Title: Do root hairs of barley and maize roots reinforce soil under shear stress?

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Abstract

Roots reinforce soil by acting as soil pins, dissipating shear stresses and anchoring the soil in place. By protruding into the soil and binding to soil particles, root hairs increase root-soil contact and aid root anchorage. However, it is not yet known whether this ability to anchor roots affects the root system's ability to reinforce soil. Using a laboratory box shearing rig, this study explores whether root hairs affect soil shear resistance. The force required to shear soil columns permeated with roots lacking root hairs (barley *brb* and maize *rth3* mutants) are compared to columns permeated with hairy roots (their respective wild types, WT) using unplanted soil columns as controls. Known root traits (e.g. root length density, root surface area density, average diameter, percentage of fine roots, and root tensile strength) were measured to ensure that differences in shear resistance could be attributed to the presence/absence of root hairs. All rooted columns required more force to shear than their respective unplanted columns but the thicker, stronger maize roots were more effective at soil reinforcement than the more numerous but weaker barley roots. After the maximum growth period, root hairs appeared to have a consistent and significant impact on peak shearing force. However, the WT root systems also produced greater root surface area density. As the rate at which peak shearing force increased with increasing root surface area density was similar for roots with and without root hairs, the increased peak shearing force of the WT columns cannot be attributed to resistance supplied by the presence of root hair but rather to a more prolific root system. Therefore, it was concluded that root diameter and root tensile strength most influenced root reinforcement of soil and as such, the relatively minute root hairs had negligible effects compared to their parent roots.

Keywords: Root hairs, shear reinforcement, soil, barley, maize

1. Introduction

Soil structural instability can pose a multitude of socio-economic problems. Small scale erosion can result in loss of fertile soils which has offsite consequences, such as sediment pollution, sedimentation of waterways, and an increase to flood risk (Boardman and Poesen, 2007; Pollen et al., 2013). Larger scale soil instability can result in mass wasting, such as landslides and soil creep, these have the potential to completely alter landscapes, destroy properties, and endanger lives (Petley, 2012). Understanding how to increase soil stability is key to developing methods that mitigate the detrimental effects of soil erosion and mass wasting.

Mass wasting ranges in scale but occurs when the frictional forces holding soil together are overcome by shearing forces caused by gravity. Soils are inherently anisotropic and are weak under shear forces (Al-Karni and Al-Shamrani, 2000). The fault line that occurs when soil fails under shear stress is called the shear plane and a soil’s shear strength is its ability to withstand these shear forces. Some soils are naturally susceptible to shear forces, either due to a layer of weakness referred to as a failure plane or because they have inherently poor particle cohesion. Most mass wasting events occur due to hydraulic pressures resulting from the increased weight of saturated soil or as a result of scouring from running water (Iverson, 2000). With decreasing scale of event, erosion from shear stress can be mitigated with increasing effectiveness by altering soil physical and biological properties.

Plant roots are widely understood to enhance soil shear strength by introducing tensile reinforcement to the soil, countering soil’s natural susceptibility to shear forces (Gyssels et al., 2005; Simon and Collison, 2002; Stokes et al., 2014, 2009; Wu and Sidle, 1995). Fine roots penetrate laterally through the soil, enmeshing and binding the surface soil, whilst deeper penetrating tap roots cross failure planes, pinning them together as well as anchoring the fine root matting (Fan and Chen, 2010; Simon and Collison, 2002; Stokes et al., 2009). A root’s ability to reinforce the soil depends on its resistance to either being pulled out or breaking. Roots dissipate shearing forces throughout the whole system, increasing the area of soil that is engaged in anchorage until the roots are either broken or pulled out (Bengough et al., 2011; Stokes and Mattheck, 1996). A root remains anchored in the soil when there is sufficient root soil contact to provide friction in excess of the opposing forces (Ennos, 1990). Further, if the root’s tensile strength is greater than the friction of its anchorage roots will slip from the soil; if it is less the root will break (Pollen, 2007). For straight roots, without forks or bends, the length of the root determines how efficiently it is anchored. Forks and bends enables a root to engage more soil and dissipate the shear forces with greater effect. Both root breaking force (Docker and Hubble, 2008; Nilaweera and Nutalaya, 1999; Pollen and Simon, 2005; Tosi, 2007; Yang et al., 2016) and the force required to pull the root from the soil (Nilaweera and Nutalaya, 1999; Norris, 2005; Stokes et al., 2009) increase with root diameter, although, root tensile strength is inversely related to root diameter (Nilaweera and Nutalaya 1999; Pollen and Simon 2005; Genet et al. 2005). Therefore, root anchorage is affected by many different root traits.

Since the strength of the root is largely dependent on its diameter, most research in this area has focused on the roots of trees and woody shrubs. Fine roots, associated with annual and perennial species, have frequently been unified into one synonymous category (Hishi, 2007; Pregitzer et al., 2002; Reubens et al., 2007). While the impact of fine roots on shear erosion has been investigated, there are gaps in our understanding of how fine roots mitigate sub-surface shear erosion, and other root traits such as root hairs, have been almost completely disregarded in studies of soil reinforcement.

Root hairs emerge just behind the root elongation zone, protruding laterally to anchor the root and enabling the root tip to penetrate the soil (Bengough et al., 2011; Haling et al., 2013) whilst preventing the growth force from deforming the rest of the root or pushing the plant from the soil (Bengough et al., 2016; Handley and Davy, 2002). Root hairs are considered a key component in root anchorage (Czarnes et al., 1999; Ennos, 1989), to the extent that root anchorage is believed to be a primary function of root hairs (Bengough et al., 2011; Gilroy and Jones, 2000). However, whether this capacity to anchor the root to the soil reinforces soils under shear stress is unknown. This paper aims to address this knowledge gap by assessing the contribution of different root traits (including root hairs) to soil reinforcement. Soil columns permeated by root systems with and without root hairs were subjected to shear force and the resistance of the columns were measured.

2. Materials and methods

2.1. Germination and growth

A root hairless mutant (*brb*) of barley (*Hordeum vulgare* L. cv. Pallas) and a root hairless mutant (*rth3*) of maize (*Zea mays* L.) were compared to their respective wild type (WT) genotypes that had root hairs. Maize seeds were initially surface sterilized using 10 % bleach for 5 minutes, then rinsed thoroughly with deionised (DI) water. Surface sterilization was not necessary for barley seeds because they had low levels of microbial contamination. All seeds were germinated on two sheets of filter paper (Whatman #3) moistened with 5 ml of DI water and sealed in petri dishes for 3-4 days at room temperature (approximately 20 °C). Once germinated, the seeds were transplanted into pots and moved to a walk-in controlled environment room, set at 24 °C during the day and 19 °C at night with a 12 hour photoperiod. Every second day the positioning of each pot was randomized in the controlled environment room and watered with approximately 100 ml. Each pot consisted of two 125 mm sections of

68 mm diameter guttering down pipe (FloPlast Ltd), making each pots total height 250 mm. During the growth stage, the two sections were held together with fabric-backed duct tape. The bottom of each pot was sealed with a section of woven wire mesh (0.70 mm Aperture, 0.36 mm Wire Diameter, SS304 Grade) to retain the soil but allow excess water to drain. Each pot was filled with a set weight (dependant on the initial water content) of a sandy loam topsoil (Bailey's of Norfolk LTD; 12 % clay, 28 % silt, 60 % sand and 3 % gravel D50 6 mm, no particles greater than 8 mm) to achieve an approximate bulk density of 1.3 g cm^3. Eighteen plants per genotype (72 plants in total) were harvested over 3 periods (denoted as Harvests 1, 2, and 3) in order to vary root density. Barley was harvested 35, 49 and 54 days after germination, while maize was harvested 23, 35, and 49 days after germination.

2.2. Soil shear strength

A laboratory shearing box rig, designed by Gould (2014), was used to measure the shear resistance of the soil columns. The shearing rig comprises a metal frame that supports two wooden inserts, each containing a hole for the experimental pots (Figure 1). The top section of the frame moves laterally on metal runners at a rate of 8-9 mm sec^{-1} (depending on the sample resistance) and extends the whole width of the pot, allowing a full displacement profile. The bottom section is held static. The displacement of the top section is measured with a linear potentiometric displacement transducer (PD13, LCM Systems Ltd, UK) and the displacement force is measured at a resolution of 0.02 kg with an S type compression load cell (STA-1-300, LCM Systems Ltd, UK). All data were recorded by a CR800 data logger (Campbell Scientific, Inc., USA) at a resolution of 200 milliseconds.

2.3. Soil water content, bulk density, and root measurements

Soil water content (WC) affects soil shear strength (Pollen, 2007), so all pots were stood in 5 cm of water overnight to standardise the WC across the treatments and replicates. Just prior to shearing, the duct tape was cut with a razor blade. Once sheared, the soil from the bottom half of the pot was weighed and then dried at 105 °C to establish soil bulk density (BD) and WC, assuming that the level of treatment variation recorded in the bottom half of the pots would also occur in the top half. The top half was sealed in a plastic bag and stored in a fridge until the roots could be harvested, no more than two days after the experiment. Only the bottom 3 cm of this section was used for root measurement as it was assumed that the root mass directly adjacent to the shear plane would most influence the soil's shear resistance. The

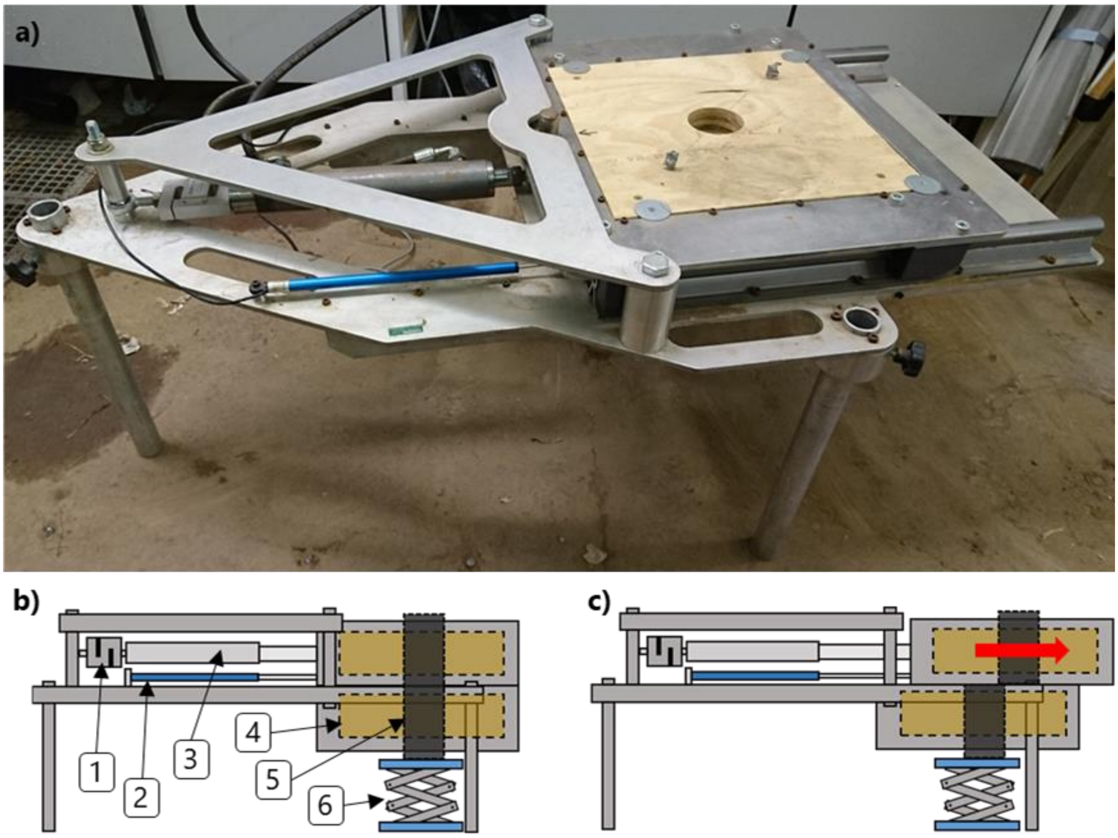

Figure 1. Depicts the shearing rig used in this experiment (a). The parts of the rig are numbered in their stationary position (a); 1. Load cell, 2. Transducer, 3. Hydraulic arm, 4. Wooden inserts, 5. Pot, 6. Adjustable platform to support the pot at the correct height so that the seam of the pot aligns with the shearing plane of the rig. The top section of the rig then extends over the bottom section shearing the pot (c).

3 cm section was measured and then cut with a razor blade. The roots were then washed out and stored at approximately 4 °C in a 50 % ethanol and DI water solution until they could be scanned using an Epson Expression 11000XL Pro with transparency unit at 600 DPI. Root parameters (diameter, length, and surface area) were analysed using WinRHIZO (2013e, Regent Instruments Inc.). Since the roots were very fine in this study (< 2 mm) it was not possible to measure root area ratio (percentage of total cross sectional area of roots per the soil cross sectional area at the shearing plane), so root length density (RLD) and root surface area density (RSAD) were used instead and are calculated as follows:

$$RLD = \frac{RL}{V_s} \tag{1}$$

$$RSAD = \frac{(\pi D) \times RL}{V_s} = \frac{RSA}{V_s} \tag{2}$$

Where RL is the total length of roots (cm) and V_s is the volume of soil sampled (cm^3). Root surface area (RSA, cm^2) is calculated using the diameter (D) of the root (excluding root hairs) and makes the assumption the root is cylindrical.

2.4. Root tensile strength

To measure the tensile strength of individual roots, four of each barley and maize genotype (16 plants in total) were grown in 4 litre pots (22 cm tall, 17 cm top diameter, 13.5 cm bottom diameter). After 35 days of growth (in the same substrate and under the same growth conditions as previously mentioned), the roots were washed out of the soil and stored at approximately 4 ℃ in a 50 % ethanol and DI water solution for two days. The roots were kept in this solution until immediately before testing to ensure each root remained saturated. Five 3 cm segments of lateral and axile roots were randomly selected from each plant and scanned using an Epson Perfection V700 at 600 DPI, and analysed using WinRHIZO (2013e, Regent Instruments Inc). Each segment of root was attached to a small plastic tab using a combination of superglue and duct tape, overlapping the plastic by 1 cm at each end; leaving a 1 cm length of unobstructed root. The plastic tabs were pre-tested to ensure their tensile strength far exceeded that of the roots and that their deformation was negligible. The plastic tabs, with the roots attached, were then secured into the clamps of a Single Column Table-top Testing Machine (series 5944, Instron, UK). The clamps were moved apart at a displacement rate of 10 mm min^{-1} and the force was recorded every 20 ms by a 100 N load cell at a resolution of 0.5 mN (Instron, UK). Tensile strength (TS) is calculated as:

$$TS = \frac{PF}{A}$$

Where PF is the peak force recorded on the displacement profile and A is the cross sectional area of the tested root. Any roots that broke at the joint of the plastic tabs were discarded.

2.5. Data and statistical analysis

Repeated measures analysis of variance (ANOVA) assessed whether the treatments (soil columns containing WT roots, root hairless mutant roots, and the unplanted control with no roots) exerted a different force over the same distance of displacement recorded from the shearing rig. However, the data violated the sphericity assumption of this method, so the p value is corrected using Greenhouse-Geisser. Pairwise comparisons with a Bonferroni correction was used as a post-hoc test. This analysis was carried out in SPSS (Version 25). The ANOVA function and multiple comparison procedures in MATLAB (R2017b) were used to estimate the genotypic means for peak force and their displacement distance and to assess which treatments statistically differed. This method was also used to assess whether there was a difference in the WC and BD of each treatment and whether the root parameters differed between genotype.

Analysis of covariance (ANCOVA) function in MATLAB assessed whether WC affected either peak shearing force or the distance at which it was reached. It was also used to assess genotypic differences in root tensile strength with increasing root diameter and whether there was any genotypic difference in peak shearing force with increasing RSAD.

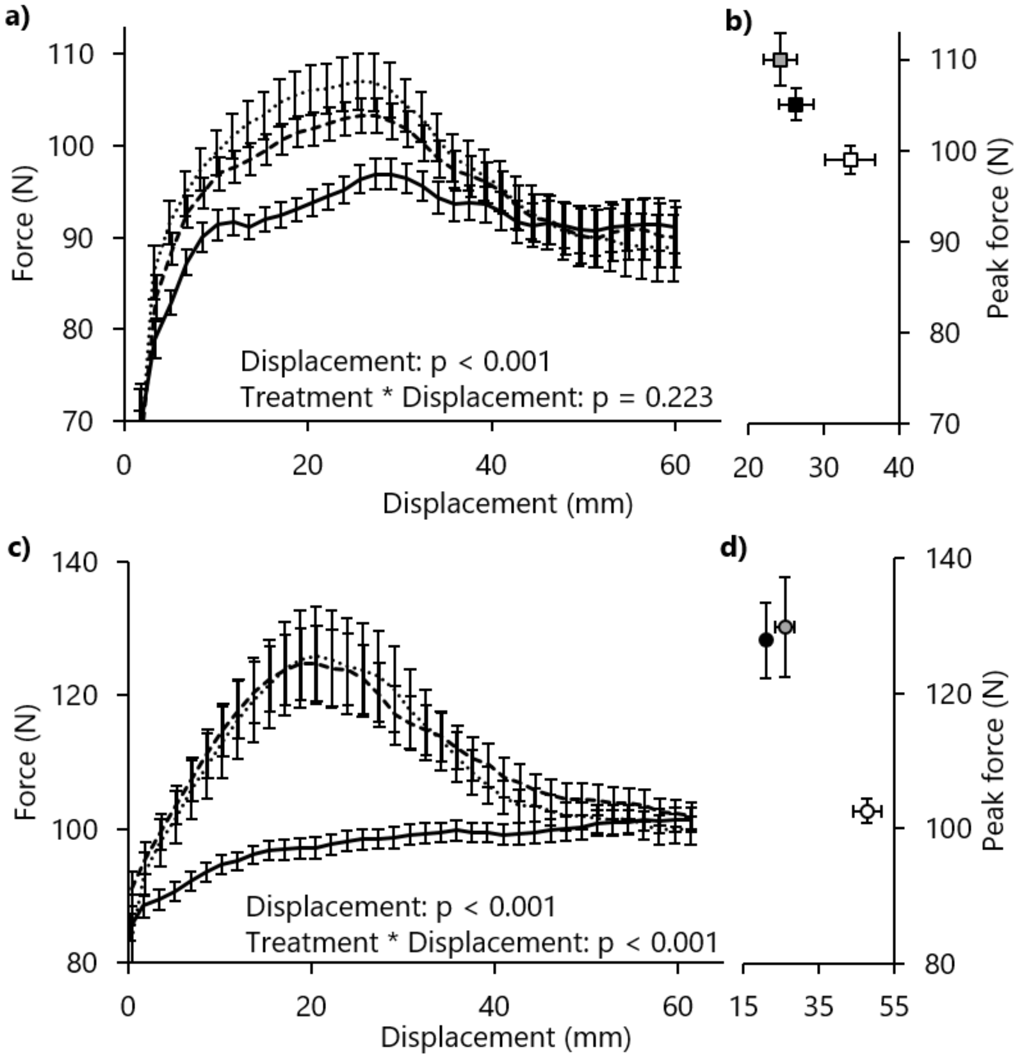

Figure 2. Displacement force (a, c) and peak displacement force (b, d) for barley (a, b) and maize (c, d) versus distance. Solid lines = unplanted control pots, dashed line = root hairless mutant (*brb* for barley and *rth3* for maize), dotted line = wild types (WT). P value represents the genotype*displacement interaction with displacement force derived from repeated measures ANOVA. White marker = unplanted, black marker = root hairless mutant, grey marker = WT.

3. Results

3.1. Displacement profile

The force required to displace all soil columns changed significantly ($p < 0.001$) over the displacement profile for both barley and maize (Figure 2a and 2c). Each rooted treatment shows an initial build-up of force to a peak which then tapers off, whereas the unplanted soil columns had a more gradual build-up and peaked much later. For barley, the initial build-up and subsequent tapering off of the rooted soil columns were similar to the unplanted soil columns, so the displacement profile was only significantly different between 6-48 mm for WT ($p < 0.05$) and 6-24 mm for *brb* ($p < 0.05$). For maize, both rooted columns had a significantly different displacement profile (over the entire width of the column) than their unplanted soil columns ($p < 0.05$ for both *rth3* and WT, respectively). Therefore, for at least part of the displacement profile, the presence of roots significantly affected the force required to shear the soil columns.

The presence of root hairs seemed to have no consistent or significant impact on the displacement profile. In barley, the WT soil columns required a greater mean force to shear than *brb* (94.85 N and 93.40 N for WT and *brb,* respectively). Whereas in maize, *rth3* required a greater mean force to shear than WT (110.76 N and 109.50 N for *rth3* and WT, respectively). For both barley and maize these differences were not significant which suggests the presence of root hairs had no impact on the displacement profile.

3.2. Peak shearing force

The peak force required to shear each soil column corresponds to the maximum amount of resistance the soil column was able to exert (Figure 3a and 3b). At Harvest 1, all rooted columns produced a greater mean peak force than their respective unplanted columns (*brb* = 6.7 %, barley WT = 5.1 %, *rth3* = 8.0 %, and maize WT = 10.7 % increase from the

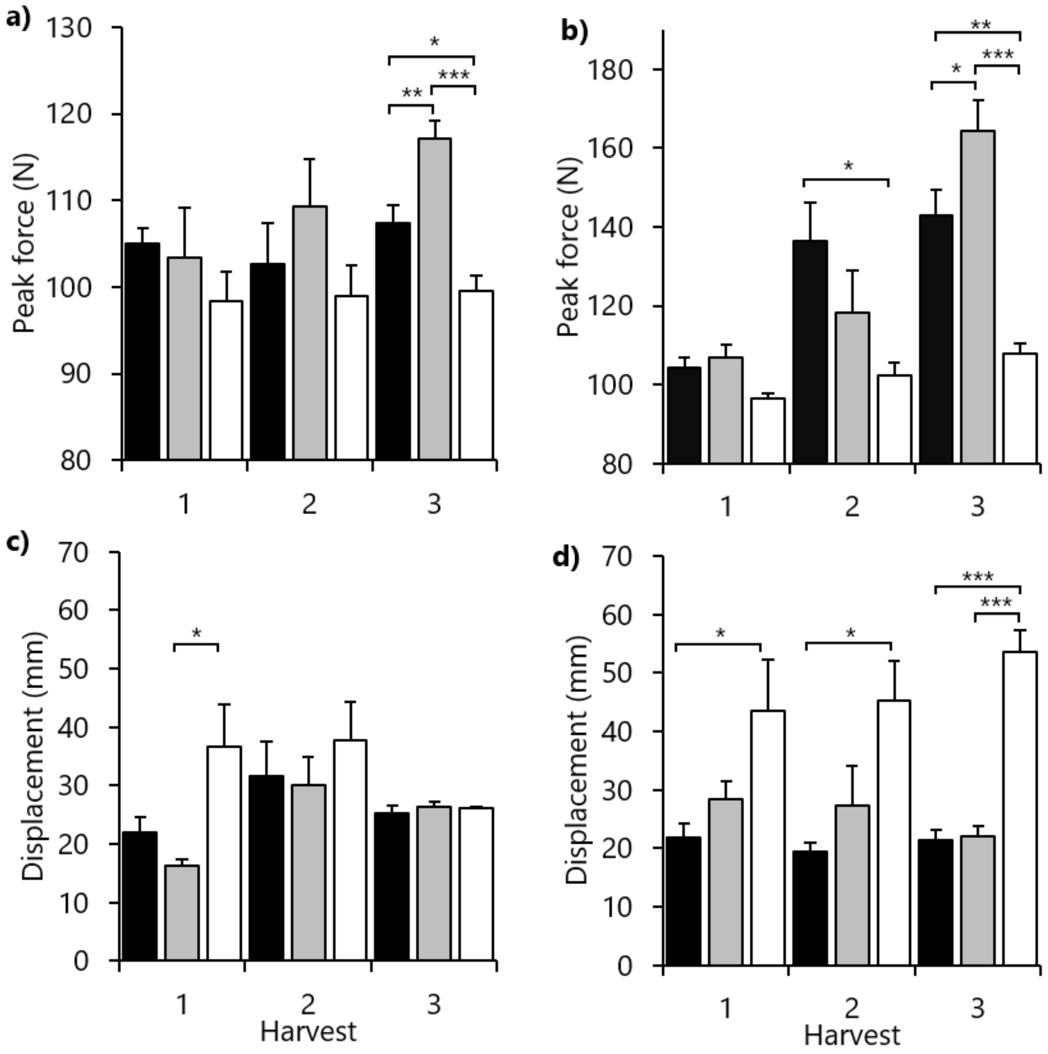

Figure 3. Peak force readings (a, b) and displacement distance (c, d) for barley (a, c) and maize (b, d) harvests (d). Black bars = root hairless mutants (*brb* for barley and *rth3* for maize), grey bars = wild types (WT) and white bars = unplanted control pots. Data are means of 6 replicates. Asterisks are derived from pairwise comparisons with a Bonferroni correction. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Error bars are equal to 1 standard error.

mean of their respective unplanted soil columns), though none of the increases were statistically significant. At Harvest 2, all rooted columns produced a greater mean peak force than their respective unplanted soil columns (*brb* = 3.7 %, barley WT = 10.4 %, *rth3* = 33.1 %, and maize WT = 15.5 % increase from the mean of their respective unplanted soil columns) but only *rth3* was significantly ($p < 0.05$) greater. At Harvest 3, both genotypes of barley ($p < 0.05$) and maize ($p < 0.01$) produced peak forces significantly greater than their unplanted columns (*brb* = 7.9 %, barley WT = 17.7 %, *rth3* = 32.8 %, and maize WT = 52.6 % increase from the mean of their respective unplanted soil columns). As peak shearing force tended to increase with each harvest differences between the rooted and unplanted treatments are likely to increase with longer periods of growth.

Water content (WC) and bulk density (BD) can both impact the peak force required to shear soil. For each treatment of barley and maize BD did not significantly differ ($p = 0.23$ and $p = 0.07$ for barley and maize, respectively). Likewise for maize, WC did not differ between the treatments (20.0 % ± 0.1 %, $p = 0.13$), however there was a significant treatment effect in barley ($p < 0.001$). Although *brb* and its WT soil columns were similar moist, both barley rooted treatments were consistently drier than the unplanted soil columns (19.8 ± 0.2 % WC and 19.0 ± 0.3 % WC for the unplanted columns and rooted columns, respectively; $p < 0.001$). There is a general consensus in the literature that increasing soil WC decreases soil shear strength (Vanapalli *et al.* 1996; Kayadelen *et al.* 2007; Fan and Su 2008; Hales and Miniat 2016; Yang *et al.* 2016) however, the variation in WC in this work was purposely small and therefore did not significantly impact peak forces ($p = 0.21$; Figure 4a). So, differences in peak force can be attributed (at least in part) to the presence/absence of roots.

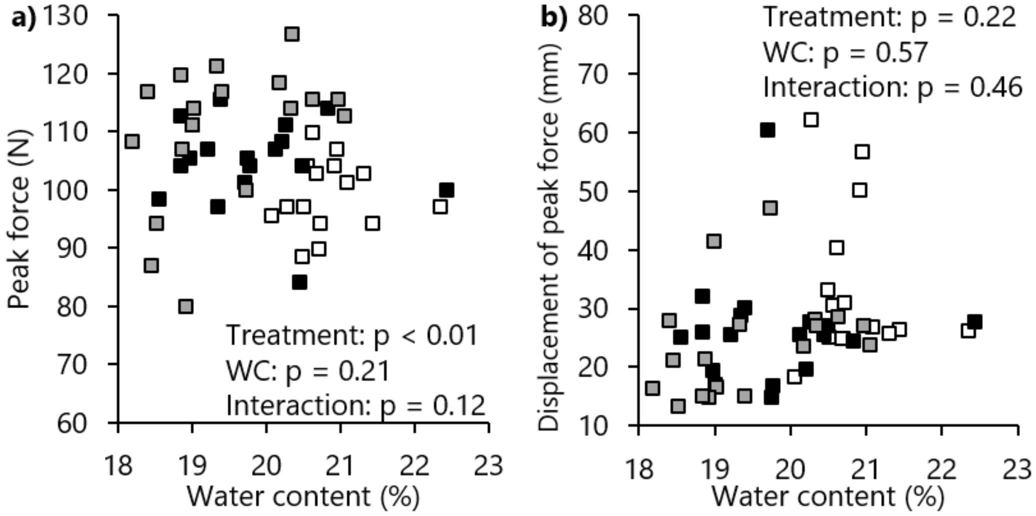

Figure 4. Water content of barley treatments against peak force (a) and the distance along the displacement profile that peak force was recorded (b). Grey markers = wild types, black = root hairless mutant (*brb* for barley and *rth3* for maize), and white = unplanted. P values are from ANCOVA.

When comparing the genotypic variation in peak forces (Figure 3a and 3b), there was no consistent or significant effect until Harvest 3. For barley, *brb* produced a peak force 1.6 % greater than its WT at Harvest 1, but at Harvest 2 the peak force required to shear the *brb* soil columns was 6.1 % less than its WT. For maize, *rth3* produced a peak force 2.5 % less than its WT at Harvest 1, and at Harvest 2 produced a peak force 13.2 % greater than its WT. However, at Harvest 3, both barley and maize WTs required significantly ($p < 0.05$ and $p < 0.01$, respectively) greater force to shear than their respective root hairless mutants (8.3 % increase for barley and 13.0 % increase for maize). Thus, the presence of root hairs only showed a consistent and significant impact at the final harvest for both barley and maize, where root hairs seemed to significantly increase the soil columns ability to resist shear forces.

3.3. Displacement of peak force

The point on the displacement scale at which the peak force was recorded (Figure 3c and 3d) corresponds to the amount of deformation the soil column can withstand. Peak forces occurring early in the displacement scale suggests brittle columns, whereas peak forces occurring later in the scale suggest higher levels of plasticity. The unplanted columns are expected to have peak forces near the end of the displacement profile, likely caused by increasing build-up of soil between the two halves of the pots, whereas the rooted columns should be more brittle. For maize, *rth3* consistently reached peak force at a displacement significantly earlier than its unplanted soil columns and the differences increased with each harvest (49.7 %, $p < 0.05$; 57.1 %, $p < 0.05$; 60.3 % $p < 0.001$ for Harvests 1, 2, and 3, respectively). The maize WT also consistently reached peak force at a displacement earlier than its unplanted soil columns (34.7 %, 39.7 %, and 58.7 % for Harvests 1, 2, and 3, respectively). Though the differences again increased with each harvest between the maize WT and unplanted columns, only Harvest 3 produced significantly ($p < 0.001$) different results. So, the increasing presence of maize roots tended to reduce the distance at which the peak force was recorded, suggesting the soil columns permeated with maize roots were more brittle than their unplanted columns.

For barley, the presence of roots did not have a consistent effect on how far along the displacement scale the peak force recorded. At Harvest 1, WT reached peak force at a mean displacement significantly earlier than the unplanted soil columns (55.6 %, $p < 0.05$), *brb* also reached peak force at a mean displacement earlier than the unplanted soil columns (40.2 % less), but the difference was not significant. Again at Harvest 2, both *brb* and its WT reached peak force earlier than their unplanted soil columns (16.4 % and 20.7 % for *brb* and its WT, respectively). At Harvest 3 however, *brb* soil columns reached peak force 2.7 % earlier than the unplanted soil columns, but WT soil columns reached peak force 1.3 % later. So, although the unplanted columns tended to reach peak force later than the rooted columns this trend was not consistent or significant. Though not significant ($p = 0.57$; Figure 4b), differences in WC could exacerbate the differences in where peak force was reached on the displacement profile. Consequently, the increasing presence of barley roots with each harvest did not seem to affect where in the displacement profile the peak force was reached.

The presence of root hairs did not significantly affect the point at which peak force was recorded (Figure 3c and 3d). For barley, *brb* reached the peak force later than WT for Harvests 1 and 2 (34.59 % and 5.51 %, respectively) but at Harvest 3, *brb* reached peak force before WT (3.93 %), though the difference was much narrower. As such, the mean difference between *brb* and WT across the harvests (*brb* reaching peak force an average of 8.56 % later than WT) was not significant for barley. For maize, *rth3* consistently reached its peak force before WT (29.73 %, 40.55 %, and 3.70 % for Harvest 1, 2, and 3, respectively) however, the mean differences across the harvests (*rth3* reached peak force 19.54 % earlier than WT) were also not significant. Therefore, as barley WT mostly reached peak force earlier than *brb*, whereas the maize WT consistently reached peak force after *rth3*, there is no consistent or significant genotypic impact of root hairs on where in the displacement profile the peak force was reached.

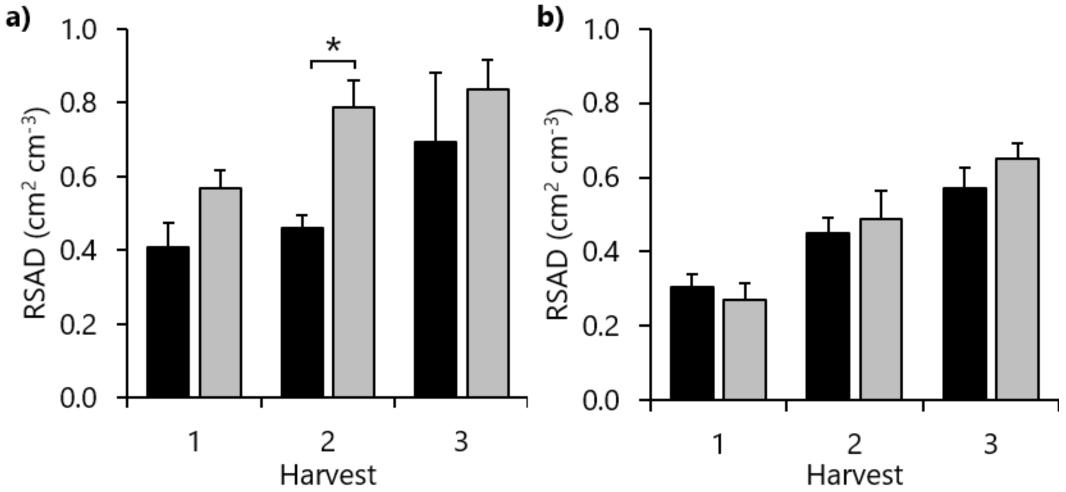

Figure 5. Root surface area density (RSAD) of the main root system (excluding root hairs) per harvest for barley (a) and maize (b). Black bars = root hairless mutants (brb for barley and rth3 for maize), and grey bars = their respective wild types (WT). Data are means of 6 replicates and error bars are equal to 1 standard error. Asterisks is from a student t test, * = $p < 0.05$.

3.4. *Root traits*

Not all root traits varied over time (Table 1). For root diameter, no genotype significantly varied between harvests, although the WT maize tended to increase with each harvest. Proportional representation of lateral roots was not significantly different between harvests for maize, though the mean percentage did tend to increase. In contrast, barley roots showed a significant ($p < 0.05$) decrease in percentage of lateral roots with each harvest. Root length density (RLD) increased with harvest for all genotypes, except barley WT where it decreased from Harvest 2 to Harvest 3, as such, only maize showed a significant ($p < 0.001$) increase with each harvest. For each genotype of each species, root surface area density (RSAD) increased significantly ($p < 0.05$ for barley and $p < 0.001$ for maize) with each harvest

(Figure 5 and Table 1), suggesting that RSAD is the most appropriate proxy for root development.

Across all harvests, the barley genotypes had significantly ($p < 0.001$; Table 1) thinner roots (by 34 %) than the maize genotypes, though the percentage of lateral roots did not differ between the two species ($p = 0.99$). Barley roots grew at a mean rate of 23.8 ± 1.5 and 31.3 ± 1.3 cm day^{-1} for *brb* and its WT, respectively, and maize roots grew at a slower rate of 16.2 ± 1.0 and 17.6 ± 0.4 cm day^{-1} for *rth3* and its WT, respectively. Thus, the RLD of maize root systems was approximately half (52 %) that of barley. Although shorter, maize roots were significantly ($p < 0.001$, Table 1) thicker (by 34 %) than barley roots. As RSAD is more responsive to increases in length, the RSAD of barley was significantly ($p < 0.001$) greater (by 27 %) than maize. Although maize produced thicker roots, barley had the greatest RSAD, as the roots were longer.

Root hair presence/absence affected some root traits, but not consistently across species or harvests. The maize WT and barley *brb* had consistently lower mean root diameter than their genotypic counterparts. The barley WT and maize *rth3* had the least percentage of lateral roots in their species at Harvests 1 and 2, but these genotypic effects were reversed at Harvest 3. Due to the differing growth rates, the RLD for *brb* barley was less than its WT at all harvests but in maize the growth rates were similar for both genotypes. The root hairless mutants of both species had lower RSADs (which does not include the surface area of root hairs) than their WTs, except for the first maize harvest. Therefore, the contribution of root hairs to soil reinforcement cannot be compared without accounting for the consistent increase of RSAD or changes in other root traits.

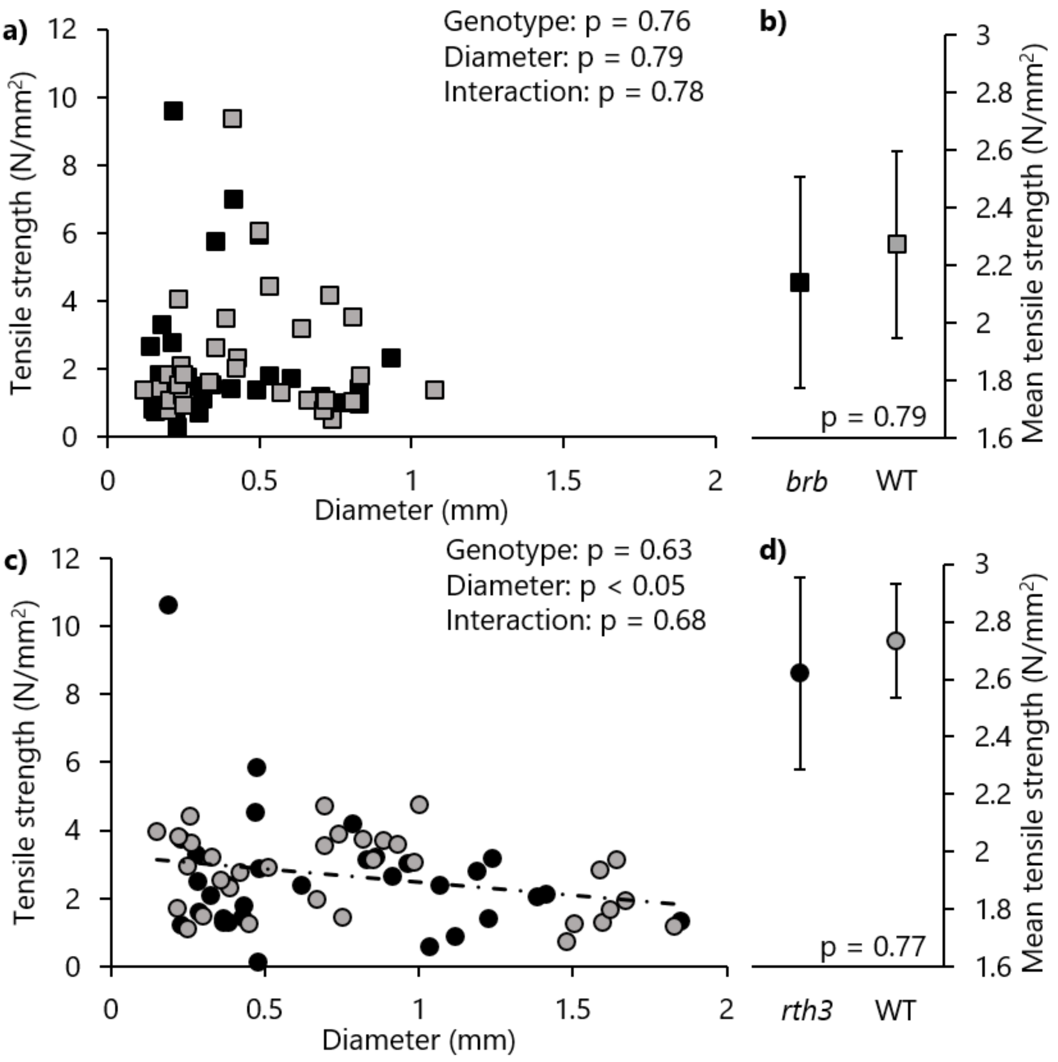

Figure 6. Root tensile strength against diameter of root for barley (a) and maize (c) and mean tensile strength of the barley (b) and maize (d). Grey = wild types (WT) and black = root hairless mutant (brb for barley and rth3 for maize). For a and c, there was no genotypic effect so a single regression line was fitted to pooled WT and mutant data where significant. p values from ANCOVA for a and c, for b and d p values are from ANOVA.

3.5. Root trait effect on peak force

The distance at which the peak force occurred was not significantly correlated to any root trait (Table 2). However, the peak force required to shear the soil columns significantly increased with RSAD for both species ($p < 0.01$ and $p < 0.001$ for barley and maize, respectively). Additionally, the presence of root hairs also appears to significantly increase the peak force at Harvest 3 ($p < 0.01$ for barley and $p < 0.05$ for maize; Figure 3). As mean root tensile strength were identical for the barley and maize genotypes ($p = 0.79$ and $p = 0.77$, respectively; Figure 6b and 6d), and the tensile strength of each genotype similarly decreased with increasing diameter as their counterpart (Figure 6a and 6c), it can be assumed there were no intrinsic differences between the strength of the root hairless mutants and their WTs. Additionally, the genotypic disparities in RSAD show that the differences in peak shearing force recorded at Harvest 3 cannot be solely attributed to the presence of root hairs (Table 1). Furthermore, the rate at which WTs peak force increased with increasing RSAD was identical to their respective root hairless mutants, for both barley and maize (Figure 7). So, root systems with the same surface area density produced the same peak force regardless of whether they had root hairs or not. Therefore, as genotype did not significantly affect root tensile strength or the relationship between RSAD and peak force ($p = 0.64$ and $p = 0.90$ for barley and maize respectively), any variations in peak shearing force should be attributed to differences in RSAD and not to the presence/absence of root hairs.

The tensile strength of both maize and barley roots tended to decrease with increasing root diameter (Figure 6a and 6c) though this trend was only significant in maize ($p < 0.05$). Maize roots were significantly ($p < 0.001$, Table 1) thicker than barley, by up to four times, and their mean tensile strength was 21.4 % greater than in barley (Figure 6b and 6d), though this difference was not significant. As such, peak shearing force increased by 17 % with each unit increase of RSAD for barley (Figure 7a), whereas for maize the rate of increase was 6.3-fold greater, with one unit of RSAD approximately doubling peak force (Figure 7b). The divergence in trends suggests that increasing presence of maize roots are more effective at reinforcing soil than the same increase in barley roots. Although barley produced a more extensive network of roots than maize, the increased tensile strength of the maize root systems were more effective at increasing the peak shearing force, suggesting that root tensile strength is more influential at reinforcing soil than root proliferation.

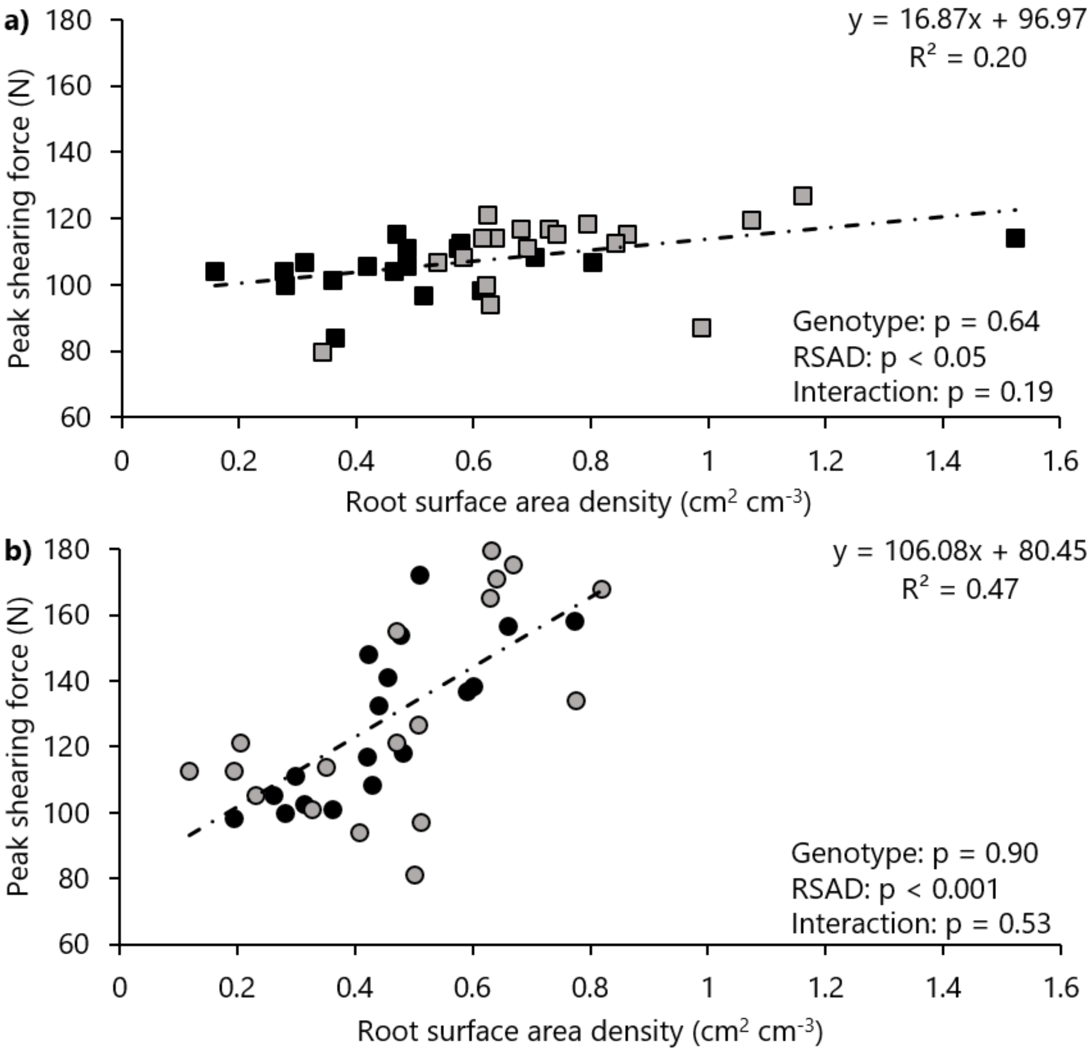

Figure 7. Peak shearing force against root surface area density (RSAD) of the main root system (excluding root hairs) for barley (a) and maize (b). Grey markers = wild types (WT) and black markers = root hairless mutant (brb for barley and rth3 for maize). For a and b, there was no significant genotypic effect so a single regression line was fitted to pooled WT and root mutant data where significant ($p < 0.05$ for barley, $p < 0.001$ for maize) p values from ANCOVA.

4. Discussion

4.1. Impact of root hairs on soil shear strength

Root hairs increase the resistance of seedling radicles to removal from the soil (Bengough et al., 2016, 2011; Ennos, 1989; Stolzy and Barley, 1968) and significantly increase the amount of soil that binds to the root system (Haling et al., 2013; Czarnes et al., 1999), however, they do not seem to contribute to a root system’s ability to reinforce soil. The root systems of the root hairless mutants of each species were equally as capable as their respective WT at reinforcing the soil, as they required equal forces to shear (Figure 7). Further to this, although barley WT roots have significantly longer and more numerous root hairs than the maize WT roots, and thus achieved greater root-soil contact (Burak, 2019), these differences in soil adhesion had no measurable effect on soil reinforcement. In contrast, barley roots consistently provided less soil reinforcement than the maize root systems.

A root's ability to withstand shear forces and contribute to a soil's shear strength is ultimately determined by its ability to stay anchored in the soil (Pollen, 2007). Root hairs are only single celled and have significantly smaller diameters than their parent roots (Figure 8), so although the tensile strength of root hairs is unknown the force required to break them is estimated to be an order of magnitude less than that of a fine root (Bengough et al., 2011). As shear force is applied to the soil, roots dissipate the force throughout the root system (Figure 8a), however as the shearing force increases the root hairs will break (Figure 8b) long before the parent root (Figure 8c). So, although root hairs can effectively reinforce singular roots against

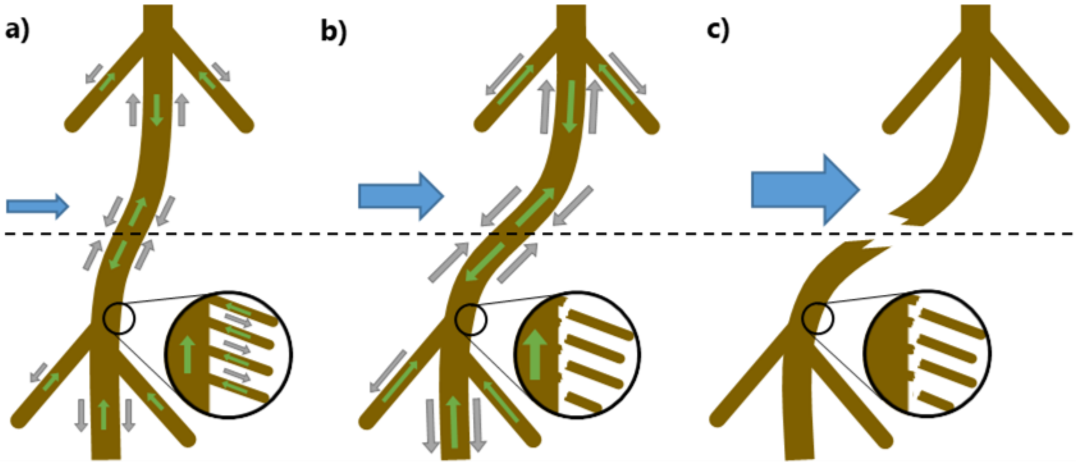

Figure 8. A conceptual diagram of the impact of root hairs on a roots ability to reinforce soil at the shear plane (dashed line). The shear resistance of roots is their ability to resist movement, thus reinforcing the soil. (a) At low shear force (blue arrows) the whole root system can resist movement (green arrows), effectively dissipating the traction (grey arrows) throughout the root system; (b) increasing shear force will reach then exceed the tensile strength of root hair causing them to break well before the parent root (c).

uprooting (Czarnes et al., 1999; Ennos, 1990, 1989) or during root penetration (Bengough et al., 2016, 2011; Haling et al., 2013; Handley and Davy, 2002), their impact on root anchorage is negligible in a more complex root system. Thus, the contribution of root hairs to soil reinforcement is overshadowed by the shear resistances exerted by the greater tensile strength and diameter of the roots themselves.

4.2. Plant species affects root contribution to soil shear strength

Rooted soil columns required considerably more force to shear than unplanted soil columns (Figure 2 and 3) and the force required to shear the soil columns increased with increasing root presence (Figure 7), as previously observed (Fan and Su, 2008; Jonasson and Callaghan, 1992; Li et al., 2013; Loades et al., 2010; Pollen and Simon, 2005). However, when roots are present, root diameter and tensile strength seem to primarily determine soil reinforcement.

Although it is widely understood that tensile strength decreases as root diameter increases, (Nilaweera and Nutalaya 1999; Pollen and Simon 2005; Yang et al. 2016), the thicker maize roots exhibited greater tensile strength than the thinner barley roots. The tensile strength of a root system can change depending on the orientation of slope and the direction of prevailing winds (Stokes et al. 1995; Norris 2005). Additionally, taller plants have extra weight to anchor, so produce roots with greater tensile strength (Nilaweera and Nutalaya 1999; Ali 2010; Sun et al. 2011; Osman et al. 2011). It is therefore rational that maize roots have greater tensile because they grow significantly taller and, thus, have more above ground matter to support.

As the two parts of the pot are displaced, the force exerted by the soil columns increase to a peak (termed the peak shearing force) and then tapers off (Figure 2). Peak shearing force increased with increasing RSAD in each species, however not at the same rate (Figure 7). Despite RSAD being significantly greater in barley than in maize (Figure 5), the force required to shear the maize soil columns far exceeded (by 66% for a RSAD of 0.8 cm^2 cm^{-3}) the force required to shear the barley soil columns (Figure 3). Thus, the increased diameter and tensile strength of the maize roots better resisted shear forces, therefore not all fine roots have the same ability to reinforce soil.

Due to the increased RSAD in barley, it can be assumed that more roots crossed the failure plane for barley than for maize. Despite this, maize still provided greater tensile resistance than barley. So, the increased root presence of barley could not compensate for their lesser tensile strength in comparison to the maize roots. This is largely because the combined tensile strength of a root bundle is not equal to the sum of each individual root. When tension is initially exerted, root loading will be unequally distributed because not all roots will be perpendicular to the shearing plane, further to this, roots will break at different points due to differences in tensile strengths. When the applied force exceeds the strength of a root (be it because force is unequally focused on it or that it is weaker than the rest), it will break. Progressive breaking compounds the forces applied to the remaining roots and exacerbates breakages (Pollen and Simon, 2005). Therefore, bundles of stronger but less numerous roots (as seen in maize) are more effective at reinforcing soil than more numerous weaker roots (as seen in barley).

5. Conclusion

By comparing genotypes with and without root hairs in two cereal species, this study investigated which root traits most influenced a root system’s ability to reinforce soil. Since the WT and root hairless mutants showed no differences in soil reinforcement, it can be concluded that root hairs have very little impact on a root systems ability to reinforce soil under shear stress, as they cannot withstand the same forces resisted by the rest of the roots. Further to this, barley roots were more than twice as numerous as maize roots, but were 21 %

weaker, on average, than maize roots and almost half as thin. As such, maize root systems (with their increased diameter and tensile strength) were six times more efficient at reinforcing soil than the barley root systems. Thus, increased root tensile strength and diameter reinforced soil more effectively than increased root length density. So, root strength appears to be the biggest factor determining a root system’s ability to withstand shear forces regardless of the presence or absence of root hairs.

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Tables

Table 1. Root parameters per genotype per harvest.

			Diameter (mm)		Lateral (%)		RLD (cm cm^{-3})		RSAD (cm^{-2} cm^{-3})	
Barley	*brb*	1	0.146 ± 0.003^{a}		96.77 ± 0.69^{ab}		8.94 ± 1.37^{a}		0.408 ± 0.064^{a}	
		2	0.143 ± 0.005^{a}		94.94 ± 0.84^{ab}		10.29 ± 0.81^{a}		0.461 ± 0.035^{a}	
		3	0.165 ± 0.006^{ab}		93.35 ± 1.35^{b}		13.62 ± 3.80^{a}		0.692 ± 0.188^{a}	
	WT	1	0.161 ± 0.003^{ab}		95.65 ± 0.38^{a}		11.18 ± 0.87^{a}		0.567 ± 0.049^{a}	
		2	0.180 ± 0.002^{b}		91.89 ± 0.82^{ab}		16.41 ± 1.35^{a}		0.787 ± 0.074^{a}	
		3	0.170 ± 0.008^{b}		93.77 ± 1.53^{ab}		15.66 ± 2.87^{a}		0.835 ± 0.079^{a}	
ANOVA			*F value*	*P value*	*F value*	*P value*	*F value*	*P value*	*F value*	*P value*
Harvest			3.12	0.058	**4.68**	**< 0.05**	2.48	0.100	**4.28**	**< 0.05**
Genotype			**17.70**	**< 0.001**	2.21	0.147	4.01	0.054	**7.40**	**< 0.05**
Maize	*rth3*	1	0.236 ± 0.006^{a}		93.40 ± 0.82^{a}		4.14 ± 0.49^{a}		0.305 ± 0.033^{a}	
		2	0.257 ± 0.010^{a}		94.43 ± 0.53^{a}		5.69 ± 0.69^{ab}		0.451 ± 0.040^{ab}	
		3	0.255 ± 0.009^{a}		95.03 ± 0.80^{a}		7.18 ± 0.74^{ab}		0.572 ± 0.053^{b}	
	WT	1	0.229 ± 0.012^{a}		93.58 ± 1.13^{a}		3.96 ± 0.80^{a}		0.271 ± 0.044^{a}	
		2	0.240 ± 0.004^{a}		95.20 ± 0.77^{a}		6.47 ± 0.98^{ab}		0.489 ± 0.074^{ab}	
		3	0.247 ± 0.012^{a}		94.66 ± 1.22^{a}		8.49 ± 0.77^{b}		0.649 ± 0.041^{b}	
ANOVA			*F value*	*P value*	*F value*	*P value*	*F value*	*P value*	*F value*	*P value*
Harvest			2.49	0.099	1.52	0.235	**12.83**	**< 0.001**	**22.03**	**< 0.001**
Genotype			2.09	0.158	0.07	0.793	1.09	0.304	0.47	0.497
Species			**293.17**	**< 0.001**	0.00	0.987	**43.36**	**< 0.001**	**9.49**	**< 0.01**

Diameter = mean diameter of the whole root system, Lateral = the proportion of the root system made up of lateral roots, RLD = root length density, RSAD = root surface area density and WT = wild type. Letters denote statistically different means ($p < 0.05$) than other harvests/genotypes within the species and are generated from a pairwise comparison with a Bonferroni correction. Data are means of 6 replicates ± 1 standard error.

Table 2. Pearson correlation coefficients for measured root parameters and displacement at which the peak force was recorded.

	Correlations	Diameter (mm)	Fine roots (%)	RLD (cm cm^{-3})	RSAD (cm^{2} cm^{-3})	Displacement (mm)
Barley	Fine roots (%)	0.05				
	RLD (cm cm^{-3})	0.13	0.22			
	RSAD (cm^{2} cm^{-3})	**0.34***	0.18	**0.90*****		
	Displacement (mm)	0.07	-0.07	-0.06	-0.08	
	Peak Force (N)	0.25	**0.39***	**0.42***	**0.44****	0.01
Maize	Fine roots (%)	**-0.66*****				
	RLD (cm cm^{-3})	-0.18	**0.54****			
	RSAD (cm^{2} cm^{-3})	0.07	**0.38***	**0.97*****		
	Displacement (mm)	-0.11	-0.19	-0.03	-0.06	
	Peak Force (N)	0.26	0.19	**0.61*****	**0.68*****	-0.24

RLD = root length density and RSAD = root surface area density.

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$