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Effects of mucilage concentration at different water contents on mechanical stability and elasticity in a loamy and a sandy soil

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Abstract

Mucilage released by plant roots affects hydrological and mechanical properties of the rhizosphere. The aim of this study was to disentangle the effects of the factors mucilage and soil moisture on a range of soil mechanical parameters in a sand and a loam. Both substrates were homogenised and filled into cylinders at bulk densities (ρ_b) of 1.26 and 1.47 g cm^{-3} for loam and sand, respectively. Chia seed (*Salvia hispanica* L.) mucilage concentrations of 0, 0.02, 0.2 and 2 g dry mucilage kg^{-1} dry soil were tested at four different gravimetric water contents in loam ($\theta_g = 0.34$, 0.19, 0.14 and 0.09 g g^{-1}) and three in sand ($\theta_g = 0.20$, 0.06 and 0.04 g g^{-1}). To quantify the influence of water content on the effect of mucilage on mechanical soil properties, two sets of samples were prepared, one for a micro penetrometer test, the other to measure bulk soil properties. Penetration tests were performed at 120 mm h^{-1} using a universal testing machine with a high-precision sensor equipped with a penetrometer conus resembling a root. Mechanical energies were determined by calculating the area of the time–force curves. The energy required for a root to grow in a loam at permanent wilting point was decreased from 0.31 J in the control to 0.26 J in the 2 g kg^{-1} mucilage treatment, whereas it increased from 0.05 J in the control to 0.08 J at the highest water content. Precompression stress (σ_{pc}), compression index (C_c), swelling index (C_s) and elasticity index were determined with a confined uniaxial compression test. σ_{pc} was increased by addition of mucilage in both substrates whereas the response on compressibility and elasticity was specific to substrate and water content. Here mucilage had a stronger impact on sand—the substrate with lower initial compressibility and elasticity. We conclude that the effect of mucilage on soil mechanical properties and subsequently on plant growth depends on the combined response of substrate and water content.

Highlights

- The influence of soil moisture on the mechanical effects of mucilage concentration was examined.
- Mucilage decreased the energy required for root growth in a loam at the permanent wilting point.
- Higher mucilage concentrations increased compressibility and elasticity in sand.
- Effect of mucilage on mechanical parameters depends on interaction between substrate and moisture.

KEYWORDS

compression index, desorption curve, elasticity index, mechanical energy, mechanical stability, mucilage, penetration resistance, pre-compression stress, root growth, swelling index

1 | INTRODUCTION

In the past decades, many studies have increased our understanding of how plants modify the soil they grow in. A growing root pushes aside soil particles (Fakih et al., 2017), modifying soil structure in the rhizosphere (Whalley et al., 2005). During its life span a root releases substances into the soil changing microbial habitats (Haichar et al., 2008), taking part in the water and nutrient cycling and affecting soil aggregation (Materechera et al., 1992). When a root decays its nutrients can be used by a wide range of soil organisms and its carbon is stored in aggregates of different stabilities (Gale et al., 2000). The site where once the root channel was is now a new habitat for soil organisms (Hoang et al., 2016) or provides easier root growth for new plants (McKee, 2001). With all these processes, plants modify the soil they grow in, changing its structure and the associated functions like nutrient and water cycling, carbon storage, gas transport or temperature response, which in turn are essential for any further growth of plants in this soil.

Taking a closer look at how plants modify the physical properties of the rhizosphere through root mucilage, the idea of amending soil with biological compounds such as polygalacturonic acid (PGA), maize root exudate, chia seed exudate and others as an analogue of the rhizosphere was adapted by numerous authors such as Czarnes et al. (2000) and Peng et al. (2011). Devices for extracting root mucilage harvest only very small amounts, far too little for the planned experiment. Zickenrott et al. (2016) harvested approximately 1.0 ml hydrated mucilage (or 5.68 mg dry mucilage) per 100 maize seeds using low centrifugal forces. Naveed et al. (2017) reported an average dry weight of 6.4 mg of root exudates for an individual maize plant. They observed that exudates of maize roots and chia seeds are chemically rather different and pointed out that chia seed mucilage has a more pronounced effect on soil physicochemical and mechanical parameters like viscosity, soil hardness and elasticity. They concluded that using chia seed mucilage as a substitute for root mucilage might not be the perfect solution but is justified as it acts in a similar way to maize root rhizodeposits increasing soil water retention (Naveed et al., 2019) and turning hydrophobic when drying (Ahmed et al., 2014).

The effect of mucilage on hydraulic properties are well studied (Ahmed et al., 2016; Benard et al., 2019; Brax et al., 2017; Carminati & Vetterlein, 2013; Kroener et al., 2014), whereas few articles examining its consequences on mechanical parameters have been published (Iijima, 2004; Oleghe et al., 2017). Mechanical soil strength influences the growth of roots into the soil and thus rhizosphere structure formation and resource exploitation. Adding mucilage reduces soil mechanical impedance to root growth (Iijima, 2004). Little is known about the interaction of water content and mucilage concentration with respect to changes in mechanical properties. To the best of our knowledge, none of the studies about the effect of mucilage on mechanical properties are dedicated to this topic but investigate the responses at a single water content (Naveed et al., 2018; Oleghe et al., 2017). By examining the impact of mucilage on penetration resistance (PR) at different water contents and combining this with the concept of energy required for root growth by Ruiz et al. (2015) this problem was addressed. We hypothesised that PR is lower at higher mucilage concentrations, but the amount of this decrease depends on the water content with stronger effects to be expected in the driest treatment.

Various authors reported improved mechanical stability of soil amended with different organic substances. Deng et al. (2015) found a stabilising effect of seed coatings of Shepherd's purse (*Capsella bursa-pastoris* L. Medik). Czarnes et al. (2000) amended soil with PGA and observed an increase of bond energy between particles resulting in higher stability. A stabilising effect of maize root mucilage and chia seed mucilage was reported by Naveed et al. (2019). Until now, only Oleghe et al. (2017) performed a compression test showing that mucilage amendment at a single defined matric potential (Ψ_m) resulted in higher compressibility. Few measurements have been done so far of the elasticity of bulk soil mixed with mucilage. The first mention of the importance of elastic components of pure maize root mucilage dates back to 1999 (Read et al., 1999). In a different context, Zhang et al. (2008) observed an increase in elasticity in clay amended soil with PGA after simulation of soil weathering. Naveed et al. (2018) reported increased elasticity of mucilage amended soil at −10 kPa.

Against this background, our hypothesis was that amendment with chia seed mucilage leads to a higher stability of bulk soil, as well as an increase in compressibility and elasticity. To test this hypothesis, another set of soil samples was measured with a confined uniaxial compression test giving information on the pre-compression stress (σ_{pc}) as a measure of mechanical stability, compressibility parameters in both the re-compression and the virgin compression curve (VCC) and the elasticity index (EI). In addition, here we expect an interplay between the factors mucilage and soil water content in their effect on mechanical soil behaviour, which, to the best of our knowledge, has not been studied so far.

2 | MATERIALS AND METHODS

2.1 | Substrates used

The two substrates used are the ones used in the DFG SPP2089 programme: (1) A loam originating from the 0 to 50 cm layer of a Haplic Phaeozem in Schladebach, Germany (51°18′31.41″ N; 12°6′16.31″ E), which was used for agricultural purposes prior to excavation. The material was pre-sieved to less than 20 mm and then stepwise sieved to less than 1 mm. (2) A sandy substrate (from now on called 'sand') consisting of 83.3% quartz sand (WF 33, Quarzwerke Weferlingen, Germany), which was mixed thoroughly with 16.7% loam. The loam consisted of 19.1% clay, 47.7% silt and 33.2% sand and the sandy substrate of 3.3% clay, 8.1% silt and 88.6% sand (Vetterlein et al., 2020). Particle density (ρ_s) of loam and pure quartz sand WF 33 was measured (VDLUFA, 1991) with five replicates and histol to permit the correction of calculations based on this value. Loam had a particle density of 2.4620 (±0.009) and WF 33 sand of 2.6558 (±0.002), resulting in a calculated value of 2.6234 for sand. Regarding calculations based on ρ_s, the importance of measuring this value becomes apparent when looking at the results. The value for loam differed substantially from the 2.65 g cm^{-3} that is generally accepted as an approximation for a wide range of soils in case ρ_s is not measured.

2.2 | Extraction of mucilage from chia seeds and sample preparation

Mucilage was extracted from chia seeds and mixed with soil, following the protocol suggested by Kroener et al. (2018) with some minor adjustments. The ratio of seeds to water (w/w) was set at 1:15 as this proved to be the most suitable ratio for the extraction device available. Deionised water and chia seeds were mixed on a magnetic stirrer for 2 h. A 10-cm diameter sieve with a mesh size of 2 mm was placed tightly on a conical flask with neck, which was connected to a sub-atmospheric pressure of up to −70 kPa. The sieve was filled with the mucilage-water mix and the mucilage extracted under continuous stirring. The procedure was repeated with the extracted substance using a mesh-size of 0.63 mm to remove any remaining seed particles. Three subsamples were dried at 30°C for 24 h to calculate the ratio of dry mucilage to wet mucilage. Subsequently, the prepared soil was mixed with the necessary amount of wet mucilage using an electrical household mixer to obtain the final mucilage concentrations 0, 0.02, 0.2 and 2 g dry mucilage kg^{-1} dry soil. In case of the control and the lowest concentration of 0.02 g kg^{-1} deionised water was added until saturation was reached to make these treatments comparable to the other two. The wet substrate was placed onto trays and left to dry at 30°C with maximum ventilation for 62 h. After determination of the residual moisture content, the substrates were crushed and sieved to 1 mm. Stainless steel cylinders with an average radius of 2.82 cm and a height of 2 cm, thus resulting in a volume of 50 cm^3 were used to keep the amount of mucilage needed as low as possible. The substrates were filled in with a spoon at a gravimetric water content of 3% and 7% for sand and loam, respectively, which resulted in a homogeneous consistency. The height of the samples was adjusted to 20 mm using a material testing machine (100 kN Allround Table Top Zwick/Roell, Ulm, Germany) at a defined speed of 0.2 mm s^{-1} to obtain standard bulk densities (ρ_b) of 1.26 g cm^{-3} for loam and 1.47 g cm^{-3} for sand, respectively.

2.3 | Adjustment of matric potentials corresponding to soil water retention curve

Samples were saturated with tap water for 48 h, as the presence of mucilage prolonged the saturation process. Soil samples were desaturated with ceramic suction plates connected to a vacuum pump and drained subsequently to the matric potentials (−0.01, −1, −3, −6, −12.5, −30 and −50 kPa) until weight consistency was reached. To prevent bacterial degradation of the mucilage, this process took place in a cooling chamber at 4°C. To obtain the water content at the permanent wilting point, disturbed samples were placed in a pressure pot at −1500 kPa for 6 weeks.

2.4 | Measurement of penetration resistance

Prior to the actual PR measurements, a pre-test was taken out to quantify the effect of insertion speed on PR values. These samples were prepared in the same way as the samples for the desorption curves but did not contain any mucilage. They were saturated with tap water and then placed on ceramic plates with a suction of −3, −12.5 or −50 kPa, respectively, until weight consistency was reached. Cylinders were placed on top of an additional sample with the same substrate, bulk density and water content to ensure that cylinder height would not influence results, especially at lower depths. A stainless-steel top with three holes for penetrometer insertion was placed on top of the cylinder for the correct placing of the measurements and to prevent evaporation. Only the hole used was left open, the others were covered with tape. Our material testing machine was equipped with a micro-sensor with a nominal force of 10 N with accuracy grade 1 according to ISO 7500-1 (ISO, 2018) down to 0.02 N. Attached to it was a stainless-steel penetrometer probe with a 15° semi-angle resembling root geometries (Ruiz et al., 2017) and a maximum radius of 0.5 mm (Oleghe et al., 2017) that was pushed into the sample at insertion speeds of 0.72, 2, 4, 60 and 120 mm h^{-1} to cover a range of different velocities, the lowest speed being a maize root growth rate observed by Ruiz et al. (2017), the highest a more practicable rate used by Oleghe et al. (2017). Each cylinder was penetrated three times at different insertion speeds randomly assigned to it, ensuring that the same speed was not repeated within one cylinder. Four replicates were measured for each combination of matric potential and penetrometer speed. Resulting forces were logged every 10 μm. As the probe's shaft was non-recessed it was drawn out at insertion speed to correct for shaft friction for all replicates at the three faster speeds and for one of the replicates at 0.72 and 2 mm h^{-1} due to time limitations. Within the range of 2–4 mm depth, the cone had full contact with the soil and values were available for all measurements. The area below the curve between 2 and 4 mm distance was calculated, representing the mechanical energy (U) according to Ruiz et al. (2017):

$$U = \int_0^l F_{Z,m} dz$$

with l being the length and $F_{Z,m}$ the measured axial penetration resistance and was subsequently related to the length of 1 m for better comparability. The null hypothesis (H_0) assumed that no differences in mechanical energy occur between penetrometer velocities and was tested using ANOVA within each substrate and matric potential.

For the measurement of PR as a function of mucilage content, samples were prepared as described above and sprayed with tap water until a defined gravimetric water content was reached. For loam, θ_g were 0.34, 0.19, 0.14 and 0.09 g g^{-1}, and for sand 0.20, 0.06 and 0.04 g g^{-1}, covering a substantial range of the water retention curve for each substrate. Subsequently, they were stored for 1 week in a dark place at 4°C to allow equal distribution of water in the sample and to prevent degradation of mucilage. Samples regained ambient temperature prior to measuring. The general technical set-up was identical to the pre-test with an insertion speed of 120 mm h^{-1}. Three measurements per sample were averaged for each of the four replicates per treatment. Axial force was measured every 10 μm and corrected with the forces obtained by withdrawing the probe at insertion speed once per sample. The measured axial penetration force ($F_{Z,m}$) was divided by the cross-sectional area of the conus to obtain PR. Mechanical energy was calculated as described above. The hypothesis that mucilage concentration alters mechanical energy per unit length was tested using ANOVA, with H_0 stating no differences between groups.

2.5 | Confined uniaxial compression test

The dimensions of the sample cylinder as well as the ratio between diameter and height of approximately 2.8 were in accordance with the International Organization for Standardization (ISO, 2017). A confined uniaxial compression test was carried out using an oedometer test ring (Eijkelcamp 08.67 Compression test apparatus, Giesbeek, Netherlands) and the software Physical soil test Version 2.0.4 (Eijkelcamp, Giesbeek, Netherlands). Samples were prepared in the same way as for PR with four replicates each. In order to use the 50 cm^3 cylinders, an adaption was made for the devices and they were re-calibrated for cylinders of this size. A special measuring protocol was devised that allowed the simultaneous acquisition of σ_{pc}, compression index (C_c), swelling index (C_s), as well as the EI. It included a sequence of 10 log-equidistant loading steps ranging from 10 to 630 kPa followed by an unloading step of 3 kPa after each loading step. The duration of each step was 20 min for loam and 5 min for sand, the amount of time needed to reach consolidation. Applied stress, vertical settlement and matric potential were logged every 2 s. Evaluation of σ_{pc} was based on the methods of Casagrande (1936) and the logistic function suggested by Gregory et al. (2006). The C_c is defined as the slope of the VCC. It was calculated as follows:

$$C_c = \frac{\varepsilon_{\text{VCC initial}} - \varepsilon_{\text{VCC final}}}{\log\sigma_{\text{VCC final}} - \log\sigma_{\text{VCC initial}}}$$

with ε being the void ratio at the beginning or end of the VCC and σ the applied stress at the beginning or the end of that curve. The slope C_s of the elastic rebound curve (ERC) was determined by

$$C_s = \frac{\varepsilon_{\text{ERC initial}} - \varepsilon_{\text{ERC final}}}{\log\sigma_{\text{ERC final}} - \log\sigma_{\text{ERC initial}}}$$

with ε and σ relating to the void ratio and applied stress within the ERC.

EI was determined for all stress steps of each sample, being defined as

$$\text{EI} = \frac{\Delta\varepsilon_{\text{rebound}}}{\Delta\varepsilon_{\text{loaded}}}$$

with $\Delta\varepsilon$ being the difference between the initial and the final void ratio by Peth et al. (2009). The EI ranges from 0 at completely plastic behaviour to 1 signifying fully elastic behaviour. After completion of measurements, soil cores were dried for 48 h at 105°C to estimate dry weight of the samples.

2.6 | Statistical analysis

To determine whether different penetration rates affect energy required for root growth, data were grouped according to substrate and Ψ_m. Subsequently, one-factorial ANOVA with penetration rate as factor was calculated. To be able to find out if mucilage concentration alters the energy required for root growth, energy data were arranged depending on water content within each substrate. For each combination of substrate and θ_g one-factorial ANOVA was applied. To disclose the combined effects of mucilage concentration and θ_g on the results of the compression tests, that is, σ_{pc}, C_c and C_s, multifactorial ANOVA was calculated for each substrate. The factors considered were mucilage concentration, θ_g and the interaction between the two. In case of significant differences, a subsequent Tukey HSD-test determined between, which groups these differences occurred. For EI significances were calculated for each loading step.

For all statistical tests carried out, H_0 was defined as no differences occurring between groups. A significance threshold (α) of 0.05 was chosen below which H_0 was refuted. The complete statistics tables are included in the Supporting Information. All statistical tests were performed with the open-source software R (R Core Team, 2020) and RStudio Team (2020). Figures were prepared using R-package ggplot2 (Wickham, 2016), the software Inkscape and Microsoft Excel Version 2008.

3 | RESULTS

3.1 | Water retention curve

Throughout the desorption curve in both substrates (Figure 1), at the same matric potentials higher volumetric water contents were retained in the samples with higher mucilage concentrations. In loam, the differences at higher matric potentials (near saturation) could not be quantified due to the swelling of the substrate. Within the range from −6 to −1500 kPa, loam with 2 g kg^{-1} mucilage added, contained between 19 and 56 vol% more water than the control, these differences were significant. In sand, the water-retaining effect of mucilage led to an increase of 21–68 vol% water contained in samples with the highest mucilage concentration compared to the control within the matric potentials measured. Differences between the highest mucilage treatment and the control were significant at all Ψ_m except −30 and −50 kPa.

3.2 | Energy required for penetration at different penetrometer rates

The influence of penetration velocity on measured penetration resistances was tested only for the control samples but at different matric potentials (Figure 2). The mechanical energy per unit length shows the influence of the matric potential and the comparability of the results at different penetrometer velocities. As expected, more energy was needed to penetrate the soil at more negative

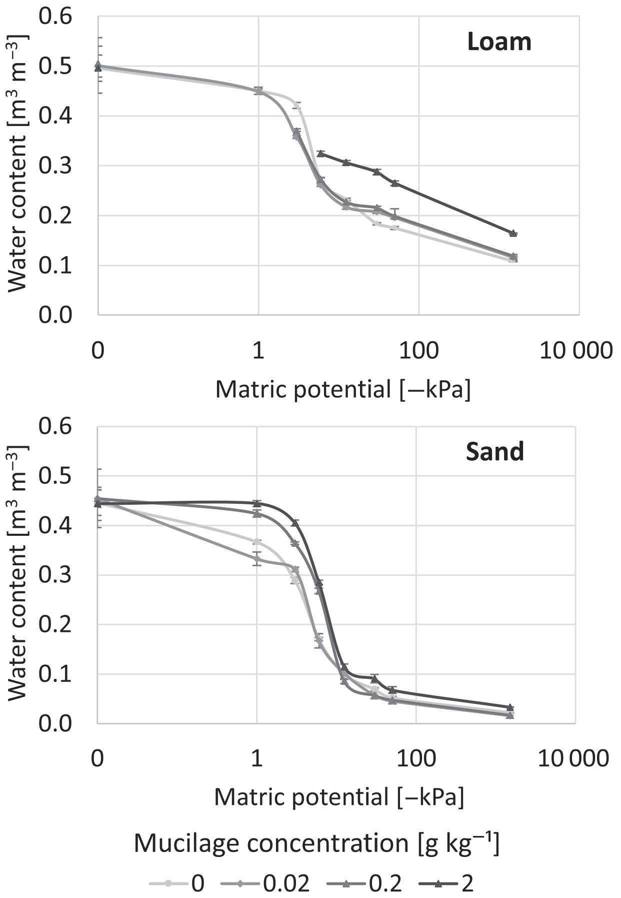

FIGURE 1 Water retention curve in loam and sand at mucilage concentrations of 0, 0.2, 0.02 and 2 g kg^{-1} dry mucilage in dry substrate. Every point is the mean (±1 SE) of four measurements. In loam at 0.2 and at 2 g kg^{-1} swelling occurred at matric potentials of −1 kPa, and at −1 and −3 kPa, respectively. As this altered the volume, and therefore, the bulk density of the samples, these values were omitted. The horizontal lines indicate Ψ_m and θ_v used in the following experiments

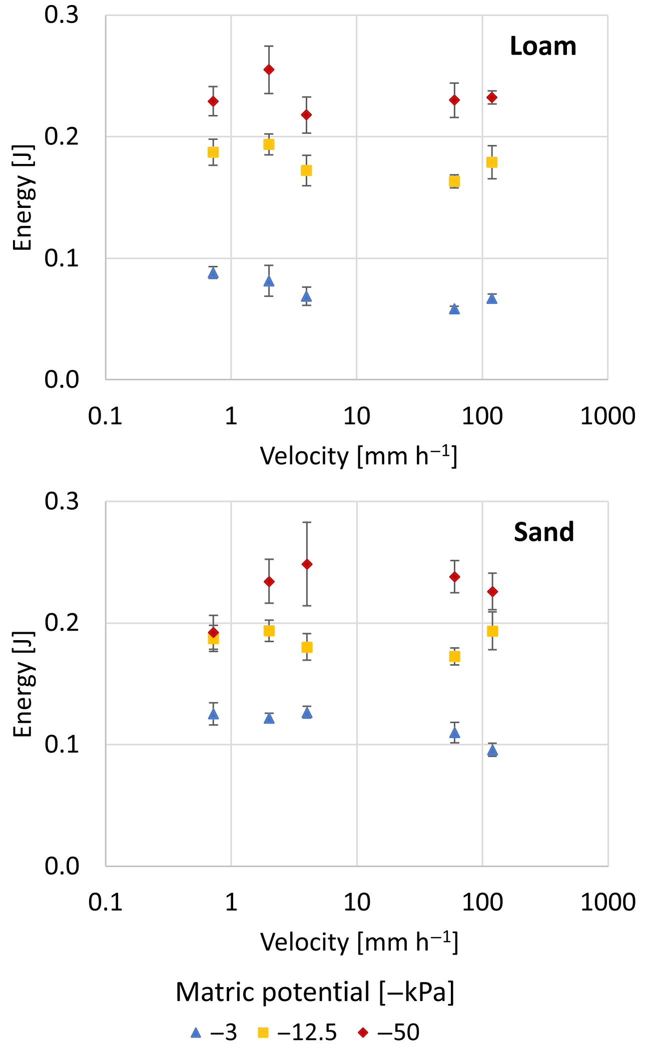

FIGURE 2 Mechanical energy (J) in relation to velocity (mm h^{-1}) at matric potentials of −3, −12.5 and −50 kPa in loam and sand. Data points are means ±1 SE [Color figure can be viewed at wileyonlinelibrary.com]

Ψ_m in both substrates. Regarding the mechanical energy (J), H_0 (no differences in mechanical energy per unit length at disparate velocities) was rejected for the samples at −3 kPa in the sandy soil but accepted for all other groups (Table S1). In sand at −3 kPa slight differences ($p = 0.025$) occurred between speeds of 120 and 4 mm h^{-1} and 120 and 0.72 mm h^{-1}. Presumably, this was caused by the faster drying of the sandy substrate around the penetrometer needle, which was kept low at higher speeds. These results implicate that faster penetration of 120 mm h^{-1} did not result in significantly different PR measurements compared to real root growth rates. Therefore, we conducted all PR measurements at penetration rates of 120 mm h^{-1} to speed up the measurement process, which also avoided a potential influence of sample desiccation during the test.

3.3 | Penetration resistance at different mucilage concentrations

The gravimetric water content played a major role in the effect of mucilage concentration on PR for both loam (Figure S1) and sand textures (Figure S2). In loam, the highest mucilage concentration of 2 g kg^{-1} caused highest PR values at wet conditions (θ_g = 0.34 g g^{-1}), whereas in dry conditions (θ_g = 0.09 g g^{-1}) PR values were lowest for the highest mucilage concentration. In sand, the highest mucilage concentration displayed a different behaviour with a relatively constant increase of PR with penetration distance, while at lower mucilage concentrations, we observed a stronger increase in PR below 2 mm and a flattening of the curves between 2 and 4 mm. Compared to loam, we measured lower penetration resistances in sand at a similar water content and a less clear trend in penetrations resistance changes depending on mucilage concentration. A complete results table of penetration resistance values can be found in the Supporting Information (Table S2).

Applying the concept of energy per unit length to the measurements, led to comparable results as shown in the PR curves (Figures S1 and S2). Significant differences in energy required for soil penetration within groups of the same substrate and water content are shown in Figure 3, the corresponding ANOVA table is Table S3. Note that here only the distance between 2 and 4 mm where the penetration needle had full contact with the soil was considered for calculation. A higher water content generated a pronounced decline in energy in loam. The treatment of 2 $g\ kg^{-1}$ mucilage resulted in significantly lower energy at a gravimetric water content of 0.09 $g\ g^{-1}$, whereas the contrary was the case at θ_g 0.34 $g\ g^{-1}$. At water contents of 0.14 and 0.19 $g\ g^{-1}$ differences between treatments were not as pronounced with lowest values in the control. Generally, in sand, less energy (≤0.1 J) was required to cover a certain distance compared to loam (≤0.3 J). Differences in energy between the water contents were very small in sand with no significant differences occurring at $\theta_g = 0.04\ g\ g^{-1}$. At $\theta_g = 0.06\ g\ g^{-1}$ the mucilage treatment of 2 $g\ kg^{-1}$ energy did not differ from the control and at $\theta_g = 0.20\ g\ g^{-1}$ it was slightly higher.

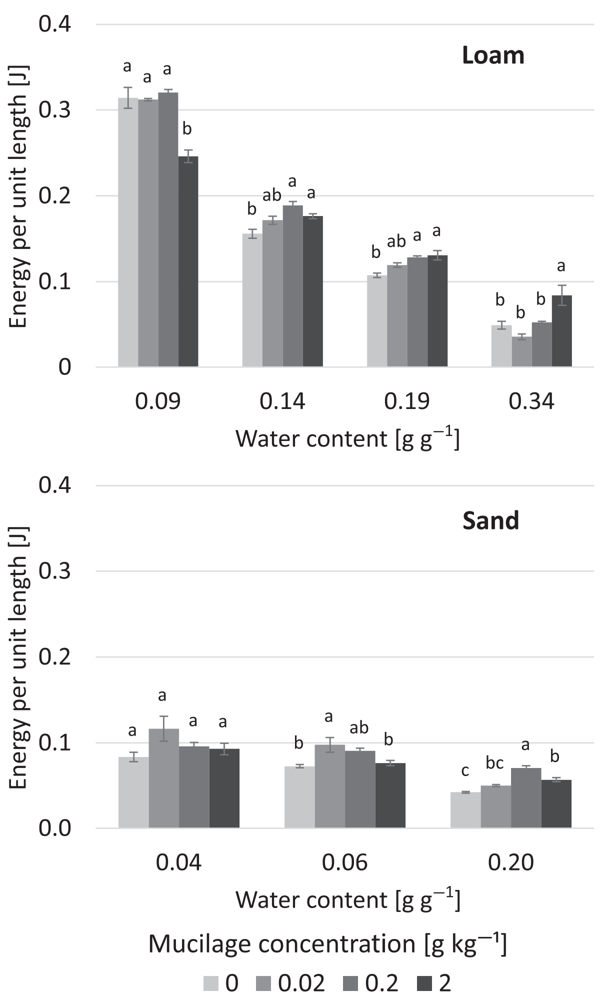

FIGURE 3 Energy (J) required for a root with 1 mm diameter to grow 1 m at different water contents and mucilage concentrations in loam and sand. Each column represents the mean of four separate measurements with an error bar of 1 SE. ANOVA with subsequent Tukey HSD-test was carried out to identify significant differences indicated by different letters within each water content

3.4 | Confined uniaxial compression test

The results of the confined uniaxial compression test are summarised in boxplots in Figures 4 and 5 for loam and sand, respectively, with the full ANOVA tables located in the Supporting Information (Table S4). In loam, all factors considered resulted in significant differences in the observations of σ_{pc}, C_c and C_s. Higher mucilage concentrations led to higher σ_{pc}, whereas higher water contents resulted in lower σ_{pc}. Only C_c was not significantly affected by mucilage, but was strongly influenced by water content with the highest compressibility at θ_g 0.14 and 0.19 $g\ g^{-1}$ (Figure 4). At 0.34 $g\ g^{-1}$ compressibility was lowest, due to excess pore water (data not shown). C_s values were highest in the control. Increasing water content produced higher C_s, with no values available for θ_g 0.34 $g\ g^{-1}$, as compressibility was too high in these samples. Regarding the compressibility of the loamy substrate both in the re-compression and the VCC, θ_g had a stronger effect than mucilage concentration. All interactions between the factors mucilage concentration and water content in loam were significant and are summarised based on mean values in Figure S3. Differences in σ_{pc} caused by mucilage concentrations were more pronounced at lower water contents, esp. at θ_g 0.09 $g\ g^{-1}$. The highest mucilage concentration increased C_c in the moistest treatment but decreased it at all other water contents. With added mucilage, C_s was strengthened with increasing θ_g, whereas the control samples displayed a much higher C_s at the lowest θ_g.

In both substrates, σ_{pc} was highest at the highest mucilage concentration and decreased with increasing θ_g (Figures 4 and 5). In sand, it increased with increasing mucilage concentration while it decreased with increasing water content though less pronounced as it was the case in loam. Adding mucilage increased the compressibility whereas water content only caused minor differences in C_c. C_s were not significantly affected by water content and the effect of mucilage on C_s remained

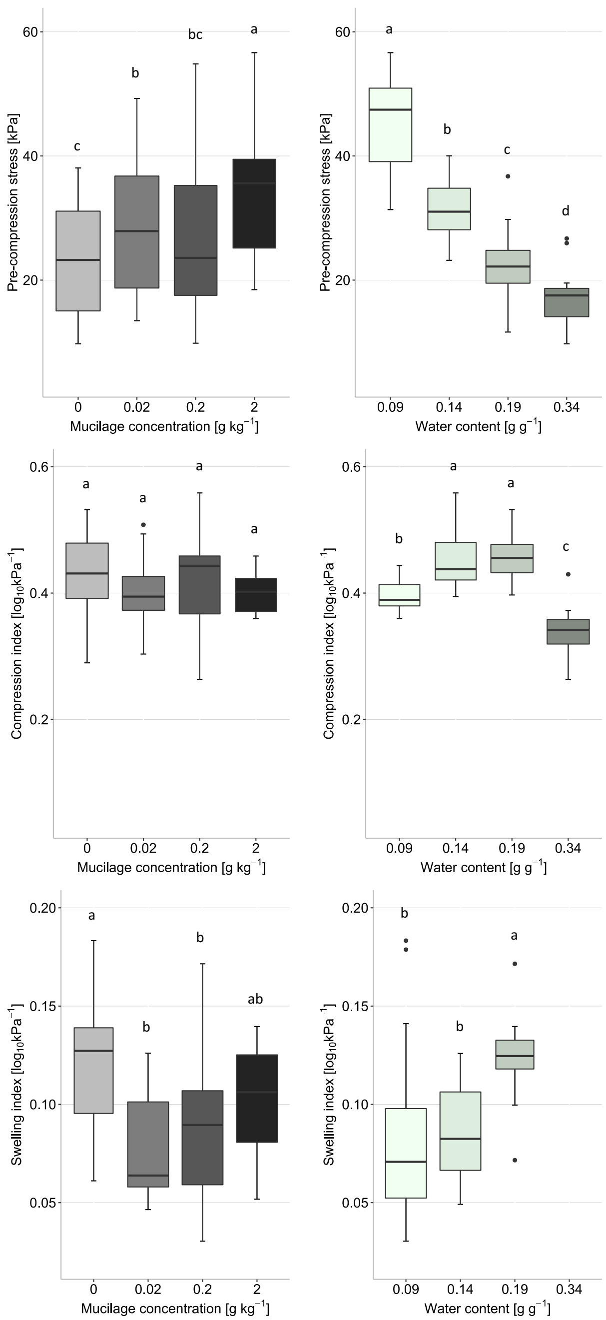

FIGURE 4 Boxplots of penetration resistance (PR, top), compression index (C_c, middle) and swelling index (C_s, bottom) values in loam. The graphs on the left are grouped by mucilage concentration ($n = 16$), the ones on the right by water content ($n = 16$). Box-cox transformation was used for σ_{pc}; C_c values were transformed with ordered quantile normalisation transformation (Peterson & Cavanaugh, 2019). Data were re-transformed for plotting. C_s values were not available for the highest water content treatment in loam, as the ERC here was not distinct enough. Three C_s-values in loam at 0.19 g g^{-1} water content (two at 0 and one at 0.02 g kg^{-1} mucilage concentration) could not be calculated as not enough data points in the respective range were available. Multi-factorial ANOVAs with subsequent Tukey HSD-tests were carried out and resulting significant differences are indicated with different letters [Color figure can be viewed at wileyonlinelibrary.com]

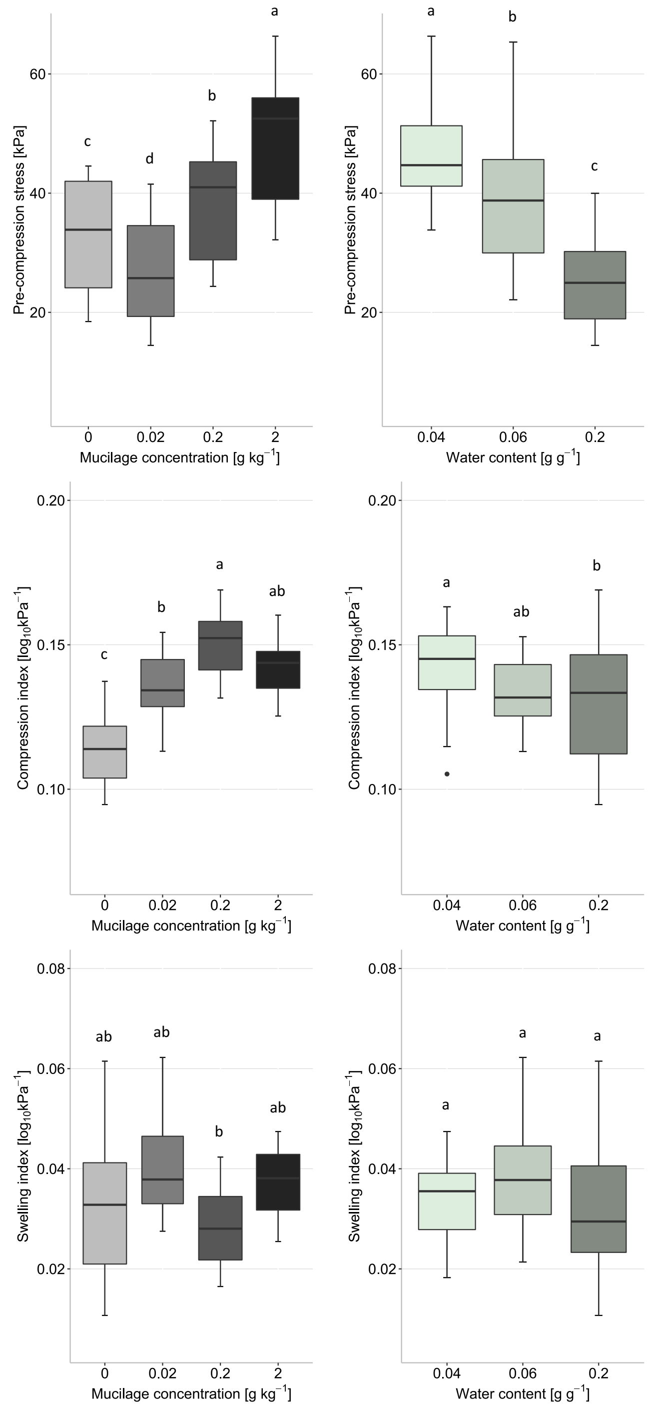

FIGURE 5 Boxplots of penetration resistance (PR, top), compression index (C_c, middle) and swelling index (C_s, bottom) values in sand. C_s values were log-transformed to obtain normal distribution of data and re-transformed for plotting. The graphs on the left are grouped by mucilage concentration ($n = 12$), the ones on the right by water content ($n = 16$). Multi-factorial ANOVAs with subsequent Tukey HSD-tests were carried out and resulting significant differences are indicated with different letters [Color figure can be viewed at wileyonlinelibrary.com]

unclear. Altogether, water content affected the compressibility both in the re-compression and in the VCC much less in this substrate than in loam. Interactions between mucilage and water content only occurred in C_c, where the higher mucilage treatments retained good compressibility at higher water contents (Figure S4) reciprocal to the results in loam (Figure S3).

For the analysis of EI, the results of the first two loading steps were discarded as they were based on minute differences in the range of μm to tens of μm. The EI for the highest pressure in loam was rejected because maximum settlement was already reached at that point, inevitably resulting in an incorrect EI, whereas in sand differences according to mucilage concentrations in the characteristic S-shape of the compression curve rendered a comparison questionable. We, therefore, focused on the range of the virgin compression line. The greatest effect of mucilage on EI could be observed in the driest loam (Figure S5 and Table S5), with higher EI mainly at the highest mucilage concentration compared to the other treatments, whereas no differences were distinguishable at the other water contents. In sand, higher EI was observed at soil moistures of 0.04 and 0.06 g g^{-1} with significant higher EI at the highest mucilage treatment compared to the control at some of the loading steps (Table S6).

4 | DISCUSSION

4.1 | General considerations and water retention curve

As discussed in the introduction, chia seed mucilage was chosen for the experiments with the next decision being the appropriate concentrations. Zickenrott et al. (2016) estimated concentrations of mucilage in the rhizosphere between 0.05 and 50 g kg^{-1}, depending on the amount produced per root tip and the length of root covered, bulk density, root radius and the distance impacted by mucilage. Holz et al. (2018) measured mucilage concentrations in the root channel and in the rhizosphere via diffuse reflectance infrared spectroscopy with averages of 0.017 and 0.003 g kg^{-1}, respectively. They calculated a theoretical value of 0.056 g kg^{-1} in the rhizosphere for their experiment, based on the literature values for exudation rates. Naveed et al. (2018) used maize root exudate concentrations of 0.46 and 4.6 g kg^{-1} and chia seed mucilage at concentrations of 0.046, 0.46, 0.92, 2.3 and 4.6 g dry mucilage kg^{-1} dry soil. Keeping in mind that chia seed mucilage has a stronger effect on viscosity than maize root mucilage, we decided on a maximum value of 2 g kg^{-1}, the same as Oleghe et al. (2017) used, thus making these studies comparable. We further used concentrations of 0.02 and 0.2 g kg^{-1} to reflect the drastic decrease of mucilage concentration with distance from the root surface (Zickenrott et al., 2016) and to include the more conservative estimates of Holz et al. (2018).

The increasing effect of mucilage on the gravimetric water content at a given matric potential was already reported by Kroener et al. (2014) and Ahmed et al. (2014). Problematic issues for the substrates and bulk densities we used were the consolidation of the sand, especially at low mucilage concentrations, the swelling of the loam in the range close to saturation and its shrinking at lower matric potentials. Kroener et al. (2018) reported comparable results for a similar (but better sorted) sand and porosity with no significant differences occurring between mucilage treatments at higher matric potentials. The slight swelling of the loam at 0.34 g g^{-1} water content, which was observed at mucilage concentrations of 0.2 and 2 mg g^{-1} might have impacted bulk densities thus affecting PR and σ_{pc}. As swelling increases, the volume of the soil, bulk density are lowered; therefore, it could be expected that PR values would be lower in the samples concerned. However, PR and energy required for root growth were higher in these samples. Regarding σ_{pc} in loam, greatest values for the highest mucilage concentration of 2 mg g^{-1}, were observed at 0.34 g g^{-1} but also at all other water contents. For these reasons, we assume the effect of the swelling in the loam on PR and σ_{pc} to be negligible. The results for the water retention curve provided the base for the interpretation of the following experiments when we had to keep in mind that equal θ_g of different mucilage treatments signify different Ψ_m.

4.2 | Penetration resistance and energy required for penetration

Evaporation right around the penetrometer needle during the measurement through the hole in the stainless-steel cover could not be entirely avoided in the present set-up. Any type of covering used in a pre-test had an impact on the measurements of the extremely sensitive sensor required and was consequently omitted in the test. This might have led to an over-estimation of PR or energy through locally dried soil, especially at lower penetration speeds. The average distance between the penetration sites, as well as between the penetration sites and the cylinder edge was 18 mm. As this distance is 18× higher than the penetration cone diameter, it can be assumed that no interference between measurements or edge effects occurred. Wang et al. (2016) concluded that a distance between penetration points of 10 times larger than the diameter of the penetration cone (here 1 mm) is sufficient to avoid interferences between the tests.

Testing the influence of penetrometer rate on resulting forces was a necessary step to make sure our results would be comparable to the penetration rates of real root systems. Ruiz et al. (2017) examined the influence of penetration rates of 18, 60 and 600 mm h^{-1} in a silt loam soil and a silty clay loam soil and did not find differences in forces resulting from the two slower rates. However, at 600 mm h^{-1} resulting forces almost doubled in the silt loam. Only in the driest samples of the silty clay loam, no rate dependency was detected. Our results showed no rate dependency in both soils, even at very low velocities close to real root growth rates. The comparability of the results at 120 mm h^{-1} to the ones at 0.72 mm h^{-1} is remarkable with faster rates having the added advantage of reducing evaporation from the samples. Penetration rates should be considered carefully prior to a PR experiment and ideally tested using the soil type and water content of the actual experiment.

We assumed that the comparability of forces derived at different velocities is also given at the slightly lower energy range in sand in the mucilage experiment. In the velocity pre-test, the energy required is in a similar range for velocities of up to 120 mm h^{-1} for both substrates, whereas in the mucilage test less energy was required in sand. The reason is found in the different procedure for preparing the samples for the pre-test, in which saturating this very homogeneous and unstable substrate at ρ_b 1.47 g cm^{-3} and putting it unto suction plates caused hydro-consolidation with resulting higher ρ_b and hence more energy required for penetration. For this reason, this approach was abandoned, and samples were adjusted to a defined water content for both the mucilage trial and the confined uniaxial compression test.

For a silt loam with similar texture and ρ_b as the loam in the present study, Ruiz et al. (2017) modelled mechanical energy required by plant roots using soil rheological parameters and calculated values from around 0.0025 J at 0.34 g g^{-1} to approximately 4 J at 0.09 g g^{-1} water content. This is a wider range than our measured results from 0.05 to 0.3 J at the same water contents but can still be considered comparable due to the different approach. Based on a root radius of 1 mm and a root extension rate of 0.1–0.2 μm s^{-1}, Ruiz et al. (2017) did not report a limiting value for root growth, whereas they defined such a limit for earthworms caused by their higher penetration rates. The combination of the energy concept with the mechanical effects of mucilage allows a statement about how mucilage makes it easier for the roots to penetrate soil. We hypothesized that mucilage reduces the energy required for root growth, which has been confirmed for the loam at dry conditions. Interestingly, this is the treatment in which root growth tends to be most restricted. Here, mucilage enables the plant to easily penetrate into the soil in search of water and nutrients thus ensuring its survival. In sand and at higher water contents very little energy is needed for the root to grow in the soil, consequently, the slightly higher energies in the highest mucilage treatment do not have a limiting effect on root growth. In this context, we should also keep in mind that at a given water content the matric potential is lower at higher mucilage concentration, especially in the loam, leading to an increment in stability for the highest concentration of 2 g kg^{-1} that has to be overcome.

In both substrates, even more prominent in sand, the curve of the PR continued to rise after full insertion of the cone at 2 g kg^{-1} mucilage, in contrast to the other treatments. The applied method of calculating energy per length might have led to an over-estimation of the forces needed for this treatment due to the continuous increase of the displacement force curves. We concluded that the method of subtracting shaft friction did not work ideally for the 2 g kg^{-1} mucilage treatment. If we had only considered the force at a travelling distance of 2–2.5 mm of the penetrometer needle, the resulting energy would have been lowest in the 2 g kg^{-1} mucilage treatment at all water contents in sand. Ruiz et al. (2017) observed a similar curve progression in a soil with greater strength and lower viscosity, especially at lower water contents. He used a penetrometer with a recessed shaft and assumed the observations are caused by elastic rebound, which is in our case likely to be amplified by the presence of mucilage.

Higher exudate concentrations resulting in lower PR were reported by Oleghe et al. (2017) for a clay loam and—to a smaller extent—for a sandy loam at −50 kPa $\Psi_{m,}$ which is consistent with our findings in loam. In contrast to this, Zhang et al. (2020) detected an increase in PR within defined water content groups when adding a synthetic root exudate, specifically at lower water contents in a sand with a similar texture to ours but a higher bulk density of 1.61 g cm^{-3}. The decisive difference in approach was the incubation of the soil samples at 18°C in Zhang et al. (2020) allowing a microbial community to develop, whereas we stored them at 4°C, largely suppressing microbial activity. Gao et al. (2017) recognised that PR depends on incubation temperature affecting the microbial community. After incubation at 4°C, he could not detect an effect of exudate treatment on PR. Comparing the technical details in Zhang et al. (2020) with Oleghe et al. (2017) and our study suggests that results also depend on boundary conditions during tests and that direct comparison of absolute values should be interpreted with caution. Cone geometries and penetration rates used might alter resulting forces to different extents depending on substrate and water contents (Ruiz, 2018). A thorough methodological comparison of the effects of different technical approaches is indispensable to bring the results of different studies together.

4.3 | Uniaxial confined compression test

The homogeneous soils had a different initial stability depending on their water content as well as on the presence of mucilage. Such an increase in soil stability through addition of exudates has been reported by numerous authors such as Naveed et al. (2018) and was one of our hypotheses. We also hypothesised higher C_c values signifying a greater compressibility of the soil in the range of the VCC, a result we found in sand already at the lowest concentration of 0.02 g kg^{-1}. In both substrates, a similar pattern of mucilage increasing C_c in the wettest samples is discerned. Oleghe et al. (2017) observed higher C_c for samples amended with mucilage even at low concentrations of 0.2 g kg^{-1} with values 17% higher in sandy loam and 9% higher in clay loam at 1.85 g kg^{-1} mucilage concentration compared to the control. They used a uniaxial compression test to evaluate the influence of mucilage concentration on C_c but used an approach in which samples were pre-loaded to 200 kPa whereas in our samples σ_{pc} was much lower with mean values between 20 and 50 kPa and different initial stability between mucilage treatments. These differences provide an explanation why we could not detect an influence of mucilage concentration on C_c in loam. Differences in C_c were here mainly driven by water content. Adding PGA leads to a strong increase of bond energy in clay (Zhang et al., 2008) thus playing an important role in rhizosphere formation and possibly making finer soils more resistant to compression. This would fit with Di Marsico et al.'s (2018) observations of a decrease in soil porosity through reduction in larger pores at higher mucilage concentrations.

Regarding the swelling index - providing information about the compressibility within the re-compression curve - no clear pattern was visible. However, to study the impact of mucilage addition on C_s a different measurement protocol with a defined pre-compression stress and more loading steps in the re-compression curve would be advisable.

Our hypothesis of higher soil elasticity in the range of the VCC for samples with the highest mucilage concentration compared to the control was confirmed for the sandy soil, but not for the moistest samples. This in turn supports our suggestion of increasing PR values with distance especially in sand being caused by greater elasticity in this treatment. These results are in agreement with Naveed et al. (2018) who performed indentation measurements with loading-unloading cycles at −10 kPa, finding increasing soil elasticity at higher concentrations of maize root mucilage and chia seed mucilage, especially in sandy loam and less in clay loam. They concluded that plant exudates have a smaller effect if soil stability and elasticity are initially greater in the unamended soil. Interesting is a similar trend in our driest loam samples: under moisture contents around the wilting point elasticity of the soil amended with mucilage increased, possibly improving soil-root contact at these harsh circumstances for the plant.

5 | CONCLUSIONS

In this study, we examined the influence of soil water content on the effects of mucilage concentration on various soil physical properties of remoulded homogeneous soil samples. Energy required by plants for the penetration of soil was substantially decreased by amending a loam at permanent wilting point with chia seed mucilage as hypothesised. So, in the substrate and at the water content where most energy is required, addition of mucilage at 2 g kg^{-1} makes it less energy-demanding for the root to grow into the soil. In contrast at higher water contents, mucilage concentration of 2 g kg^{-1} increased PR in loam suggesting that the effect of mucilage on mechanical soil resistance is not linearly changing with water content but depends on a complex interaction between mucilage and water. Results in sand were less clear but showed only an overall minor effect of mucilage on mechanical energy required to penetrate the soil. Caution has to be paid to technical and analytical procedures, especially with respect to the comparison of results with other studies that used different boundary conditions during testing. Penetration rate may be a critical factor when PR measurements are transferred to root growth conditions. A comparison of penetration rates as done in this study showed that penetration velocities of 120 mm h^{-1} gave similar results to velocities that were in the order of real root growth rates. With respect to bulk soil mechanical parameters, we found that the overall stability of both substrates increased with mucilage concentration and decreased with higher water contents according to our hypothesis. Compressibility in loam was mainly determined by water content, whereas it increased with mucilage concentration in sand with a positive effect especially at higher water contents. Higher EI within the range of the VCC was observed in the sand and in the driest loam. The effects of mucilage on compressibility and elasticity were, therefore, more pronounced in sand, a substrate in which these properties are initially low. Further studies need to be conducted using a range of texture mixtures and a focus on drier conditions, where the benefit of mucilage amendment is greatest. We have seen how an effect observed (here caused by mucilage) might not only be dependent on the substrate but also on a specific water content range and how important it is to consider all contributing factors as well as their interactions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Ulla Rosskopf: Formal analysis (lead); investigation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Daniel Uteau:** Conceptualization (equal); formal analysis (supporting); funding acquisition (equal); project administration (equal); writing – review and editing (equal). **Stephan Peth:** Conceptualization (equal); funding acquisition (lead); project administration (lead); supervision (lead); validation (lead); writing – review and editing (equal).

DATA AVAILABILITY STATEMENT

The data used in this study are available upon request from the corresponding author. The data set will be archived for at least 10 years after publication.

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