Plant mucilage increases pull-out resistance of root analogues from soil

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Abstract: The interface between plants roots and soil is strongly affected by rhizodeposits, especially mucilage, that change mechanical and hydrological behaviour. In addition to impacts to aggregation, water capture and root penetration, rhizodeposits may also affect the pull-out resistance of plant roots. Due to the complex architecture of plant roots and an inability to restrict rhizodeposit production, this study used a simplified system of wooden skewers to simulate roots, and chia seed mucilage as a model to simulate to simulate rhizodeposit compounds. Pull-out tests were then carried out to measure the impacts of mucilage, and one (WD1) or two (WD2) cycles of wetting and drying of soils. Using a mechanical test frame, the maximum pull-out resistance ($F_{\max}$) and pull-out displacement (dL) were recorded, allowing for pull-out energy (E), average pull-out force ($\bar{F}$) and bond strength ($\tau_{\max}$) to be calculated. The results showed that all pull-out parameters of the samples with added rhizodeposit compounds tended to decrease between WD1 and WD2, but they were still significantly greater than without the added mucilage. The model rhizodeposit increased all pull-out parameters by a minimum of 30%. With an additional wet-dry cycle, the mucilage tended to cause a decline in pull-out parameters relative to a single wet-dry cycle. This suggests mucilagess could enhance the mechanical resistance of roots to pull-out, but resistance decreases over time with cycles of wetting and drying. To conclude, an important role of mucilage is pull-out resistance, which has relevance to plant anchorage and root reinforcement of soils.

Highlights

- Plant mucilage could greatly increase the maximum pull-out resistance of roots in soil.
- Mucilage enhanced the binding between root system and soil.
- Cycles of wetting and drying decreased the effect of mucilage on the pull-out strength of the root-soil composition.

Keywords: rhizodeposits, pull-out resistance, cycles of wetting and drying, root-soil interaction, soil reinforcement, mucilage

Introduction

Plant anchorage is one of the major functions of plant roots (Goodman & Ennos, 1999). Root architecture is a major driver of anchorage, both at large scales by the physical dimensions of the root system and its branching properties, but also at small scales from the root-soil interface (Hamza *et al.*, 2006). At the root-soil interface, root hairs may provide tiny anchors into soil that increase pull-out resistance and provide a brace to improve root penetration (Bengough *et al.*, 2016; De Baets *et al.*, 2020). Another root-soil interface property that may influence anchorage is the deposition of mucilages and exudates by the root into soil. These rhizodeposits have a large influence on the mechanical and hydraulic properties of the soil. On the one hand chemicals such as organic acids and sugars in rhizodeposits can alter the mechanical properties of the soil, such as fracture toughness, compression resistance, hardness and resistance to penetration (Hallett, 2009; Peng *et al.*, 2011; Arnold *et al.*, 2015; Oleghe *et al.*, 2019). On the other hand, rhizodeposits can alter the fluid properties of the aqueous phase in the rhizosphere and affect the hydraulic properties of the soil, such as water absorption, rewetting rate after drying, water evaporation and hydraulic conductivity (Czachor *et al.*, 2013; Oleghe *et al.*, 2017; Cooper *et al.*, 2018). These effects are also influenced by soil type and plant species, the drying history of the soil, and other factors (Naveed *et al.*, 2017).

The properties of rhizodeposits could impact mechanical pull-out resistance of roots. Due to the variability of hydrological processes at the root-soil interface, wetting or drying of the soil have the potential to either increase or decrease pull-out resistance due to aggregation and water repellency (Carminati *et al.*, 2010; Boldrin *et al.*, 2022; Montaldo & Oren, 2022). From our current understanding, we hypothesise that mucilage in rhizodeposits will enhance the pull-out resistance of roots due to impacts on mechanical and hydrological properties at the root-soil interface. With weathering, such as cycles of wetting and drying, pull-out resistance will gradually decrease due to aggregation, water repellency, leaching and decomposition. These processes have not been studied to date, hindered by methodological limitations of controlling rhizodeposit inputs from live plant roots.

In this study we overcame this constraint by using a model system consisting of wooden skewers to simulate roots, and injected chia seed mucilage as a model rhizodeposit. The simple shape of the wooden skewer removes the impacts of root branching on pull-out resistance, while providing a model root with similar mechanical properties to woody roots (Hamza *et al.*, 2006). Because this experiment requires a large amount of rhizodeposit and it is difficult to collect rhizodeposits from live roots, we used chia seed mucilage. Whilst not identical in composition and behaviour to root rhizodeposits, chia seed mucilage has several advantages including harvestable quantity and reproducibility. It presents analogous mechanical properties to maize and lupin root exudates making it a popular model exudate in rhizosphere studies (Ahmed *et al.*, 2014; Naveed *et al.*, 2017; Oleghe *et al.*, 2017; Naveed *et al.*, 2018; Oleghe *et al.*, 2019). Pull-out tests were carried out on samples with and without the addition of chia seed mucilage after alternating wetting and drying treatments. The pull-out tests recorded the pull-out resistance and displacement, which were used to quantify the pull-out energy and bond strength. If this model rhizodeposits alters ‘root’ pull-out resistance when applied at the soil interface, then it will indicate an ability for rhizodeposits to influence root anchorage.

Materials and Methods

Preparation of rhizodeposit analogue

Chia seed mucilage extraction followed Naveed *et al.* (2017). This involved mixing 100 g distilled water with 10 g chia seeds using a magnetic stirrer for 2 min at 50 ℃. After cooling to room temperature (20 ℃) and standing for 4 hours, the seed mucilage was separated from the seeds by repeatedly pushing the mixture through a 500 μm sieve under pressure with a cut syringe. This approach harvested the easily extracted seed mucilage, with tightly bound mucilage remaining on the seeds.

Preparation of soil

A Dystric Cambisol sandy loam was sampled from the top 20 cm in south Bullion field located at the James Hutton Institute, Dundee, UK (56 °27′ 39″ N, 3 °04′11″ W). After sampling, the soil was air dried to 0.10 g g^{-1} water content and passed through a 2-mm sieve. It was then wetted to 0.14 g g^{-1} gravimetric water content. The amount of required water was calculated according to the initial moisture content of air-dried soil and the desired moisture content. The water was then sprayed onto the air-dried soil using a spray and stirred evenly. The soil was stored at 4 ℃ before any measurements started. More detailed soil properties can be found in Naveed *et al.* (2018).

Pull-out tests

The prepared soil was packed in 20 soil cores with a diameter of 5.5 cm and a height of 4 cm using a mechanical test frame (Zwick All Round Z5, ZwickRoell, Ulm, Germany) fitted with a 5 kN load cell accurate to 0.01 N. The packing pressure was 100 kPa and the speed was 10 mm min^{-1}. Application of this force resulted in a dry soil bulk density of 1.27 g cm^{-3} which was concurrent with field conditions. Packing was done in 1-cm layers to avoid edge effects. Using the mechanical test frame, wooden skewers with 3 mm diameter, as root analogs, were pushed vertically into the centre of the soil cores to a depth of 35 mm at a rate of 2 mm min^{-1}. All soil cores were saturated for 12 h and dried to -5 kPa matric potential (0.145 g g^{-1} gravimetric water content, equivalent to field capacity for the soil used), until water loss ceased (2-3 days), using a tension table (EcoTech MeBaystem GmbH, Germany) at 4 ℃. This was one cycle of wetting and drying (WD1).

In order to realistically simulate the release of rhizodeposits from the plant roots at the inter-root level, and to make the rhizodeposits as concentrated as possible in the inter-root region, we used a syringe and needle. Either 5 ml of chia seed mucilage with 0.02 mg g^{-1} solid concentrations or 5 ml distilled water was injected into a 2-cm diameter cycle around the root analogue (Fig. 1). Half the samples had pullout tests completed immediately one day after the application of root exudates (WD1), the other half were dried at 40 ℃, re-saturated and re-equilibrated to -5 kPa water potential (WD2), and tested like the samples after WD1. For the pull-out test, the root analogue was clamped in a drill chuck, and pulled vertically upwards out of the soil at a speed of 10 mm min^{-1}. There were five replicates of each treatment.

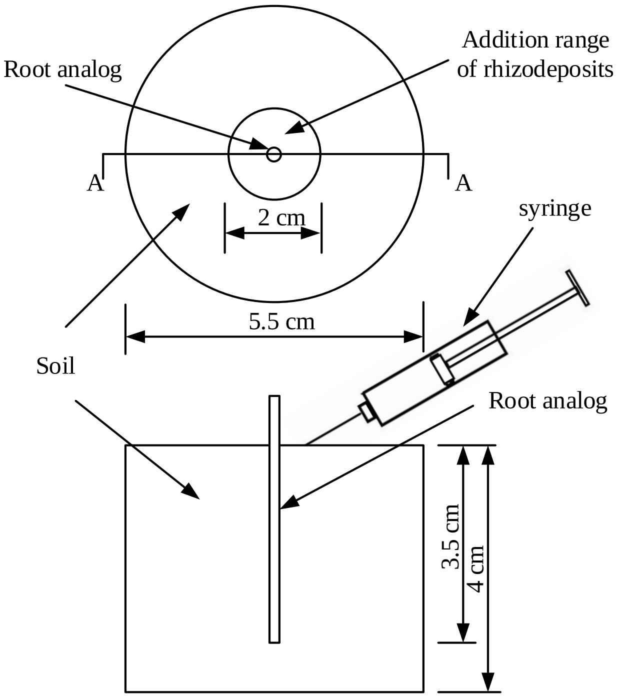

Fig. 1. Dimensions of a pullout specimen and layout of root analogues

Measurements of root analogue pull-out properties

The pull-out force (F, N) and displacement (dL, mm) of the root analogues were recorded by Zwick TestXpert software during the pull-out tests. Pull-out energy was defined as the integration of pull-out force and pull-out displacement based on the pull-out curve up to the pull-out displacement at the maximum pull-out force. It was obtained using:

$$E = \int_0^L F(x)\mathrm{d}x \tag{1}$$

where E is the pull-out energy, $F(x)$ is the pull-out force, dx is the displacement, and L is the displacement at the maximum pull-out force $F_{\max}$. The average bond strength between root analogue and soil was obtained following Chan and Chu (2004):

$$\tau_{\max} = \frac{F_{\max}}{\pi D l} \tag{2}$$

where $\tau_{\max}$ is the bond strength, D is the diameter of the root analogue (3 mm), and l is the penetrated length of root analogues (35 mm). The average pull-out force $\bar{F}$ was calculated by:

$$\bar{F} = \frac{F_{max}}{\pi D} \tag{3}$$

where is, F_{max} is the maximum pull-out force.

Statistical analysis

The data were analyzed using SPSS 20.0. The statistical differences of the variables between the rhizodeposit treatments were tested using paired-samples T-test at $P < 0.05$.

Results and Discussion

Pull-out behaviour of model roots

The pull-out curves of the model roots were affected by both model rhizodeposit application and cycles of wetting and drying of the soil (Fig. 2). At the onset of pulling, there was a linear elastic response until debonding occurred and it became plastic. Debonding occurred at a greater displacement when rhizodeposits were present (Fig. 3c), and increased further after a wetting and drying cycle. With increasing pull-out displacement, the pull-force continued to increase until F_{max}, followed by a continuous drop in force as the model root was pulled from the soil. F_{max} occurred at greater root pull-out displacements when rhizodeposits were added (Fig. 3a), while wetting and drying cycles attenuated this effect, but it was still greater than in specimens without the addition of rhizodeposits. This may be due to the fact that Chia seed mucilage contains more polysaccharides and less organic acids (Griffiths *et al.*, 2005; Goh *et al.*, 2016). The polysaccharide in the mucilage likely gelled the soil, thus creating a more stable soil structure around the roots. This would improve the pore structure and water retention of the soil and enhance the interaction of the root analogues with the soil (Song *et al.*, 2009; Ren *et al.*, 2021). However, drying and wetting cycles likely diluted the rhizodeposit at the interface with soil and induced aggregation (Zarebanadkouki & Carminati, 2014; Zhu *et al.*, 2022), resulting in decreased pull-out resistance that was still greater than without added rhizodeposit compounds.

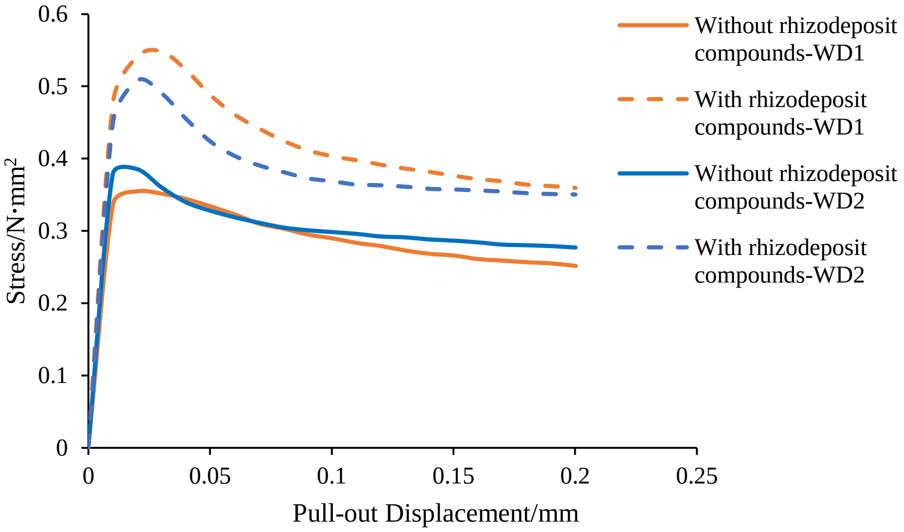

Fig. 2. Typical pull-out displacement curves of root analogues exposed to one (WD1) or two (WD2) wet-dry cycles without (solid lines) and with (dashed lines) rhizodeposit amendment.

Pull-out parameters

Rhizodeposit analogue amendment increased $\bar{F}$ and τ_{max} by 51% (WD1) and 30% (WD2), increased dL by 40% (WD1) and 33% (WD2), and increased E by 98% (WD1) and 78% (WD2) (Fig. 3). Most of the pull-out parameters, F_{max}, dL, E and τ_{max}, were significantly greater in samples with rhizodeposit compounds, apart from dL under WD1, and $\bar{F}$ and E under WD2. Without the addition of rhizodeposit compounds, $\bar{F}$ and τ_{max} increased slightly, but dL and E decreased with an additional wetting and drying cycle. This was probably due to wetting and drying cycles altering soil agglomerate structure, changing the mechanical and hydraulic properties of the soil. An additional wetting and drying cycle in the presence of rhizodeposit compounds decreased $\bar{F}$, dL, E and τ_{max}, but these parameters were still greater than for samples without rhizodeposit amendment.

This ideal experiment using controlled inputs of rhizodeposit compounds and a simple root geometery, demonstrated the importance of root-soil interface properties driven by rhizodeposits on pull-out resistance. In nature these effects will be mediated by a range of processes. Numerous studies have demonstrated that light wetting and drying of the soil promotes plant growth and the production of rhizodeposits, thus improving the rhizosphere environment of the plant (Zhang *et al.*, 2017). Moreover, with the extension of time, the rhizodeposit, as a transient organic mechanical agent, may interact with soil microorganisms, which can either decrease bonding through decomposition or increase bonding by the production of secondary exopolymeric substances (Naveed *et al.*, 2017; Bordoloi *et al.*, 2020).

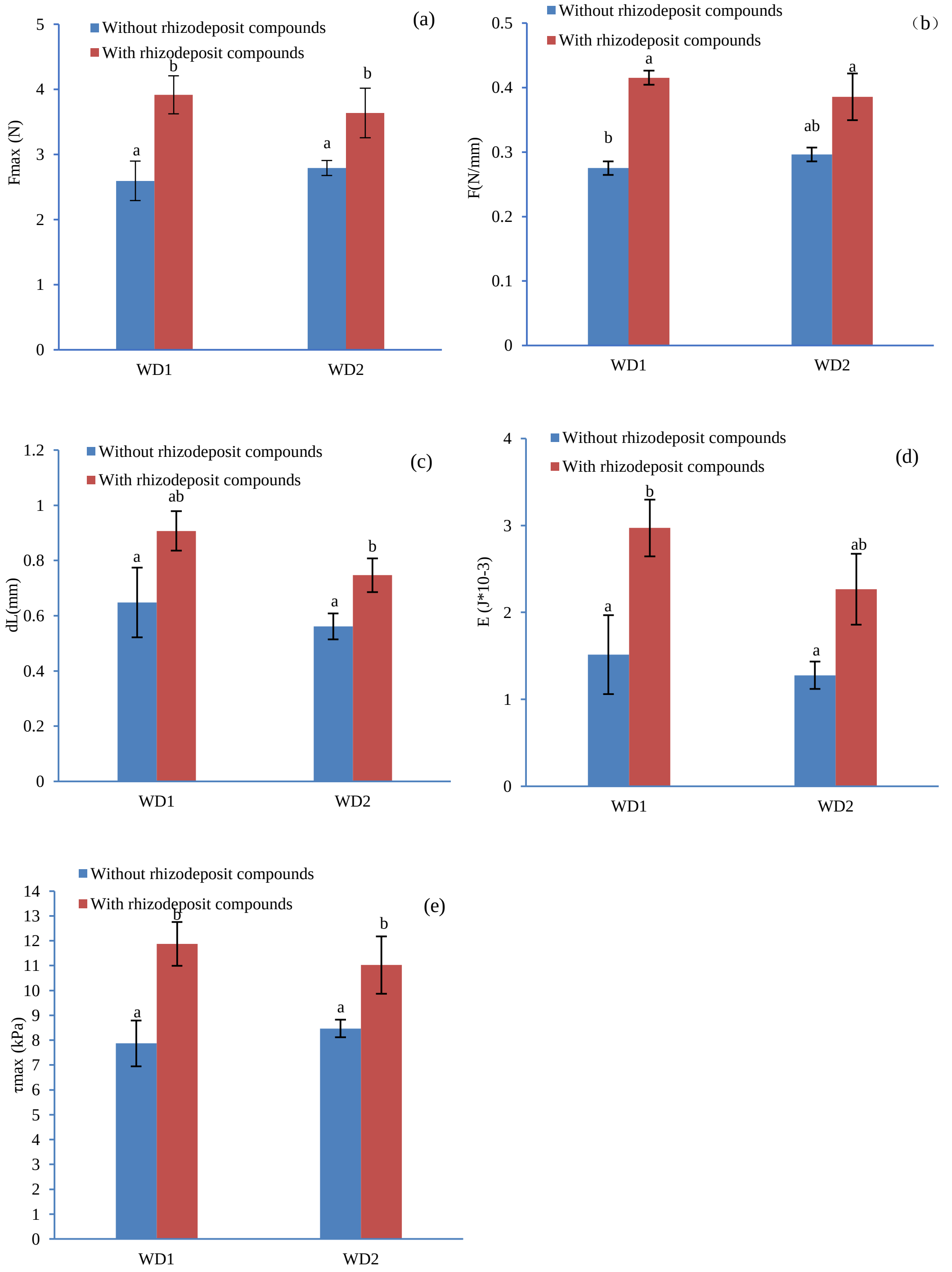

Fig. 3. Mechanical pullout properties of root analogue affected by both rhizodeposit amendment and cycles of wetting and drying (a. the maximum pull-out force, b. average pull-out force, c. pull-out displacement, d. pull-out energy, e. bond strength.)

The error bars are the standard deviation of five replicates. Different lowercase letters indicate significant differences between treatments (*P*<0.05). WD1 and WD2 indicate one cycle of wetting and drying and two cycles of wetting and drying respectively.

Conclusion

Rhizodeposit compounds, simulated here with chia seed mucilage, caused a marked increase to the pull-out resistance of a model root from soil. It is likely that the polysaccharides and other compounds in rhizodeposits caused localized bonding that increased adhesion between the root analogue and soil. With cycles of wetting and drying there was a tendency for all pull-out parameters to decrease when rhizodeposits were added, but they were still significantly greater than without the addition of rhizodeposits. This preliminary study using controlled testing conditions isolated the potential impacts of rhizodeposits on the pull-out resistance of roots. More in-depth examinations could be carried out using real root exudates or simulating root exudates having a variety of compounds. Moreover, root-soil interface impacts of rhizodeposits on bonding, water repellency and soil aggregation could be explored in greater detail to quantify anchorage mechanisms. In assessing the pull-out resistance of plant roots, the impacts of rhizodeposits have been overlooked. Experiments exploring rhizodeposition responses to mechanical surface stresses such as wind may potentially unravel another plant trait that responds to environmental conditions to improve anchorage.

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Declaration of interest

The authors declared that they have no conflicts of interest to this work.

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