the soils were at  $0.15$  g  $\text{g}^{-1}$  water content before amendments were applied, only  $0.05$  g  $\text{g}^{-1}$  additional water or exudate solution was added, so potential ionic impacts of the DW were assumed to be minimal. Barley and maize root exudates were amended with  $0.46$  and  $4.6$  mg exudate  $\text{g}^{-1}$  dry soil. Chia seed exudate was amended at five concentrations:  $0.046$ ,  $0.46$ ,  $0.92$ ,  $2.3$ , and  $4.6$  mg exudate  $\text{g}^{-1}$  dry soil. The amended soils were packed in triplicate in soil cores of 3-cm diameter and 1-cm height at  $1.3$  g  $\text{cm}^{-3}$  bulk density and  $0.2$  g  $\text{g}^{-1}$  water content. This provided homogeneous samples to both test the effects of different exudates on hydrological and mechanical properties of the soil as well as evaluate the direct measurement approaches deployed in this study.

### Measurements of Soil Hardness and Elasticity

The packed soil cores were first saturated and then drained to  $-10$  kPa matric potential on a suction plate (ecoTech, Umwelt-Meßsysteme) at  $4^{\circ}\text{C}$  to suppress microbial decomposition of the exudates. One indentation measurement was performed on each soil core using a 3-mm-diameter spherical indenter. Figure 1 shows a typical load-displacement curve obtained from a soil indentation test. A corresponding schematic cross-section of such an indentation is depicted in Fig. 2. The soil hardness parameter (Oliver and Pharr, 1992) was obtained using

$$H = \frac{F_{\max}}{A_c} \quad [1]$$

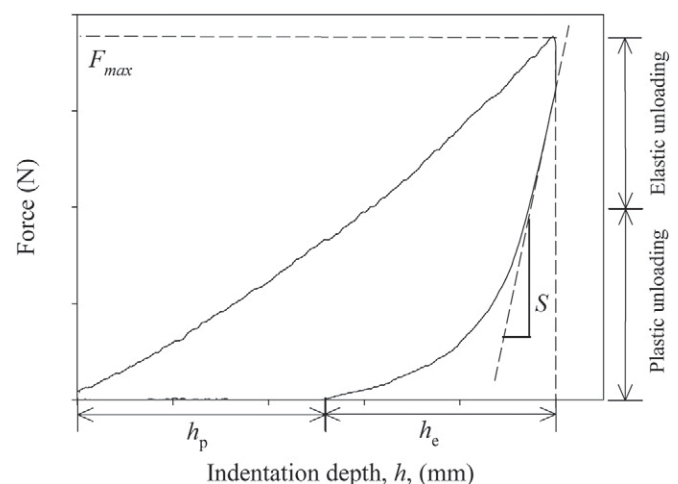


Fig. 1. Typical load-displacement curve obtained from an indentation test;  $F_{\max}$  = maximum measured force for the required indentation,  $h_e$  = elastic indentation i.e., deformation recovered on unloading,  $h_p$  = plastic indentation i.e., permanent deformation and not recovered on unloading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



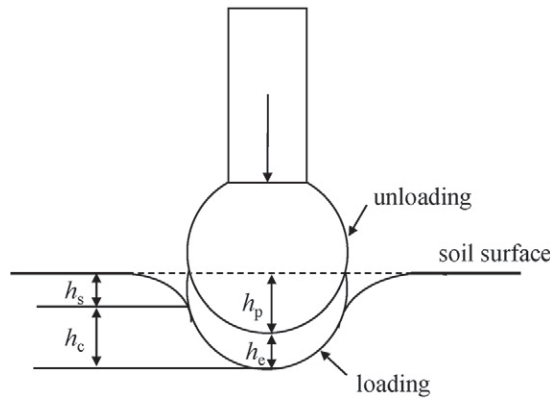


Fig. 2. A schematic of an indentation test;  $h_e$  = elastic indentation i.e., deformation recovered on unloading,  $h_p$  = plastic indentation i.e., permanent deformation and not recovered on unloading,  $h_c$  = contact depth at maximum load,  $h_s$  = displacement of the surface at the perimeter of the contact.

where  $F_{\max}$  is the maximum force applied during an indentation as shown in Fig. 1 and  $A_c$  is the projected contact area at  $F_{\max}$ , obtained using

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

where  $R$  is the radius of the spherical indenter (i.e., 1.5 mm) and  $h_c$  is the contact depth, as shown in Fig. 2 and obtained using

$$h_c = h_{\max} - h_s \quad [3]$$

where  $h_{\max}$  is the indentation depth and  $h_s$  is the displacement of the surface at the perimeter of the contact at  $F_{\max}$  before the unloading. The value of  $h_s$  was determined by

$$h_s = \varepsilon \frac{F_{\max}}{S} \quad [4]$$

where  $\varepsilon$  is a geometric constant, which for a spherical indenter is theoretically equal to 0.75 (Fischer-Cripps, 2011), and  $S$  is the stiffness,

i.e., the initial slope of the unloading curve as shown in Fig. 1. It was determined by taking the derivative of  $F$  with respect to  $h$  for the initial linear part of the unloading curve. The reduced modulus of elasticity ( $E_r$ ) was obtained following Oliver and Pharr (1992):

$$E_r = \frac{S\sqrt{\pi}}{2\beta\sqrt{A_c}} \quad [5]$$

where  $\beta$  is the tip geometry correction factor, which is equal to 1 for a spherical indenter. Because the indenter's (steel) modulus of elasticity is much higher than that of the soil, the indenter can be treated as a rigid body and the modulus of elasticity can be determined as

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

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

### Effect of Indentation Size on Soil Hardness and Elasticity

The effect of indentation depth on the soil hardness and modulus of elasticity was estimated using a series of loading–unloading cycles with increasing indentation depth as shown in Fig. 3a. The soil hardness and modulus of elasticity for each loading–unloading cycle was determined and plotted as a function of indentation depth as shown in Fig. 3b. Generally, there was large variation in the soil hardness and modulus of elasticity for an indentation depth shallower than 0.5 mm (Fig. 3b) because of surface roughness of the soil cores. In almost all soil cores, soil hardness and modulus of elasticity data became stable for an indentation depth  $>0.5$  mm. Therefore, the hardness and modulus of elasticity data were averaged at 0.6-, 0.7-, 0.8-, 0.9-, and 1.0-mm indentation depths to obtain an ultimate hardness ( $H_U$ ) and ultimate modulus

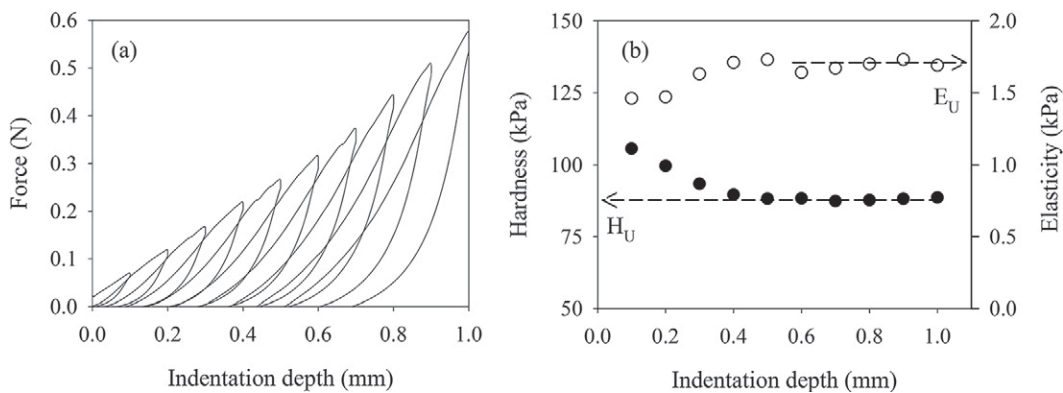


Fig. 3. (a) Ten loading–unloading cycles adopted during an indentation test and (b) corresponding soil hardness and modulus of elasticity plotted as a function of indentation depth;  $H_U$  and  $E_U$  are average hardness and elasticity for 0.6-, 0.7-, 0.8-, 0.9-, and 1.0-mm indentation depths.

of elasticity ( $E_u$ ) for each soil core as shown in Fig. 3b. The  $H_u$  and  $E_u$  data are reported here.

### Measurements of Soil Hydraulic Properties

The soil cores used for indentation measurements were air dried to measure soil hydraulic properties. Three different hydraulic properties for each soil core were obtained using a miniaturized infiltrometer with a tip radius of 1 mm: (i) water sorptivity, (ii) ethanol sorptivity, and (iii) water repellency. The complete details on the experimental setup are available in Hallett et al. (2003). Water sorptivity is the rate at which soil imbibes water, much like a wetting sponge. The soil matric potential, pore structure, and hydrophobicity all influence water sorptivity. Ethanol sorptivity was measured because the nonpolar nature of ethanol and its contact angle with hydrophobic surfaces provides a reference measurement not influenced by the hydrophobicity of the soil. All measurements were conducted at  $-10$  mm hydraulic head. Liquid uptake by the soil from the infiltrometer reservoir was logged from the balance at 1-s intervals for 90 s. After about 20 s, the flow rate,  $Q$ , was steady and used to evaluate water sorptivity ( $S_W$ ) and ethanol sorptivity ( $S_E$ ):

$$S_W \text{ or } S_E = \sqrt{\frac{Qf}{4br}} \tag{6}$$

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

The water repellency index ( $R$ ) was determined from the sorptivity measurements of water ( $S_W$ ) and ethanol ( $S_E$ ) at  $-10$  mm pressure

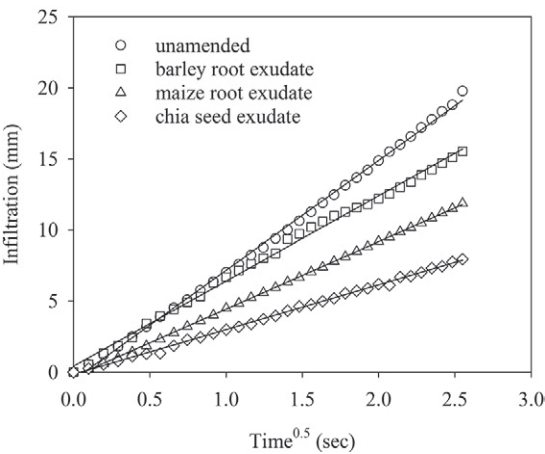


Fig. 4. An average water infiltration of three soil cores plotted as a function of the square root of time for an unamended soil and soils treated with barley root, maize root, and chia seed exudates at a concentration of 4.6 mg exudate g<sup>-1</sup> dry soil.

head (Tillman et al., 1989). Accounting for differences in surface tension and viscosity of the two liquids (water and ethanol),  $R$  was obtained as

$$R = 1.95 \frac{S_E}{S_W} \tag{7}$$

The larger the value of  $R$  the more water repellent is the soil, with  $R = 1.0$  signifying a totally non-water-repellent soil. The calculation of  $R$  assumes that ethanol wets the soil perfectly (contact angle = 0°) and that different effects of the wetting liquids on the hydrated behavior of the exudates are negligible. In reality, swelling of exudates by water and subsequent mixing effects on viscosity, pore clogging, and surface tension may influence the result. Ethanol may also dissolve organic compounds, so the interpretation of  $R$  needs to take into account these potential artifacts.

### Statistical Analysis

All the statistical analyses were performed using SigmaPlot 13. To test for significant differences in soil hardness, modulus of elasticity, water sorptivity, ethanol sorptivity, and repellency index among the different exudate-treated soils as well as the control soil, a one-way analysis of variance was performed. To test for a significant difference between the regressions, an analysis of covariance was performed using SigmaPlot 13 at the  $p < 0.05$  level. Pairwise comparison of means was performed using the Holm-Sidak method at the 95% confidence level to test for a significant difference. Linear, logarithmic, or exponential models were fitted to the measured data to show trends depending on the fitting efficiency, i.e.,  $R^2$  value.

## Results

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

The soil water content of the sandy loam soil at  $-10$  kPa matric potential decreased by 4.6% for barley root exudate, whereas it

Table 1. Carbon, nitrogen, and C/N ratios for barley root, maize root, and chia seed exudates.

Exudate	C	N	C/N ratio
	g kg <sup>-1</sup>		
chia seed	407.7 ± 1.4	10.9 ± 0.1	37.44 ± 0.52
barley root	148.9 ± 3.2	61.5 ± 0.8	2.42 ± 0.04
maize root	166.2 ± 7.9	32.5 ± 3.2	5.23 ± 0.63

Table 2. Physical properties of the studied soils.

Soil	Clay	Silt	Sand	C	N	Soil pH in CaCl <sub>2</sub>	Texture class
g kg <sup>-1</sup>							
South Bullion	160	240	600	22.5 ± 1.4	1.6 ± 0.3	5.48 ± 0.07	sandy loam
North Bullion	260	300	440	29.5 ± 1.2	2.3 ± 0.2	5.15 ± 0.04	clay loam

increased by 5.25% for maize root exudate and 7.29% for chia seed exudate at 4.6 mg g<sup>-1</sup> concentration compared with the unamended soil. The soil water content for the clay loam soil at -10 kPa matric potential decreased by 2.09% for barley root exudate but increased by 2.33% for maize root exudate and 6.52% for chia seed exudate at 4.6 mg g<sup>-1</sup> concentration (Table 3). The sandy loam soil hardness was not significantly impacted by barley root exudate at the 0.46 mg g<sup>-1</sup> concentration, whereas at 4.6 mg g<sup>-1</sup> concentration it significantly increased ( $p < 0.05$ ) by 28% compared with the control (Fig. 5). Soil hardness was increased by 20% for maize root exudate at 0.46 mg g<sup>-1</sup> concentration ( $p < 0.05$ ) and 62% at 4.6 mg g<sup>-1</sup> concentration ( $p < 0.05$ ) for the sandy loam soil (Fig. 5). The clay loam soil showed significantly greater hardness than the sandy loam soil. However, this difference was

Table 3. Volumetric soil water contents at -10 kPa matric potential for soils treated with different exudates.

Soil	Exudate treatment	Concentration	Water content
		mg g <sup>-1</sup> †	m <sup>3</sup> m <sup>-3</sup>
Sandy loam	control	0	0.343 ± 0.010
	chia seed	0.046	0.345 ± 0.014
		0.46	0.351 ± 0.012
		0.92	0.361 ± 0.004
		2.3	0.364 ± 0.011
		4.6	0.368 ± 0.001
	barley root	0.46	0.327 ± 0.002
		4.6	0.323 ± 0.013
	maize root	0.46	0.341 ± 0.009
		4.6	0.361 ± 0.009
Clay loam	control	0	0.429 ± 0.016
	chia seed	0.046	0.451 ± 0.009
		0.46	0.451 ± 0.001
		0.92	0.452 ± 0.001
		2.3	0.461 ± 0.006
		4.6	0.457 ± 0.007
	barley root	0.46	0.420 ± 0.009
		4.6	0.419 ± 0.008
	maize root	0.46	0.443 ± 0.018
		4.6	0.439 ± 0.027

† Milligrams exudate per kilogram dry soil.

overcome by the addition of barley root exudate at 4.6 mg g<sup>-1</sup> concentration and maize root exudate at both 0.46 and 4.6 mg g<sup>-1</sup> concentrations. Neither barley nor maize root exudates at any of the studied concentrations improved soil hardness for the clay loam soil (Fig. 5).

Soil hardness was sharply increased at lower chia seed exudate concentrations for the sandy loam soil ( $p < 0.05$ ), whereas more like a linear increase in soil hardness as a function of chia seed exudate concentration was observed for the clay loam soil ( $p < 0.05$ ). Soil hardness was significantly increased ( $p < 0.05$ ) by 33% at 0.46, 61% at 0.92, 69% at 2.3, and 86% at 4.6 mg g<sup>-1</sup> chia seed exudate concentrations for the sandy loam soil (Fig. 5). Soil hardness was only significantly increased ( $p < 0.05$ ) by 32% at 2.3 and 60% at 4.6 mg g<sup>-1</sup> chia seed exudate concentration for the clay loam soil. Similar to soil hardness, the modulus of elasticity of the sandy loam and clay loam soils was influenced by barley root exudate, maize root exudate, and chia seed exudate at various concentrations (Fig. 5).

On air-dried samples, water sorptivity was significantly decreased ( $p < 0.05$ ) for the sandy loam soil treated with barley root exudate at 0.46 and 4.6 mg g<sup>-1</sup> concentrations. The clay loam soil had significantly lower water sorptivity than the sandy loam soil ( $p < 0.05$ ), and it was not influenced by the barley root exudate (Fig. 6). Water sorptivity was significantly decreased by maize root exudate at the 4.6 mg g<sup>-1</sup> concentration for both sandy loam and clay loam soils ( $p < 0.05$ ). Water sorptivity exponentially decayed for both soils amended with increasing chia seed exudate concentrations ( $p < 0.05$ ). Water sorptivity was reduced by 30% for the sandy loam soil and 37% for the clay loam soil treated with chia seed exudate at the 4.6 mg g<sup>-1</sup> concentration (Fig. 6). Ethanol sorptivity was also significantly less for the sandy loam soil treated with both barley and maize root exudates at 0.46 and 4.6 mg g<sup>-1</sup> concentrations. Ethanol sorptivity of the sandy loam soil was unaffected with increasing chia seed exudate concentration. Ethanol sorptivity of the clay loam soil was significantly lower than that of the sandy loam soil ( $p < 0.05$ ), and it remained unaffected by barley root exudate, maize root exudate, and chia seed exudate treatments (Fig. 6). Water repellency of the clay loam soil was significantly higher than that of the sandy loam soil ( $p < 0.05$ ). Water repellency of both the sandy loam and clay loam soils was not influenced by barley root exudate at 0.46 and 4.6 mg g<sup>-1</sup> concentrations ( $p < 0.05$ ). Water repellency was significantly greater by 23% for the sandy loam soil treated with maize root exudate at the 4.6 mg g<sup>-1</sup> concentration ( $p < 0.05$ ). Treating the clay loam soil with maize root exudates did not impact its water repellency. Water repellency linearly increased in the sandy loam soil treated with increasing concentrations of chia seed exudate ( $p < 0.05$ ). Water repellency was increased by 48% for the sandy loam soil and 46% for the clay loam soil treated with chia seed exudate at a concentration of 4.6 mg g<sup>-1</sup>. Water repellency increased logarithmically

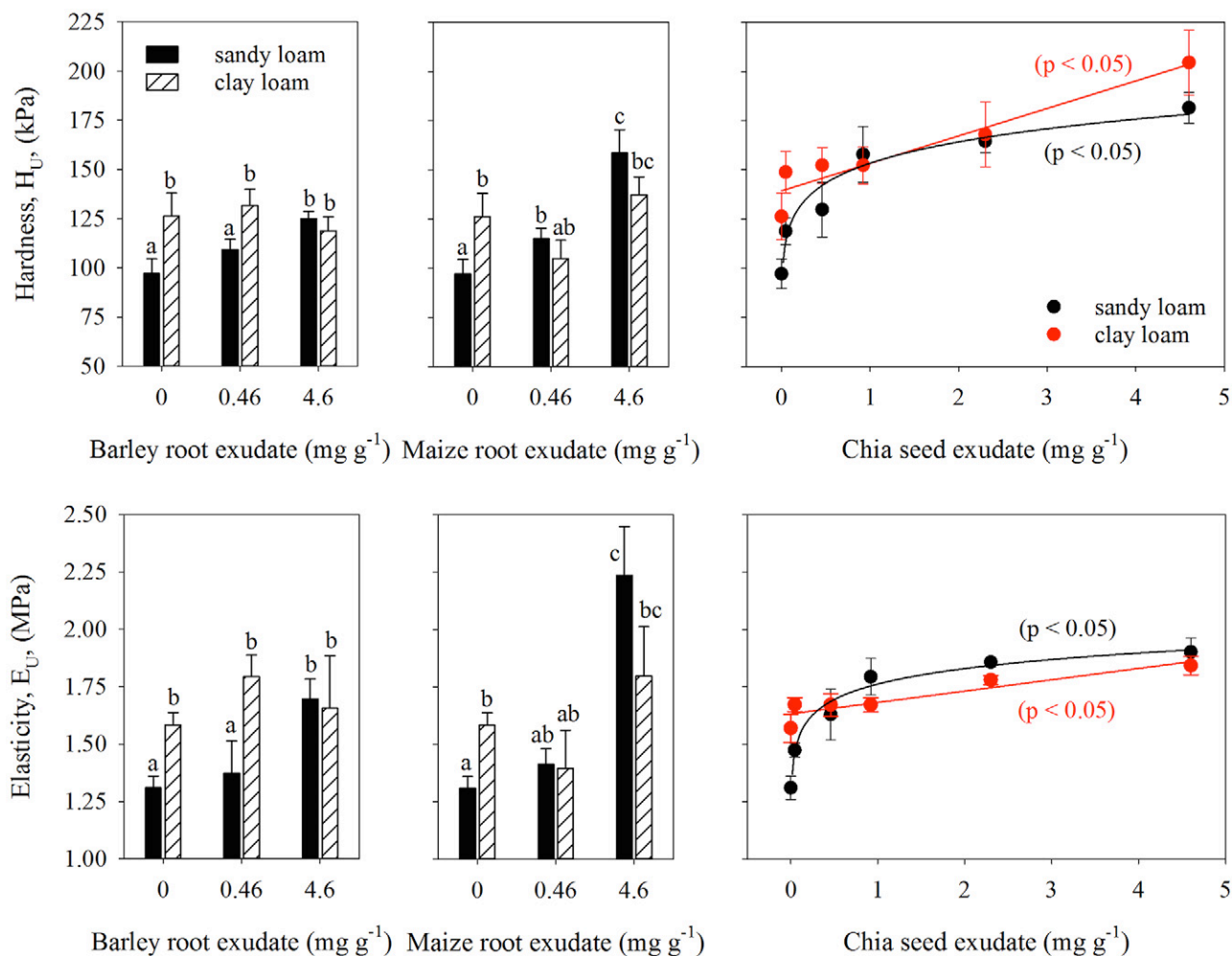


Fig. 5. Hardness and modulus of elasticity of sandy loam and clay loam soils treated with barley root, maize root, and chia seed exudates at different concentrations (in mg exudate g<sup>-1</sup> dry soil) measured at -10 kPa matric potential. Different letters indicate a significant difference between treatments at  $p < 0.05$ ; regressions were significant at  $p < 0.05$ , and regressions between sandy loam and clay loam soils were not significantly different.

with increasing chia seed exudate concentration for the clay loam soil ( $p < 0.05$ ), and it was significantly higher than in the sandy loam soil (Fig. 6).

## Discussion

### Mechanical Properties of Soils Influenced by Plant Exudates

Soil mechanical properties, quantified by soil hardness and modulus of elasticity, were greatly enhanced by barley root exudate, maize root exudate, and chia seed exudate for the sandy loam soil. The smallest impact was observed for barley root exudate, moderate for maize root exudate, and greatest for chia seed exudate at a particular exudate concentration in the soil. A possible explanation lies in the physicochemical characteristics of the exudates (Naveed et al., 2017). The barley root exudate contains the greatest amount of organic acids, followed by maize root exudate and chia seed exudate (Naveed et al., 2017). The largest concentrations of (combined polysaccharide and free) sugars

were observed in chia seed exudate, followed by maize and barley root exudates, respectively (Naveed et al., 2017). The anionic forms of organic acids present in root exudates may increase the net negative charge of clays, which would cause particles to disperse, whereas the amount of polysaccharides (sugars) present in root exudates, which can function as stabilizing materials, may offset this effect by gelling of soil particles (Shanmuganathan and Oades, 1983). This suggests that barley root exudates can have a net dispersing effect on soil, whereas maize and chia seed exudates have net stabilizing effects.

Following this explanation, the enhanced mechanical stability of the sandy loam soil following the addition of barley root exudate at the 4.6 mg g<sup>-1</sup> concentration could be explained by two factors. The first is increased drainage of the soil at -10 kPa (Table 3) driven by a lowering of the surface tension of the soil solution by the exudate (Table 4). Second, although care was taken to minimize microbial decomposition of the added exudates by storing and equilibrating the samples at 4°C, some compounds may have been metabolized to produce longer chain compounds. We plan to



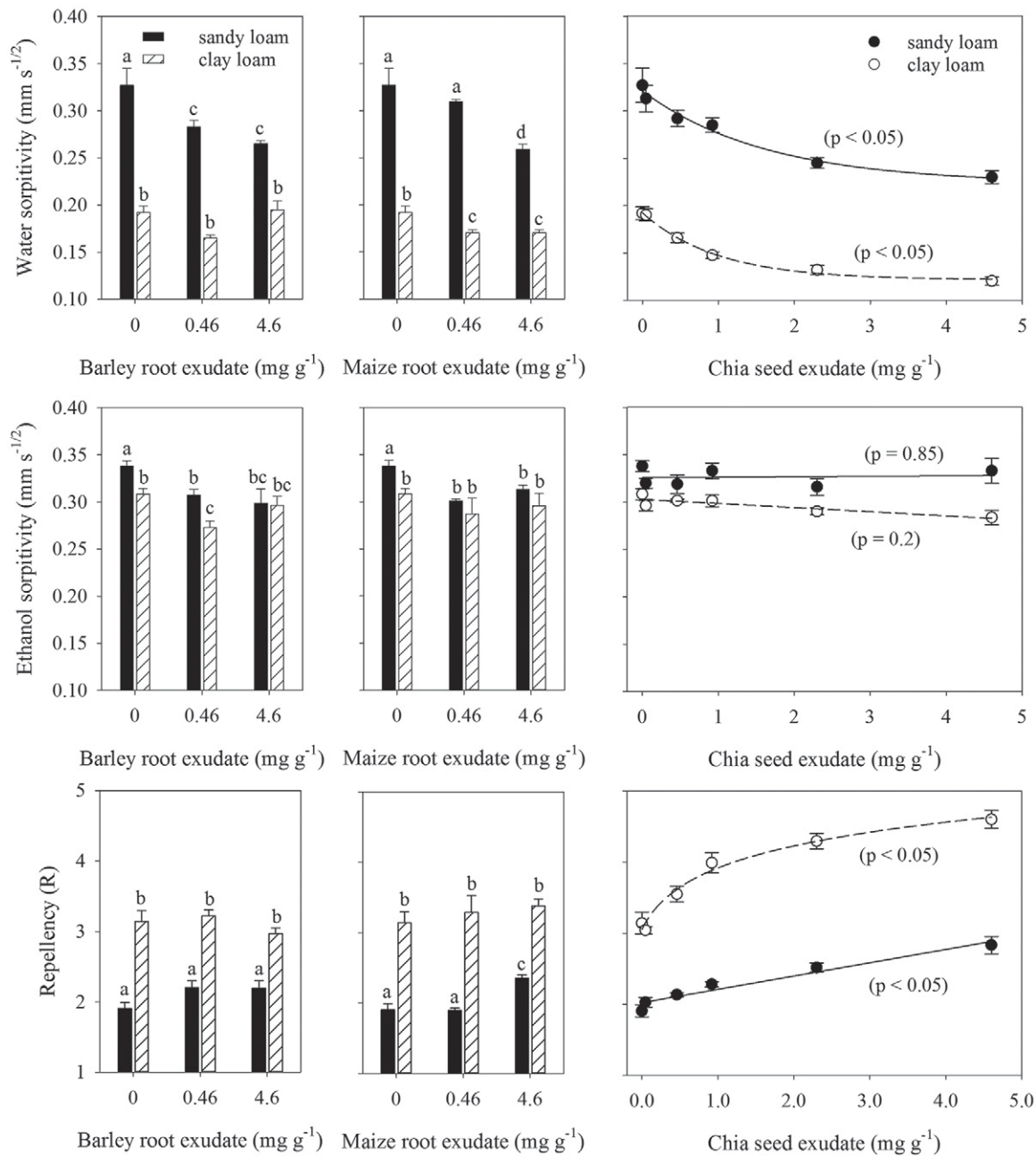


Fig. 6. Water sorptivity, ethanol sorptivity, and water repellency of sandy loam and clay loam soils treated with barley root, maize root, and chia seed exudates at different concentrations (in mg exudate g<sup>-1</sup> dry soil) measured under air-dry conditions. Different letters indicate significant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

study microbial decomposition impacts of plant exudates on soil physical properties in future research.

Viscosity of the exudates tended to be in the order barley root exudate < maize root exudate < chia seed exudate, reflecting their gelling potential with individual soil particles (Table 4). The greatest viscosity of the chia seed exudate thus resulted in the greatest mechanical stability of the soil, followed by maize root exudate and barley root exudate. Our results are in agreement with studies exploring the impacts of maize root exudate on soil aggregate stability (Morel et al., 1991; Traoré et al., 2000).

Similarly, most of the studies available in the literature have reported improved mechanical stability of soil amended with model root and microbial exudates such as polygalacturonic acid, xanthan, scleroglucan, and *Capsella* sp. seed exudate (Traoré et al., 2000; Czarnes et al., 2000; Barré and Hallett, 2009; Peng et al., 2011; Deng et al., 2015). It should be noted that model root exudates, such as the chia seed exudate used in the present study, showed an exaggerated effect on soil mechanical properties compared with real root exudates from barley and maize roots. Further, it was also evident from our findings that the impact of exudation on soil mechanical stability depends on

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

Exudate 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

initial soil properties, such as texture. An insignificant effect of both barley and maize root exudation on the soil hardness and modulus of elasticity for the clay loam soil (Fig. 5) was probably driven by the greater impact of clay minerals and possibly soil organic matter on interparticle bonding compared with the sandy loam soil. If soil was already in a stable condition, such as the clay loam soil in the present study (Fig. 5), plant root exudation would be expected to have less of an impact on its mechanical stability.

## Hydraulic Properties of Soils Influenced by Plant Exudates

Both water and ethanol sorptivities for sandy loam soil significantly decreased with increasing barley root exudate concentration (Fig. 6). Water repellency was not significantly different from the control (Fig. 6), suggesting that a marked difference in pore structure drove decreased sorptivities. This difference in pore structure might originate from either pore clogging due to exudates swelling during measurements or different levels of shrinkage between control and exudate-treated soil cores from air drying. If we assume that all of the exudate remains in the pore water, then at a 4.6 mg g<sup>-1</sup> barley exudate concentration, it has a surface tension of 45.59 mN m<sup>-1</sup> (Table 4). Sorption of exudates to soil surfaces may decrease the exudate concentration in solution and therefore increase surface tension, but even at smaller concentrations, surface tension would be less than for water (Read and Gregory, 1997). This lower surface tension of the soil solution as a result of barley root exudation might overcome soil water repellency by acting as a surfactant. Strong negative correlations were observed between the surface tension of barley root, maize root, and chia seed exudate solutions and water sorptivity of exudate-treated soils, i.e., sandy loam ( $R^2 = 0.97$ ) and clay loam ( $R^2 = 0.99$ ). With maize root and chia seed exudates at the 4.6 mg g<sup>-1</sup> concentration, the marked decrease in water sorptivity and greater repellency index (Fig. 6) for the sandy loam soil was probably due to exudates creating a hydrophobic coating on soil particles. Moreover, the greater viscosity of these exudate solutions at 4.6 mg g<sup>-1</sup> compared with barley root exudate may affect water infiltration by retarding capillary flow and potentially clogging pores (Table 4). Similar to surface tension, strong negative correlations were observed between viscosity of the exudate solutions and water sorptivity of exudate-treated soils, i.e., sandy loam ( $R^2 = 0.97$ ) and clay

loam ( $R^2 = 0.90$ ). Ethanol sorptivity was significantly lower for the sandy loam soil treated with barley and maize root exudates, but no effect of chia seed exudate was observed (Fig. 6). This suggests a different interaction of ethanol with different types of exudates, which could be explored further by quantifying time-dependent dissolution, swelling, and viscous clogging by exudates as affected by either water or ethanol. Because the hydraulic measurements were conducted on air-dried soils, greater sorption of exudates onto soil surfaces may have exacerbated the impacts of exudates compared with the mechanical tests that were done at -10 kPa water potential. It is feasible to use the same miniature infiltrometer setup at wetter water contents.

Our findings for barley root exudate treated soils agree with in situ measurements of the barley rhizosphere by Hallett et al. (2003), who found only a slight impact. The maize root exudate hydraulic measurements follow trends reported by Ahmed et al. (2015), where the rhizosphere of maize stayed temporarily dry after irrigation. Both this study and the investigation of Carminati et al. (2010) of the lupin rhizosphere observed the development of water repellency when the rhizosphere dried beyond a critical threshold, which was attributed to root exudates. While maize root exudates influenced the development of water repellency in the sandy loam soil, for the clay loam soil the greater initial water repellency was probably driven by past soil management accumulating organic matter (Fig. 6). However, chia seed exudate, which is strongly hydrophobic in nature, significantly increased soil water repellency with increasing concentrations for both sandy loam and clay loam soils.

## Interaction between Mechanical and Hydraulic Properties of Soils

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

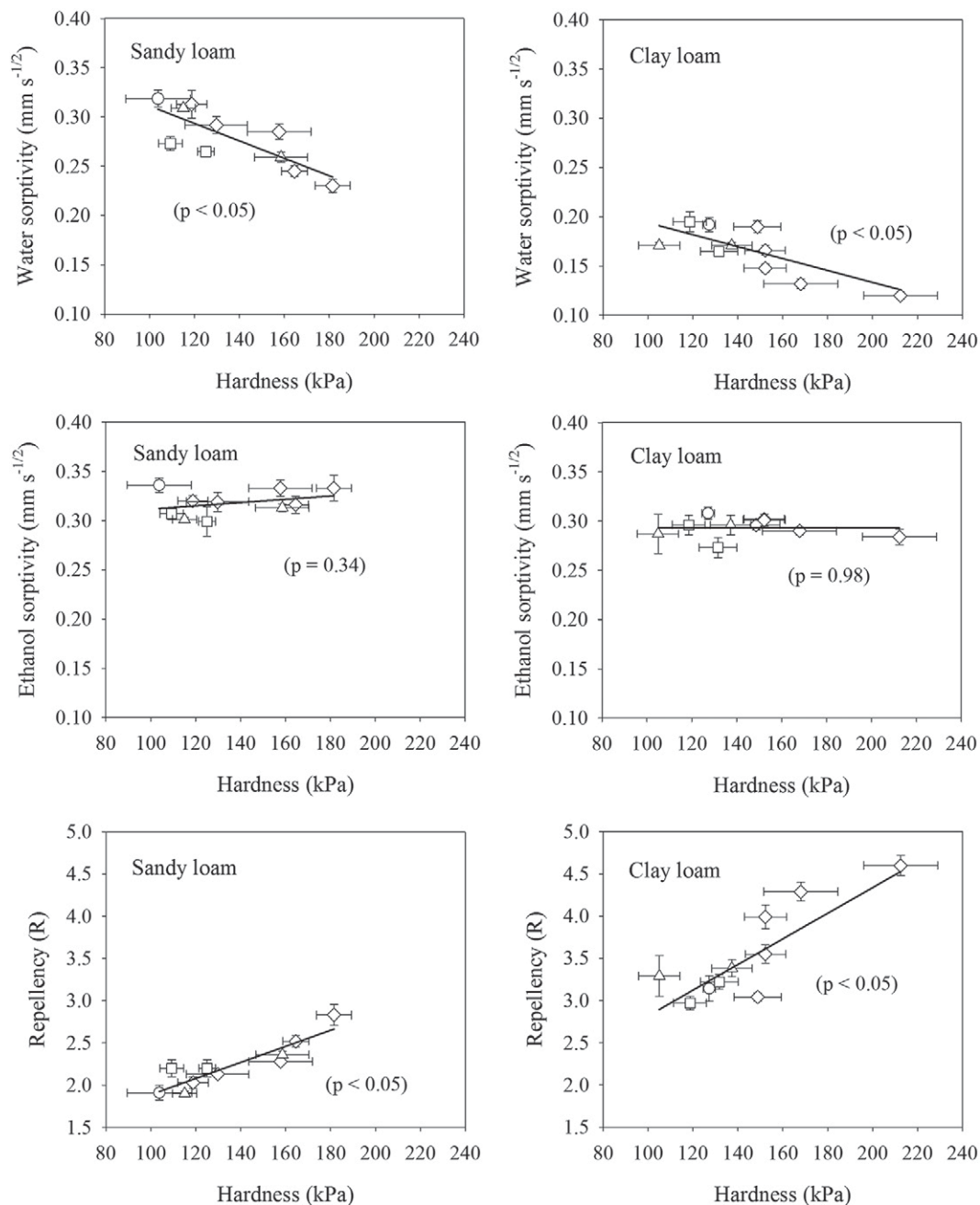


Fig. 7. Water sorptivity, ethanol sorptivity, and repellency index plotted as a function of soil hardness for sandy loam and clay loam soils treated with water (circles), barley root exudate (squares), maize root exudate (triangles), and chia seed exudate (diamonds).

## Rhizosphere-Scale Mechanical and Hydrological Tests

The rhizosphere-scale quantification techniques successfully measured the impact of plant exudates on soil mechanical properties and water transport. This could open up future research on direct in situ measurements of mechanical and hydraulic properties in the rhizosphere of different plant species. Hallett et al. (2003) have already discussed rhizosphere-scale hydrological measurements, including limitations to the approach caused by experimental artifacts such as soil contact and the development of erratic wetting bulbs over such a small area. Nevertheless, with

adequate replication, both Hallett et al. (2003) and our current study found realistic sorptivity values and elucidated the effects of plant exudates.

The spherical indenter also obtained realistic mechanical properties of the soil. In a different study (unpublished), we found from unconfined compression tests of the sandy loam soil tested at the same water potential that the modulus of elasticity ranged from 1.1 to 1.9 MPa and failure stress ranged from 37 to 73 kPa depending on packing stress. There is scope to enhance the indentation approach to estimate the fracture mechanics properties of the soil

(Chen et al., 2016), which would be a useful technique given that traditional approaches with notched bars require remolded soil and have fragile samples that are difficult to handle (Yoshida and Hallett, 2008).

## Conclusions

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

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

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

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