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

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Abstract

Using rhizosphere-scale physical measurements we test the hypothesis that plant exudates gel together soil particles and on drying they enhance soil water repellency. Barley and maize root exudates were compared with chia seed exudate, a commonly used root exudate analogue. Sandy loam and clay loam soils were treated with root exudates at 0.46 and 4.6 mg exudate g^{-1} dry soil, and chia seed exudate at 0.046, 0.46, 0.92, 2.3 and 4.6 mg exudate g^{-1} dry soil. Soil hardness and modulus of elasticity were measured at -10 kPa matric potential using a 3 mm diameter spherical indenter. 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^{-1} concentration for sandy loam soil. For a clay loam soil, root exudates did not affect soil hardness, whereas chia seed exudate increased soil hardness by 48% at 4.6 mg g^{-1} 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^{-1} concentration for sandy loam soil. For clay loam soil, chia seed exudate increased water repellency by 45%, whereas root exudates did not affect water repellency at 4.6 mg g^{-1} concentration. Water sorptivity and repellency were both correlated with hardness, presumably due to the combined influence of exudates on hydrological and mechanical properties of soils.

Key words

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

Introduction

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

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nutrients fluxes from soil to roots (Carminati et al., 2017; Oleghe et al., 2017; Hinsinger, 2009). It has been well documented in a number of species such as sorghum, wheat, and rice that root exudation decreases with the age of plants and increases with soil stress such as compaction, drought, and limited nutrient supply (Neumann et al., 2014; Aulakh at 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 polymerised 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., 2010).

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 over time due to microbial degradation. Traoré et al. (2000) also observed a significant increase in soil aggregate stability of soil by different substrates i.e. polgalacturonic acid, modelled soluble exudates, and maize root exudate. Czarnes et al., (2000) found that adding polgalacturonic acid and xanthan to soil increased tensile strength and stability against 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 stabilisation more rapidly in soils with swelling versus 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 the above-mentioned studies have either 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 behaviour, by coating soil particles and influencing soil water surface tension, they may also influence hydrological behaviour. The flow of water from soil to plant roots is controlled by the properties of soil in close contact with roots, known as the rhizosphere. Exudation is believed to strongly influence soil moisture dynamics in the rhizosphere (Bengough, 2012; Carminati et al., 2010 & 2016). Currently there are two concepts in the literature regarding hydraulic properties of the rhizosphere compared to bulk soil. The first is that polymeric gels present in root exudates increase the water holding capacity of soil on drying, but become significantly water repellent on re-wetting (Carminati et al., 2016; Ahmed et al., 2015; Moradi et al., 2011, Carminati et al., 2010). The second case is decreased water holding capacity of the rhizosphere on drying and more rapid re-wetting due to surfactants in root exudates (Dunbabin et al., 2006; Whalley et al., 2004; Read et al., 2003, Hallett et al., 2003). Soil water retention and 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 of known physico-chemical 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

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quantifies the mechanical properties Youngs 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 behaviour 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 as it concentrates stress over a very small area (Zhang and Li 2014), which in soil could be a few interacting particles.

We employed these rhizosphere-scale mechanical and hydrological tests to measure soil mechanical stability, soil water sorptivity, and water repellency index influenced by barley (*Hordeum vulgare L. cv. Optic*) and maize (*Zea mays L. cv. Freya*) root exudates. The impact of barley and maize root exudates were then compared with chia (*Saliva hispanica*) seed exudate, a commonly used root exudate analogue (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. This combined data will improve our understanding of rhizosphere development and function, and allow for the inter-dependence 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 (*Hordeum vulgare* L. cv. Optic) and maize (*Zea mays* L. cv. Freya) seeds were surface-sterilised in sodium hypochlorite solution (2%) for 10 min, then rinsed thoroughly in sterile deionised water. Sterilised seeds were pre-germinated on 0.5% distilled water (DW) agar until the radicals were approximately 1 cm long (2-3 days post germination). After discarding poorly germinated seeds, 180 individuals each of barley or maize plants were grown, successively, in a 60 litre aerated hydroponic tank. Nutrient solutions used in the aerated hydroponic tank were changed every 3 days beginning with ¼ strength, followed by ½ strength, and continuing to full strength until harvest. Plants were harvested after 2 weeks of growth. Exudates were collected for 12 hours in 150 ml pots containing 75 ml DW with a set amount of plants per pot (barley = 5 or maize = 3). Plants were removed from the pots the following morning (12 hour collection period) and the remaining liquid in the collection pots was first frozen at −20 °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 tested 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 ± 0.9 and 6.4 ± 1.7 mg individual^{-1}, 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 amount of carbon and nitrogen present in

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

Extraction of chia (Salvia hispanica) 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 Ahmed et al. (2014), by mixing 100 g of distilled water with 10 g of Chia seeds using a magnetic stirrer for 2 min at 50^{o}C, followed by cooling to room temperature (20^{o}C) over four hours in sealed containers. The exudate was separated from the seeds by repeatedly pushing the mixture through a 500 µm 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 5 repeated extraction attempts. The extracted chia seed exudates were freeze-dried so that its concentration was obtained i.e. 9.2 mg dry exudate 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 ± 0.03 g g^{-1} total exudate on seeds, only 0.10 ± 0.02 g g^{-1} of seed exudate was harvested, so the extraction efficiency was 77 ± 8 %.

Selection and preparation of soils

Soils were sampled from Bullion field, The James Hutton Institute, Dundee, UK (56^{o} 27′ 39″ N & 3^{o} 04′ 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. North Bullion soil is a Haplic Cambisol and was under fallow. The bulk soils were sampled from both locations at 0-100 mm depth. The soils were partially air-dried to 15 g water 100g^{-1} dry soil, and then passed through a 2 mm sieve and stored at 4 ^{0}C before any measurements started. The soil texture was determined by the combination of wet sieving and hydrometer methods. The amount of carbon and nitrogen present in soils were measured using CNS elemental analyser (CE Instruments). The pH_$CaCl_2$ of the soils was measured using a pH meter (Hanna Instruments, Bedfordshire, UK).

The sieved soils were mixed with either distilled water (control) or each of the exudates: barley root exudate, maize root exudate, and chia seed exudate, bringing all to 0.2 g g^{-1} water content. As the soils were at 0.15 g g^{-1} water content before amendments were applied, only 0.05 g g^{-1} additional water or exudate solution was added, so potential ionic impacts of distilled water were assumed to be minimal. Barley and maize root exudates were amended with 0.46 and 4.6 mg exudate g^{-1} dry soil. Chia seed exudate was amended at five concentrations i.e. 0.046, 0.46, 0.92, 2.3, and 4.6 mg exudate 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 cm^{-3} bulk density at 0.2 g g^{-1} water content. This provided homogeneous samples to test both the effects of different exudates on hydrological and mechanical properties of 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-Bsysteme) at 4^0C to suppress microbial decomposition of the exudates. One indentation measurement was carried out 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 Figure 2. The soil hardness parameter (Oliver and Pharr, 1992) was obtained using Eq. 1:

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

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

$$A_c = \pi\,(2h_cR - h_c^2) \qquad (2)$$

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

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

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

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

where ε is a geometric constant, which for a spherical indenter is theoretically equal to 0.75 (Fisher and Cripps 2011). S is the stiffness i.e. the initial slope of the unloading curve as shown in Figure 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) using Eq. 5:

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

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

$$E = (1-v_s^2)E_r$$

where v_s is the Poisson ratio of soil, assumed as 0.30. The E was reported in addition to hardness as 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 Figure 1.

Figure 1

Figure 2

Effect of indentation size on soil hardness and elasticity

The effect of indentation depth on soil hardness and modulus of elasticity was estimated using a series of loading-unloading cycles with increasing indentation depth as shown in Figure 3a. The soil hardness and modulus of elasticity for each loading-unloading cycle was determined and plotted as a function of indentation depth as show in Figure 3b. Generally there was large variation in soil hardness and modulus of elasticity for an indentation depth shallower than 0.5 mm (Figure 3b) because of surface roughness of soil cores. In almost all soil cores soil hardness and modulus of elasticity data became stable for an indentation depth deeper than 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 of elasticity (E_u) for each soil core as shown in Figure 3b. The H_u and E_u data are reported in rest of the paper.

Figure 3

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 of tip radius 1 mm: (1) water sorptivity, (2) ethanol sorptivity, and (3) 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 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

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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) using Eq. 6:

$$S_W \; or \; S_E = \sqrt{\frac{Qf}{4br}} \qquad (6)$$

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

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

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

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 behaviour 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 appreciate these potential artefacts.

Figure 4

Statistical Analysis

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

Results

The carbon and nitrogen 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 significant difference between soils in carbon contents ($p < 0.05$).

Table 1

Table 2

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

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4.6 mg g^{-1} concentration ($p < 0.05$) for sandy loam soil (Fig. 5). Clay loam soil showed significantly greater hardness compared to sandy loam soil. However, this difference was overcome by the addition of barley root exudate at 4.6 mg g^{-1} concentration and maize root exudate at both 0.46 and 4.6 mg g^{-1} concentration. Both barley and maize root exudates at any of the studied concentrations did not improve soil hardness for clay loam soil (Figure 5). Soil hardness was sharply increased at lower chia seed exudate concentrations for sandy loam soil ($p < 0.05$), whereas a 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^{-1} chia seed exudate concentrations for 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^{-1} chia seed exudate concentration for the clay loam soil. Similar to soil hardness, modulus of elasticity of sandy loam and clay loam soils were influenced by barley root exudate, maize root exudate and chia seed exudate at various concentrations (Figure 5).

Table 3

Figure 5

On air-dried samples, water sorptivity was significantly decreased ($p < 0.05$) for sandy loam soil treated with barley root exudate at 0.46 and 4.6 mg g^{-1} concentrations. Clay loam soil has significantly lower water sorptivity compared to sandy loam soil ($p < 0.05$), and it is not influenced by the barley root exudate (Figure 6). Water sorptivity was significantly decreased by maize root exudate at 4.6 mg g^{-1} concentration for both sandy loam and clay soils ($p < 0.05$). Water sorptivity was exponentially decayed for both sandy loam and clay loam soils amended with increasing chia seed exudate concentrations ($p < 0.05$). Water sorptivity was reduced by 30% for sandy loam soil and 37% for clay loam soil treated with chia seed exudate at 4.6 mg g^{-1} concentration (Figure 6). Ethanol sorptivity was also significantly less for sandy loam soil treated with both barley and maize root exudates at 0.46 and 4.6 mg g^{-1} concentrations. Ethanol sorptivity for sandy loam soil was unaffected with increasing chia seed exudate concentration. Ethanol sorptivity for clay loam soil was significantly lower compared to sandy loam soil ($p < 0.05$), and it remains unaffected by treating soils with barley root exudate, maize root exudate and chia seed exudate (Figure 6). Water repellency for clay loam soil was significantly higher compared to sandy loam soil ($p < 0.05$). Water repellency for both sandy loam and clay loam soils were not influenced by barley root exudate at 0.46 and 4.6 mg g^{-1} concentrations ($p < 0.05$). Water repellency was significantly greater by 23% for sandy loam soil treated with maize root exudate at 4.6 mg g^{-1} concentration ($p < 0.05$). Treating clay loam soil with maize root exudates did not impact its water repellency. Water repellency was linearly increased for sandy loam soil treated with increasing concentrations of chia seed exudate ($p < 0.05$). Water repellency was increased by 48% for sandy loam soil and 46% for clay loam soil treated with chia seed exudate at a concentration of 4.6 mg g^{-1}. Water repellency was increased logarithmically with increasing chia seed exudate concentration for clay loam soil ($p < 0.05$), and it was significantly higher compared to sandy loam soil (Figure 6).

Figure 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 soil. A possible explanation lies in the physico-chemical characteristics of the exudates (Naveed et al., 2017). The barley root exudate consists of the greatest amount of organic

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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 exudate, respectively (Naveed et al., 2017). The anionic forms of organic acids present in root exudates may be increasing the net negative charge of clays that 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 sandy loam soil upon the addition of barley root exudate at 4.6 mg g^{-1} concentration could be explained by two factors. The first is increased drainage of the soil at -10 kPa (Table 3) driven by lowering of surface tension of soil solution by the exudate (Table 4). Secondly, although care was taken to minimise microbial decomposition of added exudates by storing and equilibrating samples at 4°C, some compounds may be metabolised to produce longer-chain compounds. We plan to 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 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 chia seed exudate, used in the present study, showed an exaggerated effect on soil mechanical properties compared to 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 initial soil properties, such as texture. An insignificant effect of both barley and maize root exudation on soil hardness and modulus of elasticity for clay loam soil (Fig. 5) was most likely driven by the greater impact of clay minerals and possibly soil organic matter on interparticle bonding compared to the sandy loam soil. If soil was already in a stable condition, such as 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.

Table 4

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 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 it is assumed that all of the exudate remains in the pore water, then at 4.6 mg g^{-1} barley exudate concentration, it has a surface tension of 45.59 mN m^{-1} (Table 4). Sorption of exudates to soil surfaces may decrease 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 soil solution as a result of barley root exudation might overcome soil water repellency by acting as a surfactant. Strong negative correlations were observed

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between surface tension of barley root, maize root and chia seed exudates solutions and water sorptivity of exudates 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 4.6 mg g^{-1} concentration the marked decrease in water sorptivity and greater repellency index (Fig. 6) for 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^{-1} compared to 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 exudates solutions and water sorptivity of exudates treated soils i.e. sandy loam (R^2 = 0.97) and clay loam (R^2 = 0.90). Ethanol sorptivity was significantly lower for 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. As the hydraulic measurements were conducted on air-dried soils, greater sorption of exudates onto soil surfaces may have exacerbated the impacts of exudates compared to 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), that the rhizosphere of maize stayed temporarily dry after irrigation. Both this study and Carminati et al.'s (2010) investigation of the lupin rhizosphere observed the development of water repellency when the rhizosphere dries beyond a critical threshold, which was attributed to root exudates. Whilst maize root exudates influenced the development of water repellency in the sandy loam soils, for the clay loam soil the greater initial water repellency was likely driven by past soil management accumulating organic matter (Fig. 6). However, chia seed exudate, that is strongly hydrophobic in nature, significantly increased soil water repellency with increasing concentration for both sandy loam and clay loam soils.

Interaction between mechanical and hydraulic properties of soils

Significant negative correlations were observed between water sorptivty and soil hardness for both 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 decrease water transport through either pore clogging or water repellency. Pore clogging was less likely as 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 to 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 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; (1) coating of soil particles to form water repellent surfaces and (2) binding soil particles and thus making soil more stable.

Figure 7

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

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rhizosphere-scale hydrological measurements, including limitations to the approach caused by experimental artefacts 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 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 a scope to enhance the indentation approach to estimate fracture mechanics properties of the soil (Chen et al. 2016), which would be a useful technique given traditional approaches with notched bars require remoulded 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 upon their origin, have differing impacts on water transport and mechanical stability of the rhizosphere. Soil water repellency was measured at air-dry condition 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 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 stabilisation 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 stabilisation between 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/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 on 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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Figures Captions

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.

Figure 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.

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.

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

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 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.

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^{-1} 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).

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

Exudates	Carbon	Nitrogen	Carbon/Nitrogen
–	($g\ 100g^{-1}$)		–
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

Table 2: Physical properties of the studied soils.

Soils	Clay	Silt	Sand	Carbon	Nitrogen	Soil pH_Cacl_2	Texture class
	($g.\ 100g^{-1}$)						
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

Table 3: Volumetric water contents at -10 kPa matric potential for soils treated with different exudates (concentration is in mg exudate g^{-1} dry soil).

Soil	Exudate treatment	Concentration (mg g^{-1})	Volumetric water content (m^3 m^{-3})
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

Table 4: Surface tension and apparent viscosity at zero-shear rate of exudates solution at a concentration of 4.6 mg exudate g^{-1} water.

Exudates solution	Surface tension (mN m^{-1})	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

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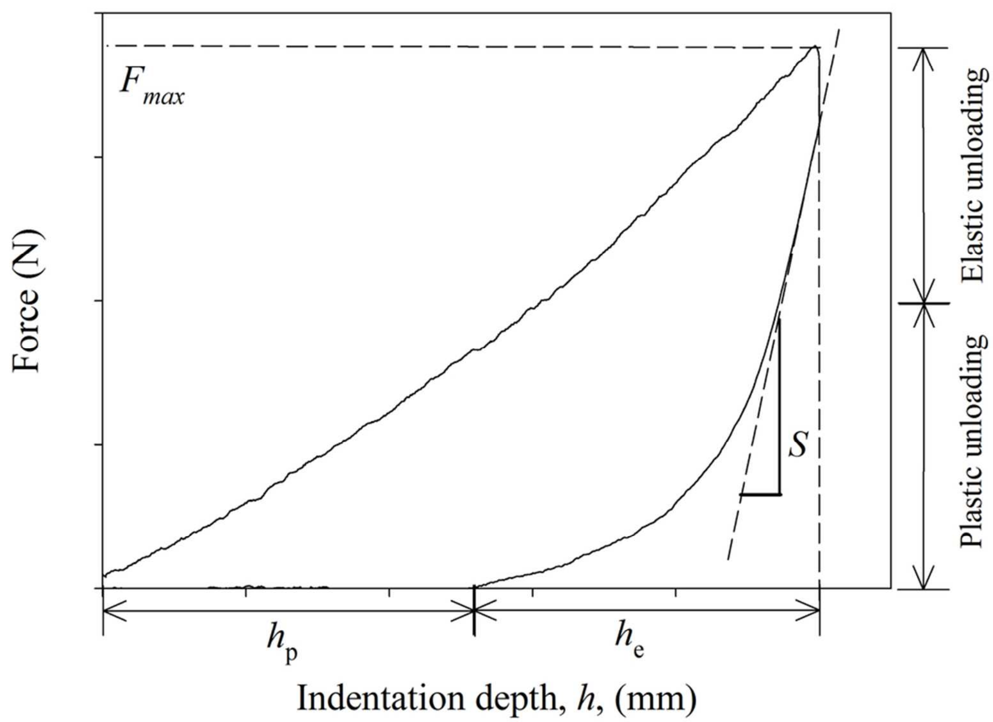

Figure 1: Typical load-displacement curve obtained from an indentation test, Fmax = maximum measured force for the required indentation, he = elastic indentation i.e. deformation recovered on unloading, hp = plastic indentation i.e. permanent deformation and not recovered on unloading and S = stiffness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

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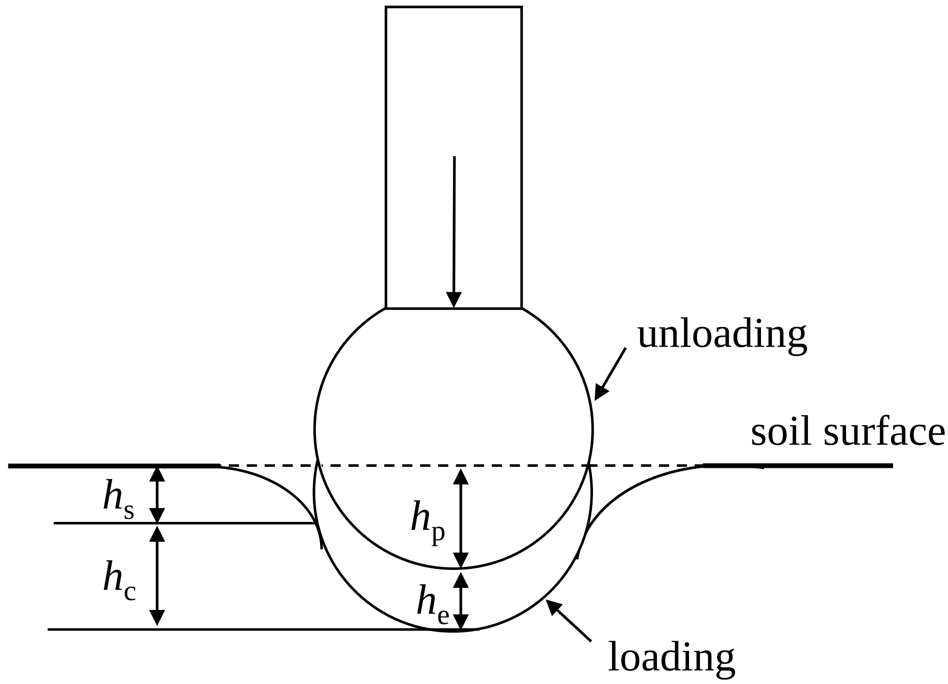
unloading
soil surface
h_s
h_p
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loading

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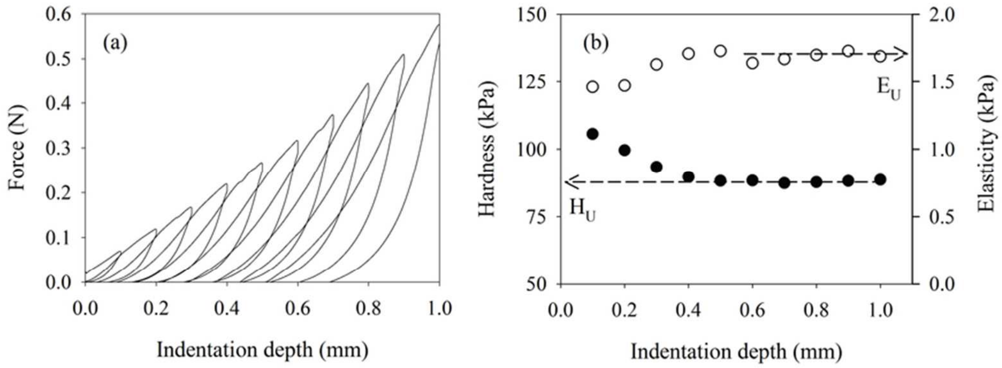

Figure 3: (a) 10 loading-unloading cycles adopted during an indentation test and (b) corresponding soil hardness and modulus of elasticity were plotted as a function of indentation depth, Hu and Eu are average hardness and elasticity for 0.6, 0.7, 0.8, 0.9, and 1.0 mm indentation depths.

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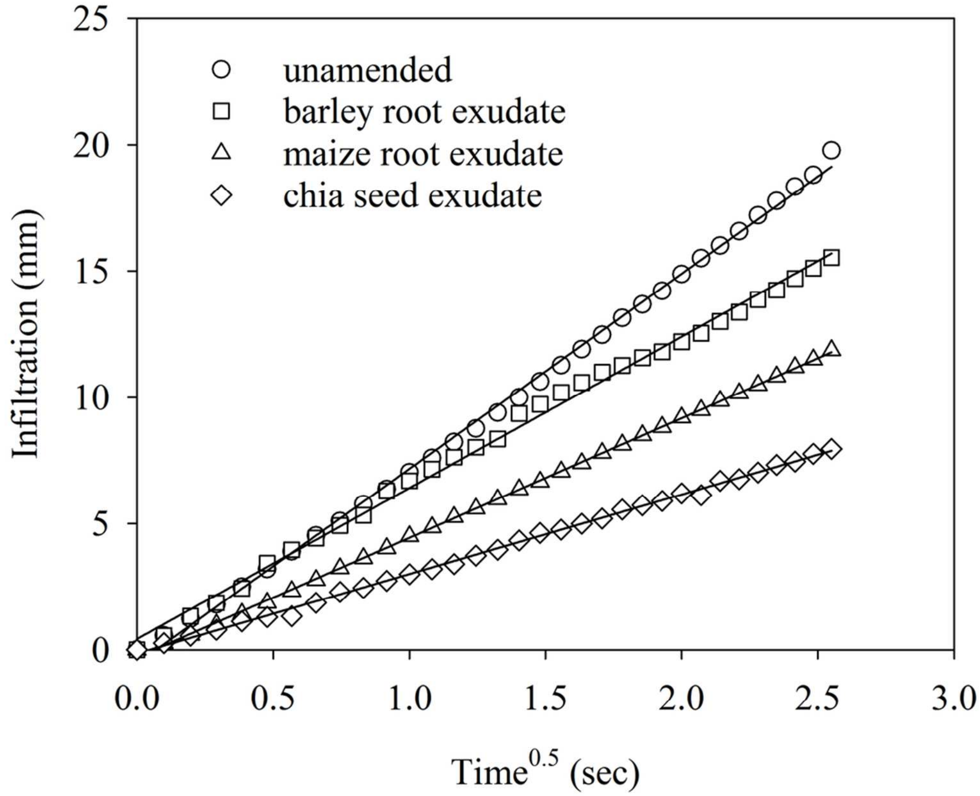

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93x76mm (300 x 300 DPI)

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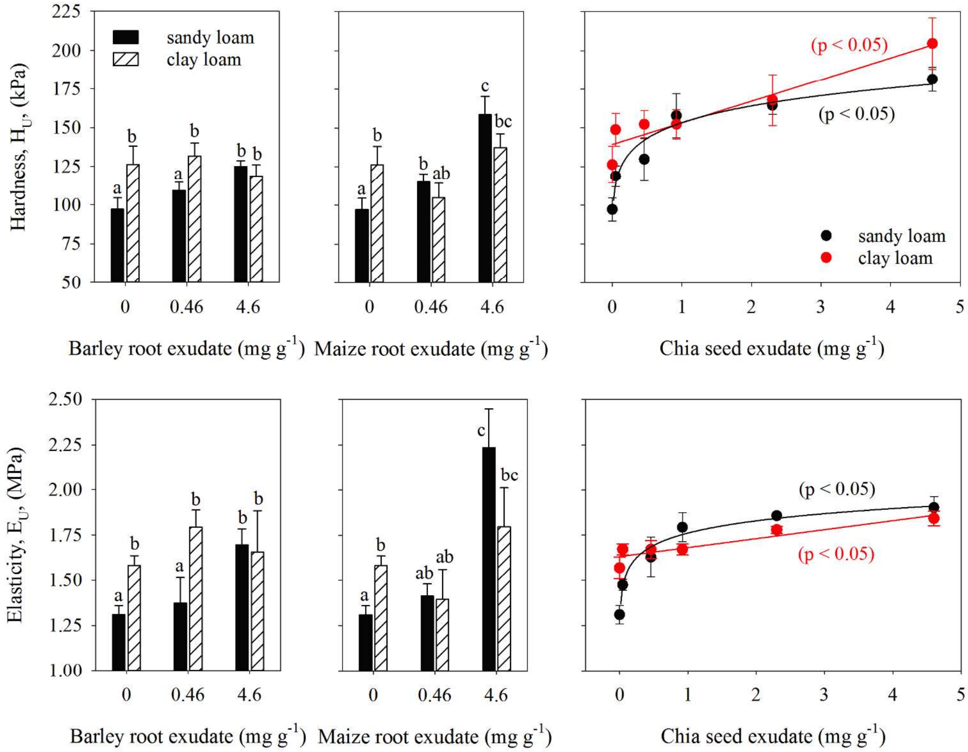

Figure 5: Hardness and modulus of elasticity of sandy loam and clay loam soils treated with barley root, maize root, and chia seed exudates at different concentrations in mg exudate g-1 dry soil measured at -10 kPa matric potential; different letters indicate significant difference between treatments 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.

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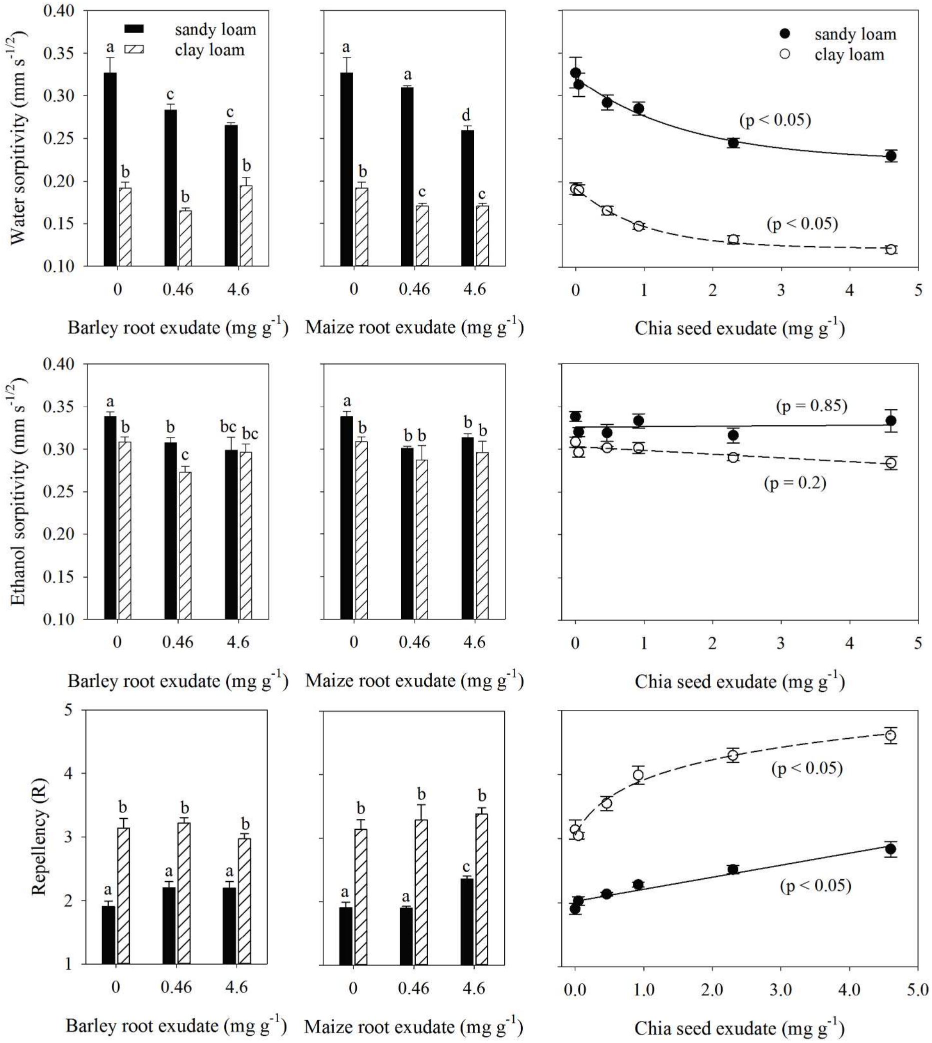

Figure 6: Water sorptivity, ethanol sorptivity, and water repellency of sandy loam and clay loam soils treated with barley root, maize root, and chia seed exudates at different concentrations in mg exudate g-1 dry soil measured at air dry condition; different letters indicate significant difference between 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.

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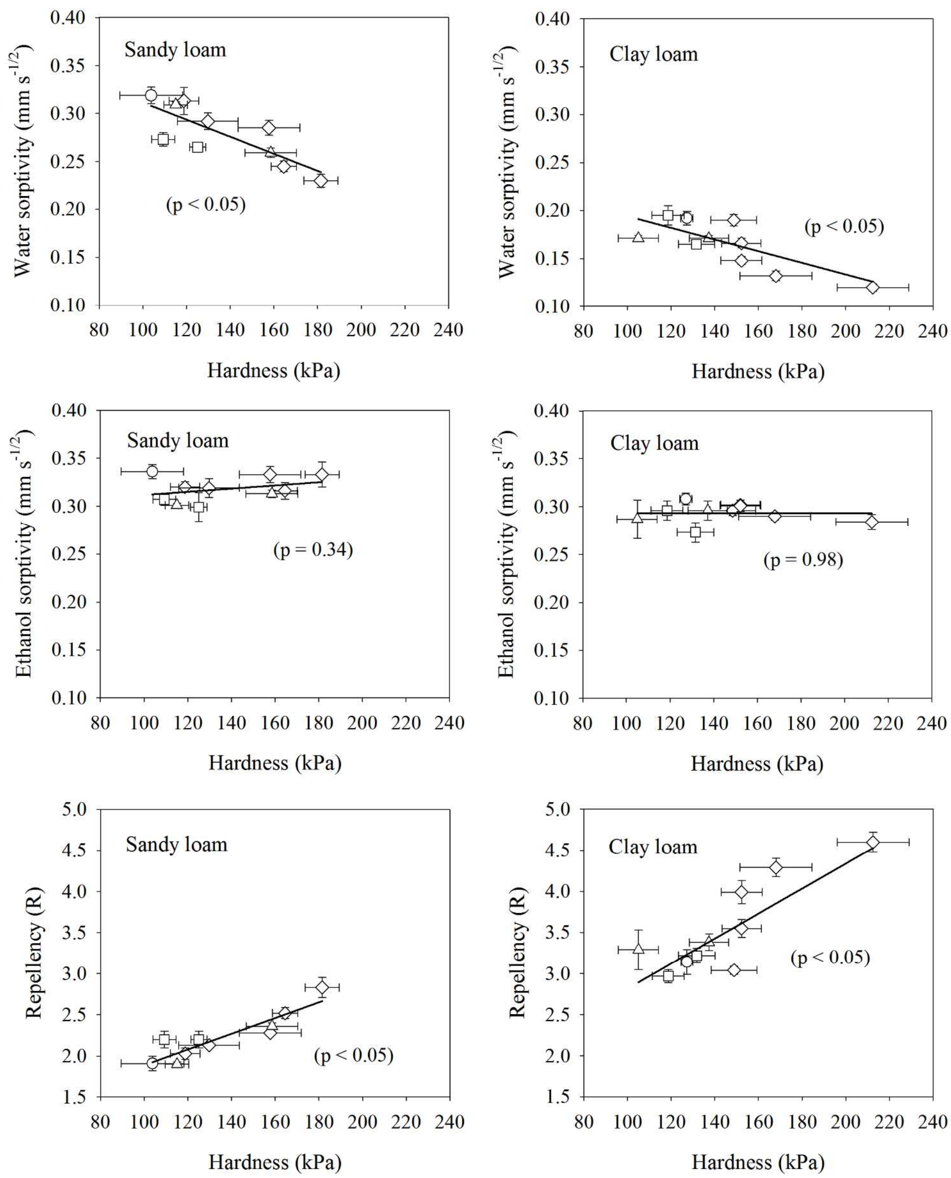

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