Revised manuscript with changes accepted:

Title: The ability of maize roots to grow through compacted soil is not dependent on the amount of roots formed

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Abstract

Highlights:

- Soil compaction influenced the proportion of coarse roots (i.e. > 1 mm diameter) of maize in a genotype-dependent manner
- Rooting depth was reduced and root distribution within the soil profile changed when grown in compacted soil
- Rooting depth and total root length, total coarse root length and total fine root length were not correlated with each other in compacted soil
- The ability of roots of different genotypes to reach a certain depth was not related to the amount of roots formed

Keywords

Impedance; compaction; root length; root distribution; compensatory growth

Abstract

Mechanical impedance is a primary constraint to root growth and hence the capture of soil resources. To investigate whether rooting depth and root length under mechanical impedance caused by compaction are correlated we evaluated 12 maize lines at two field sites. To distinguish between lateral and nodal roots, roots were sorted into different diameter classes. Coarse roots had diameters >1 mm and represent nodal root axes. Greater proportions of coarse roots on compacted plots were found at both field sites however results were driven by genotypic variation. Soil compaction reduced total rooting depth (in all diameter classes) and coarse rooting depth at both sites compared to non-compacted plots. Root distribution was influenced by compaction with greater root length densities closer to the soil surface. Root length and root depth were not related to each other under impeded conditions. Coarse roots of some genotypes became obstructed on the compacted plots, while other genotypes were capable of growing through the impeding soil and reached deeper soil strata resulting in differential distribution of roots through the soil profile. On compacted plots we observed genotypes with similar root depths but with contrasting coarse root lengths. The ability of roots to grow through compacted soils is therefore not dependent solely on the coarse root length formed by the root system.

1. Introduction

The ability of plants to acquire nutrients and water is dependent on soil exploration. Mechanical impedance can lead to reduced total root length and/or a redistribution of root length within the soil profile (Pfeifer *et al.*, 2014a; Shierlaw and Alston, 1984), which could affect the acquisition of water and nutrients. As soils get denser and stronger with depth, due to overburden pressure (Gao *et al.*, 2012, 2016), mechanical impedance will often restrict deeper rooting root phenotypes more than topsoil foraging root phenotypes. Periodic droughts are common in many ecosystems and drier soils are generally harder (Gao *et al.*, 2012; To and Kay, 2005; Vaz *et al.*, 2011; Whalley *et al.*, 2005; Suralta *et al.,* 2018). However, plants with root systems that grow deeper are in general better adapted to drought (Chimungu *et al.*, 2014a; Lilley and Kirkegaard, 2016; Lynch, 2013; Zhan *et al.*, 2015). Certain soils offer very large mechanical impedance to roots, for example hard-setting soils in Australia (Mullins *et al.*, 1987) or rainfed lowland rice cultivation systems (Suralta *et al.,* 2018). Different agricultural management approaches can also introduce compaction and plough pans by wheeled traffic or trampling (Batey, 2009; Hamza and Anderson, 2005). Depending on the soil textural characteristics, suboptimal soil conditions during trafficking (such as high moisture contents) will exacerbate compaction (Horn et al., 1995; Raper, 2005). Roots can become confined to surface soil strata when not capable of penetrating through a hard soil layer such as a plough pan (Barraclough and Weir, 1988; Ehlers *et al.*, 1983). Root systems are able to compensate root growth by exploiting the lesser impeded regions of the soil, as illustrated by split pot experiments (Bingham and Bengough, 2003; Pfeifer *et al.*, 2014a) or layered pot systems (Shierlaw and Alston, 1984). Roots of maize (Chimungu *et al.*, 2015), rice (Chandra Babu *et al.*, 2001; Clark *et al.*, 2000, 2002; Yu *et al.*, 1995), wheat (Botwright Acuña and Wade, 2005; Kubo *et al.*, 2006) and common bean (Rivera *et al.*, 2019) show substantial genotypic variability for penetrating strong wax layers simulating mechanical impedance.

Root systems consist of distinct root classes which vary by taxa, for example many dicot taxa have a dominant taproot, while monocots, such as cereals, form nodal roots from shoot nodes (Hochholdinger *et al.*, 2004; Lynch and Brown, 2012; Rich and Watt, 2013). Adult maize root systems consist of primary, seminal, crown (belowground nodal) and brace (aboveground nodal) roots, all these classes form lateral roots. For monocotyledons, nodal roots are the main parent axes of lateral roots present at depth as these laterals proliferate from nodal roots (Cairns *et al.*, 2004; Nagel *et al.*, 2012).

Genotypic variation for lateral root phenotypes has functional consequences in maize (Postma *et al.*, 2014; Zhan *et al.*, 2015; Zhan and Lynch, 2015; Jia *et al.*, 2018). Root classes have different elongation rates that vary greatly as a function of time. For maize, lateral roots have been found to elongate at 2.2 cm day^{-1} for 2.5 days, while nodal roots elongated at a rate of 3 cm day^{-1} over a 5 week period (Cahn *et al.*, 1989). Under non-impeded conditions primary roots of maize elongated at 4.8 cm day^{-1}, while seminals only elongated at 3.2 cm day^{-1} (Veen and Boone, 1990). Differences in elongation rates between root types can lead to soils being differentially explored with time by each root type and could affect the volume and depth of bulk soil that can be explored within a certain time by different root types. Biomechanical properties also vary according to root class, with seminal roots being stronger than lateral roots (Loades *et al.*, 2013). Whether this translates to specific penetration ability under impeded soil conditions according to root class remains to be investigated. It has been hypothesised that the contrasting phenotypes of distinct root classes adds to a plants' plasticity and flexibility when interacting with different environments (Chochois *et al.*, 2015; Wu *et al.*, 2016) but the functional implications of the differential effects of mechanical impedance on distinct root classes are poorly understood.

Root system size differs among genotypes and different soil conditions (Gao and Lynch, 2016; Nakhforoosh *et al.*, 2014). Root system size, expressed as total root length or root length density, can be split between coarse and fine roots (Cahn *et al.*, 1989; Steinemann *et al.*, 2015; Varney *et al.*, 1991). Small grain cereals such as wheat or barley are characterised by fine axial roots, maize has thicker axial roots, while dicots and perennials have very coarse axial roots. But for all these species, a distinction between a main root axes and smaller diameter lateral roots can be made. Coarser roots are needed in order to deploy finer roots within the soil profile. Studies on wheat suggest that wheat genotypes with more root axes had greater penetration of wax layers (Whalley *et al.*, 2013).

Mechanical impedance not only affects root growth, it also has an impact on shoot growth. Root to shoot ratios can decrease under compaction (Andrade *et al.,* 1993; Hoffmann and Jungh, 1995; Pfeifer *et al.*, 2014a). Aboveground plant growth is impacted as leaf elongation rates can be reduced (Andrade *et al.,* 1993; Young *et al.,*

1997) and the rate of leaf appearance decreases (Beemster and Masle, 1996) when roots experience mechanical impedance. The reduction of shoot and root growth due to mechanical impedance can result in decreasing yield (Kirkegaard *et al.*, 1992 ;Tuzzin de Moraes *et al.,* 2020).

Better root growth under mechanical impedance can be attributed to different traits. For instance, the frictional component of mechanical impedance is reduced when roots produce mucilage or border cell sloughing (Iijima *et al.* 2000, 2004; Bengough and McKenzie, 1997). Smaller root tip radius to length ratios are linked to greater elongation rates under mechanical impedance (Colombi *et al.,* 2017b). Another beneficial trait is the presence of root hairs which can provide anchorage for roots to cross from loose to harder soil layers (Bengough *et al.*, 2011; Haling *et al.,* 2013). Root hairs also maintain water uptake when soils dry (Carminati *et al.*, 2017). Root anatomical traits such as greater cortical cell diameter have been linked to reduced energy costs under impeded conditions (Colombi *et al.*, 2019). It has been suggested that smaller outer cortical cells prevent buckling, which facilitate penetration of harder layers (Chimungu *et al.,* 2015).

Genotypes can adjust their root distribution with depth in response to compaction (Barraclough and Weir, 1988) however few studies have compared different genotypes and their redistribution of roots under compaction. Little is known about root system size for those root systems that do manage to grow deeper in compacted soils. The hypothesis that rooting depth and root length are not related to each other on compacted plots was tested for deeper rooting genotypes.

2. Material and Methods

2.1. Plant material and growth conditions

Twelve maize (*Zea mays* L.) recombinant inbred lines from a study by Chimungu *et al.* (2015) were selected for different levels of root penetrability of a wax layer. These genotypes were planted in a split-plot design in order to study their root growth in compacted conditions at two field sites. Seeds were obtained from Dr. Shawn Kaeppler (University of Wisconsin, Madison WI, USA – Genetics Cooperations Stock Center, Urbana, IL, USA). Genotypes were grown at the Apache Root Biology Centre (ARBC), Willcox Arizona, USA (32º01'N, 109º41'W), planted on June 16, 2016, and the Russell E. Larson Agricultural Research Center in Rock Springs (further referred to as PSU), Pennsylvania, USA (40º42'N, 77º57'W), planted on July 10, 2017. Field sites differed in soil texture, the ARBC site has a soil classified as a Grabe series (coarse-loamy, mixed, superactive, calcareous thermic Torrifluvent) and has a clay loam texture, while the PSU site is classified as a Hagerstown series (silt-loam, fine, mixed, semiactive, mesic Typic Hapludalf) and has a silt-loam texture. Compaction was introduced by passing over the treated plots with heavy machinery. At ARBC a 4 wheel tractor (4 tonnes with 8 passes) and at PSU a 3-axle truck (20 tonnes with 4 passes) were used. Penetration resistance (Figure 1) as well as an increase of dry bulk density were measured over the soil profile in order to verify increased soil strength and soil density. A FieldScout SC900 Compaction Meter (Spectrum Technologies Inc., Aurora, IL, USA) fitted with a 1/2 inch cone was used to measure the penetration resistance. Dry bulk density was calculated as the mass of oven dried soil per unit soil sample volume (Blake and Hartge, 1986). Soil was oven dried at 105ºC until a constant weight was achieved. Irrigation was managed on the basis of soil moisture content to avoid water deficit stress (PR2/6-tubes at ARBC (Delta-T Devices Ltd, Cambridge UK) and multiplexed TDR-100 probes at PSU (Campbell Scientific Inc., Logan, UT, USA). PR2/6-tubes were installed on both the compacted and non-compacted plots at ARBC, while TDR-100 probes were installed at a nearby field site on non-compacted conditions to determine if irrigation was needed (Figure S1: gravimetric water contents were calculated from volumetric water contents and soil dry bulk density). As the ARBC field site was based in the desert, the plots were heavily irrigated, while PSU plots did not require any supplemental irrigation. Nutrients and pesticides were applied based on standard agronomic practices (Table S1).

2.2. Root sampling

When all genotypes were tasselling (55 and 51 days after planting for ARBC (coarse-loam) and PSU (silt-loam) respectively) one soil core was taken from each subplot. Coring tubes (60 cm deep, 5.1 cm diameter) fitted with a plastic sleeve (4.5 cm diameter) were driven into the soil between 2 plants in a row (Trachsel *et al.*, 2013). Cores were stored at 4º C until root washing could be carried out, up to a maximum of 2 weeks. Cores were divided into six 10 cm increments and roots were washed out of the soil over a 850 µm sieve for each depth profile. Roots were temporarily stored in 75% ethanol in water (v/v). Root length per section was measured by scanning roots on a flatbed scanner (Epson Perfection V700 photo, Epson America, Inc., Long Beach, USA) and analysis was carried out with WinRHIZO Pro 2013e system (Regent Systems Inc., Quebec, Canada). Each core increment was captured by images taken at a resolution of 400 dpi (15.75 pxls/mm), speed priority setting off and dust removal on high. Axial roots (nodal, primary, seminals) and lateral roots have been identified as having a diameter >0.6 cm and <1.0 cm for maize respectively (Cahn *et al.*, 1989; Varney *et al.* 1991, Hund *et al.* 2009) and are therefore used instead of the Böhm classification (Böhm, 1979). Using root diameter classes 0 – 0.5, 0.5 – 1.0, 1 – 1.5, 1.5 – 2.0, 2.0 – 2.5, 2.5 – 3.0, 3.0 – 3.5, 3.5 – 4.0, >4 mm permitted discrimination of coarse (> 1 mm diameter) and fine (< 1 mm diameter) roots and attributed coarse roots to nodal root classes from the third node and upward. However individual nodal root classes could not be distinguished from cores as there is no reference to root crown position. Root length measurements (total, coarse and fine) and proportions (coarse and fine) were made for the entire soil core. Root distributions were compared on the basis of root length densities measurements within 10 cm increments. D_{95} and D_{75} are the rooting depth above which 95 and 75% of the total root length within a core were located. These rooting depth measurements were calculated by linear interpolation (Schenk and Jackson, 2002). When applied to the coarse root fraction in the core, in order to calculate the coarse rooting depth, these measurements are indicated as D_{95c},

D_{75c}. An overview of the different measurements directly measured or calculated from the winRHIZO scans and their definition can be found in Table 1.

2.3. Plant sampling

Two plants per subplot (4 replicate subplots per compaction treatment) were sampled at tasselling using the 'shovelomics' method (Trachsel *et al.*, 2011). Subsequent measurements per subplot were obtained by averaging between the two harvested plants per subplot. Root crowns were carefully washed and removed from the stem above the brace roots, brace roots not reaching the soil were clipped off at the base of the stem to expose the crown roots. Root crowns were then imaged to obtain information about the root angle in order to establish that root angle did not affect rooting depth (Figure S2). We used a Nikon D70s camera with a sigma DC 18-50mm lens which produced 4000 x 6000 pxl images. Illumination was achieved by white fluorescent indoor lighting with a white cloth cover for light diffusion. Root angle from the horizontal was manually measured in ImageJ (Schneider *et al*., 2012). Above ground plant parts were dried at 60° C for 3 days and dry weight of the biomass recorded (Figure S3).

2.4. Statistical Analysis

Genotypes were planted in a completely randomised split-plot design with compaction treatment at the whole-plot level (167.26 m^2) and twelve genotypes as subplots, replicated four times in each field site. Each subplot was 3.05 m x 4.57 m and ordering of genotypes (subplots) was randomised within each whole-plot. Every subplot was then planted with 4 rows of the appropriate genotype, with 23 cm within row spacing and 76 cm between row spacing reaching a planting density of approximately 57500 plants per hectare. This planting density is sparser than standard agronomic practices which reduced intra-plant competition and aided sampling. All root distribution variables derived from coring (1 soil core per subplot) were transformed using a Box-Cox transformation to achieve normality before analysing the data in a split plot ANOVA. Total root length per genotype was plotted against averaged penetration resistance across both field sites. Root proportions per genotype were plotted per field site and post-hoc comparisons between compacted and non-compacted treatments were carried out using a Tukey HSD test. The same was done for coarse and total rooting depth, where additionally a linear regression was tested between these measurements. A generalised linear model was applied to assess the effect of field site, compaction and genotype and coarse and total root lengths on rooting depth. Relationships between variables were first assessed by correlation plots on pooled data across all genotypes. For the relationship between D_{75} and D_{75c} an analysis of covariance (ANCOVA) was performed, followed by linear regression. As genotypic effects were identified by the split plot analysis on certain variables, individual linear regressions were made on the averaged genotypic values. Rooting depth data (coarse and total) and total root length averaged per genotype were normally distributed within impedance level and field site datasets. Root proportional data was analysed by using a beta regression (Cribari-Neto and Zeileis, 2015). To further analyse the variable relationships among each other, a principal component analysis (PCA) was conducted within field site – compaction treatment combinations. Principal components were retained based on eigenvalues greater than 1. In order to investigate different types of root distributions under compacted conditions rooting depth data was plotted against total root length data and genotypes with either similar root length and contrasting depth or with similar depth and contrasting root length were identified. An ANOVA was used to test the effect of genotype, compaction, total rooting depth (D_{75}), total coarse rooting depth (D_{75c}), total root length (TRL) and total fine root length (TRL_f) on aboveground biomass. Graphpad Prism (Graphpad Software, 2017) was used for visualising data and R version 3.5.0 (R Core Team, 2018) was used for data analysis.

3. Results

3.1. Decrease in root length on compacted soil depends on field site

Total root length (TRL) from ARBC (coarse-loam) soil cores was greater than the total root length in PSU (silt-loam) cores in both compacted and non-compacted plots (Figure 2, S4). On coarse-loam (ARBC) total root length was reduced by 47.4% on average across all genotypes when grown on the compacted plots and total root length was clearly reduced for each genotype (Figure 2, Table 2). As total coarse root length represents only a small part of the total root length (Figure 2), total root length reduction on coarse-loam (ARBC) is mainly due to reduced total fine root length (Figure 2, Table 2). In contrast, on silt-loam (PSU), compaction did not significantly alter total root length (Table 2), possibly due to greater penetration resistance on both compacted and non-compacted plots in comparison with the coarse-loam (ARBC) plots (Figure S4).

Total coarse root length (TRL_c) was differentially affected by the compaction treatment at both field sites (Figure 2, Table 2). A compaction x genotype interaction was present on coarse-loam (ARBC), but not on silt-loam (PSU) (Table 2). The overall average of total coarse root length decreased from 48.9 cm ± 3.4 (se) to 39.0 cm ± 3.3 (se) under compaction on coarse-loam (ARBC), while it increased from 16.3 cm ± 2.2 (se) to 23.7 cm ± 3.3 (se) under compaction on silt-loam (PSU). Total fine root length (TRL_f) was negatively affected by the compaction treatment on coarse-loam (ARBC) (decreasing from 1755.9 cm ± 77.9 (se) to 809.1 cm ± 37.3 (se)), but was not affected on silt-loam (PSU) (Table 2). At both field sites no genotypic differences were present for total fine root length (Table 2). A positive effect of compaction was noted on overall coarse root proportion (P_c) at ARBC (coarse-loam) with an increase from 2.8% to 4.4% under compacted soil conditions (Table 2, Figure 3). At PSU (silt-loam) we observed an increase from 3.4% to 4.4% for P_c, (Figure 3) although no compaction treatment effect was noted (Table 2). Genotype had significant effect on the proportion of coarse roots (P_c) and fine roots (P_f) at both field sites and for coarse-loam (ARBC) there was an interaction between compaction treatment and genotype present (Table 2). Compaction increased the proportion of coarse roots for most genotypes (Figure 3). The only genotype that had greater P_c under impeded conditions at both field sites was IBM051. Other genotypes manifesting increased P_c under impeded conditions were OhW122, OhW119, NyH126, IBM146 and IBM059 at ARBC (coarse-loam), but these did not show increased P_c in silt-loam (PSU). In silt-loam (PSU) other genotypes such as OhW128 and IBM284 increased their P_c, while they did not in coarse-loam (ARBC). A genotype that did not have greater coarse root proportions in response to compaction in either location was IBM086, this genotype had similar coarse root proportions in coarse-loam (ARBC), while in silt-loam (PSU) the coarse root proportions appeared smaller under compaction.

3.2. Total rooting depth versus coarse rooting depth

Absolute and relative measures of root length density per depth increment provided insight into how roots were growing within the soil profile and how root distributions change in response to compaction (Figure 4). Root distribution changes became clearer when relative root length density measures were considered (Figure 4B-D). Distributions of coarse root length density and root length density differed (Figure 4) illustrated by differences in values of D_{95} and D_{75} (rooting depth considering all roots diameter classes) versus D_{95c} and D_{75c} (rooting depth considering coarse roots) (D_{75} and D_{75c} shown in figure 4, 5). Total rooting depth and coarse rooting depth measurements were correlated (Figure 5F). D_{75} and D_{75c} were significantly reduced by compaction at both locations, while D_{95} and D_{95c} were only reduced at ARBC (coarse-loam) (Table 2). A genotypic effect on rooting depth was present in coarse-loam (ARBC), but absent in silt-loam (PSU) (Table 2). On coarse-loam (ARBC) some genotypes had significantly shallower total and coarse rooting depths under impeded conditions (IBM014, IBM059, IBM146, OhW119, OhW122) (Figure 5A-C). Other genotypes such as OHW128 had shallower total root length under impeded conditions, but coarse rooting depth was not significantly reduced (Figure 6C). In contrast, IBM323, IBM178, IBM284 and IBM086 had shallower coarse rooting depth under compaction, but total rooting depth was not reduced (Figure 5A-C). On silt-loam (PSU) an effect of compaction was present on D75c and D75 (Table 1), however only genotype, IBM059, showed significantly shallower coarse root distributions (Figure 5B).

3.3. Relationships between root distribution variables

Relationships between the different variables can be further explored through the correlation plot across all genotypes (Figure S5, S6, S7) as well as the PCA plots per field site with treatment combination (Figure S8). Individual linear regressions between root distribution variables depicting the different genotypes can be found in Figures 6, S6 and S7. Across all field sites and levels of compaction rooting depth variables (D_{95}, D_{75}, D_{95c} and D_{75c}) positively correlated to each other (Figure S8). Likewise root length variables total root length (TRL), total fine root length (TRL_f) and total coarse root length (TRL_c) correlated strongly with each other (Figure S8). Relationships between rooting depth and the other root distribution variables are discussed below.

3.3.1. The relationship between total rooting depth and other root distribution variables

A negative relationship between total root length and total rooting depth was found under ARBC (coarse-loam) non-compacted conditions at a probability of 0.10 (Figure 6A, S5). General linear modelling indicated integrative effects of field site and compaction on the relationship between total root length and total rooting depth (Table 3). As total root length mainly consists of fine roots, a relationship persists between total fine root length and total rooting depth (Figures S6). No such relationship was seen on silt-loam (PSU) or under compaction (Figures 6B, S6). No relationship was found for total rooting depth and total coarse root length (Figure S6C-D) and coarse root proportion (Figure S6E-F) under any scenario.

3.3.2. The relationship between coarse rooting depth and other root distribution variables

A positive relationship between coarse root proportion and coarse rooting depth ($D75_c$) was present under non-compacted conditions at the coarse loam (ARBC) field site (Figure 6C). This correlation was not observed under compacted conditions, nor at the other field site (Figure 6C-D). Coarse rooting depth was also not correlated with total root length, total coarse root length or total fine root length under any of the field site with compaction treatment combinations (Figures 6E-F, S7). This could also be deduced from the general linear model (Table 3).

3.4. Root length density distributions show field-site dependent genotypic adjustments to compacted conditions

Genotype had an effect on coarse rooting depth on coarse-loam (ARBC) but on silt-loam (PSU) (Table 2). Coarse (Figure 7) and total (Figure S9) root length distributions over the soil profile at PSU (silt-loam) had smaller root length densities than on coarse-loam (ARBC). Distribution differences with depth between genotypes were less evident on silt-loam (PSU) (Figure 7), no significant statistical effect of genotype alone was noted on D_{75} or D_{75c} (Table 2), which could be attributed to larger standard errors at PSU (Figure 5D) and generally less roots found. The general linear model (Table 3) shows that genotype in combination with other factors did have a significant effect on $D75_c$.Different genotypes were found from each field site that showed similar coarse root length but contrasted in root depth or showed similar coarse root depth but contrasted in total root length. (Figure S10). For these measurements we observed genotypes with similar total coarse root length, but different rooting depths representing shallow and deeper root systems with similar root system sizes (IBM284 versus IBM323 for coarse loam (ARBC) and IBM051 and OhW122 for silt-loam (PSU)) (Figure 8A). A similar analysis was carried out based on total root length and depth (Figure S11, S12).

3.5. Relationship between root distribution variables and aboveground biomass

Compaction influenced aboveground biomass significantly (Figure 9, S2) but could also be related to other root measurements derived from coring. For both coarse-loam (ARBC) and silt-loam (PSU) soils, plant biomass was higher when coarse roots were able to grow deeper due to being less impeded (Figure 9). In coarse-loam (ARBC) however TRL_f also played a role, while in silt-loam (PSU) greater plant biomass was reached on non-compacted plots without TRL_f having a role.

4. Discussion

In this study, on two different soils with compacted and non-compacted plots, we found total root length reduction due to compaction f was field site dependent (Figures 2, Table 2). Coarse root proportions were influenced by genotype at both field sites (Figure 4, Table 2). Rooting depth of coarse and total roots were strongly correlated (Figure 5). Root length and rooting depth variables were not correlated when plants were grown on compacted plots (Figures 6, S5, S6, S7, S8). Our results support the hypothesis that the ability of roots to grow to depth through compacted soils is not dependent on the amount of roots formed by the root system. Furthermore, we propose that root length density distributions are either characterised by avoidance or by adaptive strategies for different genotypes when grown in compaction.

4.1. Root phenotypes show high levels of plasticity

4.1.1. Field site effects on root systems

Total root lengths (TRL), total fine root lengths (TRL_f) and total coarse root lengths (TRL_c) were greater on coarse-loam (ARBC) than on silt-loam (PSU) (Figures 2, S6). The significant reduction of the fine root length due to compaction on coarse-loam (ARBC) could influence the proportions of fine and coarse roots. Greater changes in coarse root proportions were observed at ARBC (coarse-loam) versus PSU (silt-loam) (Figure 3), which could potentially be driven by a disproportionally greater reduction of total fine root length versus that of total coarse root length (causing a shift towards greater proportion of coarse roots). Rooting depths D75 and $D75_c$ were different at the two field sites (Figures 5, S6, S7, Table 3). Differences between field sites for observations considering root length, root proportions and root depth could be related to differences in soil parameters. Maize seedlings had significantly longer seminal roots in a sandy loam versus a sandy clay loam (Panayiotopoulos *et al.*, 1994) while rooting depths of grapevines were deeper in coarse textured soils than fine textured soils (Nagarajah, 1987). Greater root length was possibly attained on coarse-loam (ARBC) because of the greater sand fraction in the soil versus silt-loam (PSU). Another possible explanation for the root length differences between coarse- and silt-loam could be a difference in root-soil contact between the field sites. On the non-compacted plots of PSU (silt-loam), smaller dry bulk densities could mean reduced levels of root-soil contact, which in turn reduces water and nutrient uptake (Veen *et al.*, 1992). The coarse-loam field site (ARBC) consisted of a more uniform, less structured soil, while the silt-loam field site (PSU) had more pronounced soil structure in terms of aggregation observed in the field. Roots can take advantage of cracks or bio-pores from earthworms or old root channels present to bypass compacted layers (Atwell, 1993; Hatano *et al.*, 1988; Stirzaker *et al.*, 1996). Cracks and pores will impose lower axial pressures on roots than bulk soil (Jin *et al.*, 2013). It is likely that the presence of such low-resistance channels in the soil structure at PSU (in silt-loam) could have permitted deeper rooting than at ARBC (coarse-loam).

4.1.2. Compaction influences root system distribution

Compaction influenced root growth at both field sites, but more significantly at ARBC (coarse-loam), where all rooting variables were significantly affected (Table 2). At both field sites the compaction treatment influenced the average total coarse root length across genotypes in different ways (Figure 3, Table 2). Total coarse root length decreased on coarse-loam (ARBC), which could be due to the effect compaction had on root system size in general. Total and fine root length were more significantly reduced than total coarse root length under compaction (Table 2, Figure 2). Reductions in root length in compacted soil has been reported for different species including maize (Grzesiak, 2009; Iijima and Kono, 1991). At PSU (silt-loam) total and fine root length were not significantly affected by compaction (Table 2) and total coarse root length increased (Figure 3, Table 2). Increased total coarse root length could potentially be caused by radial expansion as roots generally increase in diameter when experiencing mechanical impedance. Elongation is slowed compared to elongation rates at lower levels of mechanical impedance, which in turn decreases root length (Bengough *et al.*, 2006; Bengough and Mullins, 1991; Bengough and Young, 1993). While all root length measures decreased on coarse-loam (ARBC), these observations on root length were different on silt-loam (PSU) where fine and coarse roots were differentially affected by the compaction treatment. Coarser roots such as seminal or nodal root axes were more impeded than lateral roots possibly reflecting the fewer macropores present under compacted conditions. Such effects have been found in barley growing in glass ballotini of different sizes, with larger pores only restricting seminal growth and smaller pores restricting both laterals and seminal growth of barley (Goss, 1977). Laterals capable of growing in pores larger than their own diameters would encounter less impedance than those laterals forced to grow through bulk soil or smaller pores (Iijima and Kono, 1991).

Under compaction both rooting depth (D_{75}) and rooting depth of coarse roots (D_{75c}) decreased at both field sites (Tables 1, 2; Figures 5, 6, S6, S7). Reduction of $D75_c$ due to compaction could be linked to reduced aboveground biomass (Figure 9). Shallower rooting depths probably reflect slower root elongation rates, so it will take longer for a root to reach deep soil strata. Smaller differences in rooting depth of compacted and non-compacted plots at PSU (silt-loam) (Figure 5) could be due to the smaller differences in penetration resistance with increasing depth versus ARBC (coarse-loam) (Figure 1). Roots at the PSU field site (silt-loam) would initially experience greater levels of mechanical impedance, but once they pass this zone should be able to elongate more normally. The reduction in rooting depth under compaction is in agreement with observations with wheat (Barraclough and Weir, 1988; Chen *et al.*, 2014). Compaction altered root distribution, generally shifting root distribution to shallower strata (Figures 4, 5, 7, S9). Multiple studies have described similar redistributions of roots under impeded field conditions for various crops (Barraclough and Weir, 1988; Brereton *et al.*, 1986; Chen and Weil, 2011; Chen *et al.*, 2014). For maize specifically, roots of 2-3 week old plants were confined to surface layers under compaction (Veen and Boone, 1990). A similar observation was made during a 4 week growing period for maize grown in root boxes (Iijima *et al.*, 1991) and in the field up to tasselling (Laboski *et al.*, 1998). Soil compaction reduces soil porosity, hydraulic conductivity and air permeability and this increases the risk of hypoxia (Laboski *et al.*, 1998; Kuncuro *et al.,* 2014; Xiong *et al*., 2020). In this set-up of compacting entire field sites, the stress caused by impedance and hypoxia cannot be mutually excluded. Therefore hypoxia might also have played a role in reducing growth of the root system. A recent studies by Xiong *et al.* (2020) illustrates that higher moisture contents negatively influenced root growth in compacted soils.

4.1.3. Compaction influenced genotypes differently

Under compaction at both field sites most genotypes had a greater proportion of coarse roots (Figure 3) and genotypes differed in this response (Table 3). An increased proportion of coarse roots could either be attributed to (1) the reduction of the fine root proportion, (2) the increase in diameter of roots grown under impeded conditions due to thickening or (3) a combination of the two. On coarse-loam (ARBC), total fine root length was significantly reduced (Table 3, Figure 2), which in turn would influence root proportions. However, as there was no such reduction of total fine root length on silt-loam (PSU), root thickening, which is the increase in radial diameter of the roots, is probably the main cause of a shift in root proportions on silt-loam.

Genotypic differences were found for total and coarse rooting depth variables (Figures 5, 7, 8, S6, S7, Tables 2, 3). No relationship between root length variables existed (with the exception of negative relationship between total root length and total rooting depth under non-compacted conditions at ARBC (coarse-loam)). Deeper rooting was not associated with root system size. Root phenes that have been found to contribute to overcoming impedance include anatomical traits such as reduced cell file number and increased levels of aerenchyma (Lynch and Wojciechowski, 2015; Vanhees *et al.*, 2020) and is thought to be linked to reduction of metabolic costs, reduced energy and/or facilitating O_2 diffusion (Hanbury and Atwell, 2005; Lynch, 2015; Colombi *et al.*,2019). It has also been suggested that anatomical traits such as smaller outer cortical region cells will stabilize a root during the penetration of a harder soil layers (Chimungu *et al.* 2015). Other phenes are sharper root tip shape, the presence of root hairs, the production of mucilage, root cap sloughing and steeper growth angles (Bengough *et al.*, 2011; Colombi *et al.*, 2017b; Haling *et al.*, 2013; Iijima *et al.*, 2000, 2004; Jin *et al.*, 2013, Lynch and Wojciechowski, 2015). How all these phenes can synergistically work together is worthy of further investigation. Other studies have shown that plasticity could play a role, for instance for rice it was observed that upon rewetting after drought differences in nodal root elongation through a hardpan were present between genotypes (Suralta *et al.,* 2018).

4.2. The relationship between root length and root depth varies among genotypes

Root length and rooting depth are not related under impeded conditions (Figures 6, S6, S7, S8). Coarse rooting depth, representing nodal roots, responded differently to compaction among genotypes. Genotypes such as IBM059 or IBM323 at ARBC (coarse-loam) or IBM086 at PSU (silt-loam) grew deeper under non-compacted conditions but reduced their coarse root length under compaction (Figure 5A-B). IBM178 grew intermediately deep in coarse-loam (at ARBC), and deep in silt-loam (at PSU) but did not reduce its coarse rooting depth to the same extent as the aforementioned genotypes (Figure 5A-B). This suggests that coarse roots of some genotypes were obstructed by the compaction treatment while coarse roots of other genotypes were capable of growing through.

We found genotypes with similar root system size that reached different rooting depths (Figure 8A, S12) as well as genotypes with similar deeper coarse rooting depth but with different total coarse root length (Figure 8B, S12). Coarse rooting depths can thus be reached in different ways as the root system with smaller values for coarse root length densities were able to grow as deep as the root systems that have greater coarse root length density at depth. Shoots can be sustained by different root system sizes and rooting depths as long as water and nutrients are available. Therefore rooting depth under compaction is not simply related to the amount of roots formed. In the following section we discuss how each type of root distribution with depth could sustain plant growth.

4.2.1. Root systems with equal coarse root length reach different depths

Coarse roots of some genotypes were obstructed, while others managed to grow through impeded soil domains and reached deeper strata (Figure 8A). If nodal roots are sufficiently impeded, these and any laterals roots emerging from them will automatically be located within the upper soil strata. However laterals may grow downwards from a shallow starting point when they experience less impedance than nodal roots by, for instance, making use of smaller pores (Goss, 1977). Increased lateral branching has been observed in the non-impeded parts of the soil (Montagu *et al.*, 2001) and will enable a plant to extract water and nutrients when root length is maintained and sufficient soil resources are available in the unimpeded soil (Barraclough and Weir, 1988). Compensatory root growth introduces more roots in the less impeded domains, often in the upper soil strata (Barraclough and Weir, 1988; Materechera *et al.*, 1993; Nosalewicz and Lipiec, 2014; Pfeifer *et al.*, 2014a). A similar redistribution can be seen in the compacted plots (Figures 7, S9). Compensatory mechanisms may influence nutrient and water acquisition.

Rooting depth has been linked to water acquisition, especially under drought conditions where deeper rooting increases yield (Gao and Lynch, 2016; Hund *et al.*, 2009; Lynch, 2013, 2018; Chimungu *et al.*, 2014a, 2014b; Lynch *et al.* 2014; Zhan *et al.*, 2015). Our study did not employ a water deficit, but it has been shown that compaction can make water deficit stress more severe (Grzesiak *et al.*, 2014): even without the presence of water deficit stress, increased water uptake from the topsoil can be present on compacted soils. This in turn will increase the penetration resistance within the topsoil and further limit access to the deeper soil layers (Colombi *et al.*, 2018). A root system that is limited to shallow soil strata will thus be more at risk for water deficit both in terms of reduced access, as well as increased water depletion in its local soil environment. It is likely that in the coarse soil (ARBC) compacted treatment the air-filled porosity was often <10% in the surface 20cm, but generally >10% in the other treatments. The root system might therefore have been influenced by poor oxygen availability (hypoxia) however this can be seen as common feature of compacted soils in the field where porosity is reduced. Clear shifts in root distribution occurred in our field sites, we observed changes in root proportion, changes in rooting depth, and changes in root distribution (Figures 3, 5, 7, S9). How these shifts influence resource acquisition under impeded field conditions merits further investigation.

Stresses such as waterlogging have been found to have a more severe impact in impeded soils (Grzesiak *et al.*, 2014). Environmental effects such as temperature fluctuations or soil drying by direct evaporation pose additional threats to more shallow root systems (Lynch, 2018). Overall compensatory root growth can be seen as a stress avoidance strategy as plants come less into direct contact with the impeded soil regions and grow where impedance is lower. This can be considered as an indirect adaptation or response to the impeding conditions. As soils are a typically heterogeneous, roots could take advantage of cracks or pores present to bypass compacted layers (Hatano *et al.*, 1988; Atwell, 1993; Stirzaker *et al.*, 1996) which would be another avoidance strategy. Rasse and Smucker (1998) showed that maize can make use of root-induced macropores from a previous alfalfa crop. Preferential growth towards artificial pores has been observed in compacted soils (Stirzaker *et al.*, 1996; Pfeifer *et al*., 2014b; Colombi *et al.*, 2017a; Atkinson *et al*., 2020) but whether this is due to oxytropism or locally reduced penetration rates is still under debate, although promising attempts have been made to model the process (Landl *et al*., 2017). Roots adapted to impedance are characterised by traits that help them overcome impedance, enabling them to grow better in harder soils. Those genotypes capable of rooting deeper and of overcoming impedance stress are at less at risk of nutrient deficiencies, of lack of access to water and of other environmental stresses.

4.2.2. Equal depths can be reached by root systems of different sizes

We observed genotypes that contrasted in root system size (measured as total coarse root length) were able to reach similar depths on compacted plots (Figure 8B, S12). Greater amounts of coarse roots (measured as greater TRL_c) would be found when a maize plant forms more root axes per nodal position, additionally greater amounts of coarse roots may also be caused by root thickening. We found that no rooting depth measure correlated with TRL_c (Figures 6E-F, S6A-D, S7). The ability of a root system to grow deeper in compacted soils is therefore not dependent on the amount of roots formed as both large and parsimonious root systems can reach similar depths on compacted plots at both field sites. This is in contrast with observations on wheat, where penetrability of a harder wax layer was related to amount of root axis formed (Whalley *et al.*, 2013), or that denser root systems of lupin are deeper rooting (Chen et al., 2014). On the other hand, comparisons between species show that species with a larger number of roots in the top layers of a layered medium did not automatically have greater penetration rates through the compacted layer (Materechera *et al.*, 1993). A field study with two rice varieties showed that varieties with a greater root density were able to root deeper under control conditions, but under greater penetrometer resistances became more strongly affected than others with lower rooting densities (Cairns *et al.*, 2004).

The formation of more roots can have benefits such as increased foraging for water and nutrients or reduced risk of root loss due to pests and diseases (Lynch, 2003, 2018, 2019). Increased root formation can however come at a substantial costs (York *et al.*, 2013, Lynch, 2003). Greater elongation rate, greater root diameter, increased branching or greater formation of axial roots increase the metabolic cost of the root system (York *et al.*, 2013, Lynch, 2018). Second, the formation of too many roots will introduce competition for internal and external resources (Lynch, 2018). Excessive root formation not only induces intraplant competition for resources, it also increases root maintenance and formation costs. Other traits, such as increased aerenchyma formation, large cortical cell size, reduced cortical cell file number or reduced crown root number bring costs down (Lynch, 2003, 2018) which would enable these plants to allocate resources elsewhere. For instance, it has been shown that maize with fewer crown roots are able to allocate roots deeper (Saengwilai *et al.,* 2014; Gao and Lynch, 2016, Lynch 2018). Recent experiments by Guo and York (2019) showed excising nodal roots stimulated greater shoot biomass and root length at depth under low N conditions as biomass was reallocated to lateral and early nodal roots. Under impeded conditions, metabolic cost reduction might be significant. A recent study by Colombi (2019) found energy costs were linked to cortical cell diameters, with greater cell diameters reducing the metabolic cost under impeded conditions. As both large as well as parsimonious root systems were able to reach similar coarse root depth (Figures 8B, S12B) we suggest that parsimonious phenotypes could potentially allocate more resources to shoot growth. This effect could be apparent under high input systems, where improved conversion of soil resources to yield would be greater for parsiminous phenotypes (Lynch and Brown, 2012; Lynch, 2018).

Conclusions

Rooting depth and root length were not correlated under impeded conditions. Different coarse rooting depths were reached by genotypes characterised by similar root system sizes. We suggest genotypes better adapted to impedance (and therefore rooting deeper) are less at risk of additional stresses such as nutrient deficiency, soil drying, lack of access to water and other environmental conditions. We hypothesise that excessive root formation will introduce greater competition for internal and external resources, furthermore larger root systems have greater metabolic costs associated with them. We also suggest that parsimonious phenotypes will be able to steer resource allocation to shoot growth and improved yields. We found that the amount of roots formed by the root system does not determine the ability of those roots to grow deeper under impeded conditions.

Acknowledgements

This research was supported by the Howard G. Buffett Foundation, the University of Nottingham and the James Hutton Institute. The James Hutton Institute receives funding from the Rural & Environment Science & Analytical Services Division of the Scottish Government. We thank Hannah Schneider, Stephanie Klein, Chris Strock, Kimo Jin and other members of the PSU roots lab for their help and support during the execution of these field trials.

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Artwork

Figures

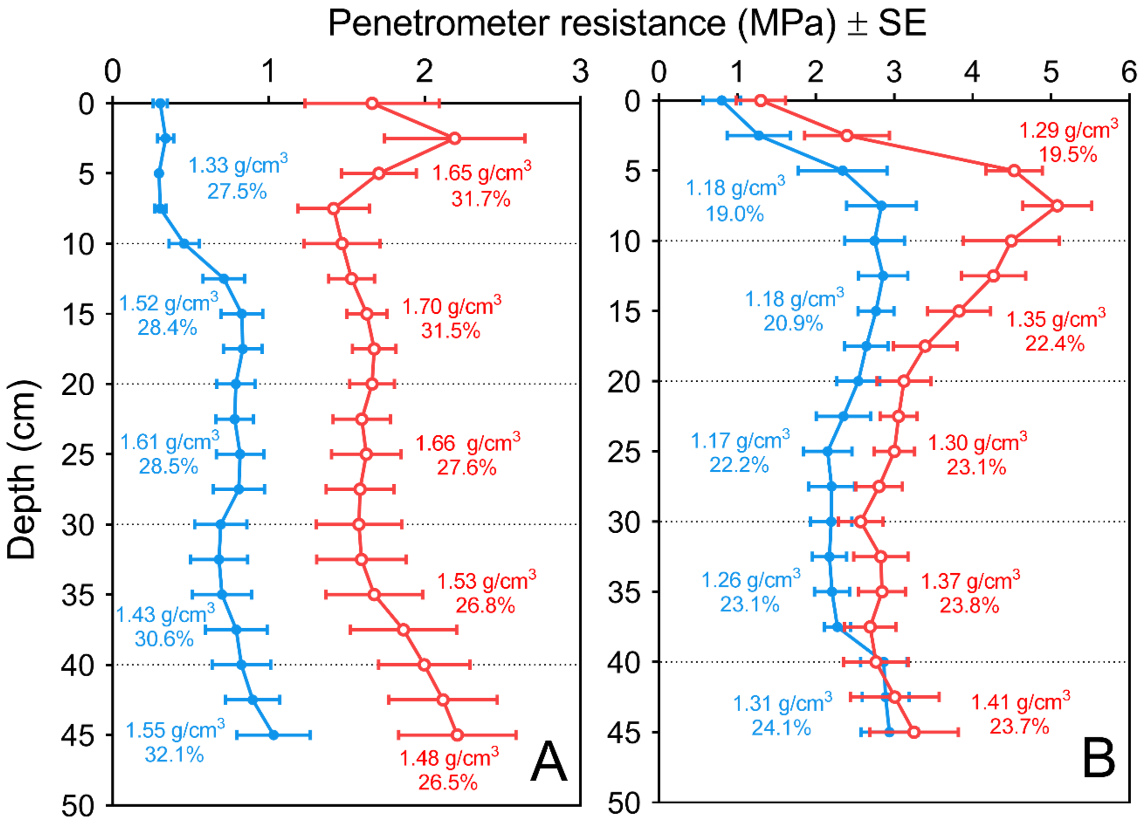

Figure 1 – Average penetrometer resistances ± SE for compacted (red) and non-compacted (blue) treatments at (A) the ARBC field site (coarse-loam) and (B) the PSU field site (silt-loam) before planting. Mean soil moisture content (v/v) was measured for each 10 cm increment, together with soil dry bulk density, both written on graph within relevant depth ranges. Figure adopted from Vanhees *et al.* (2020).

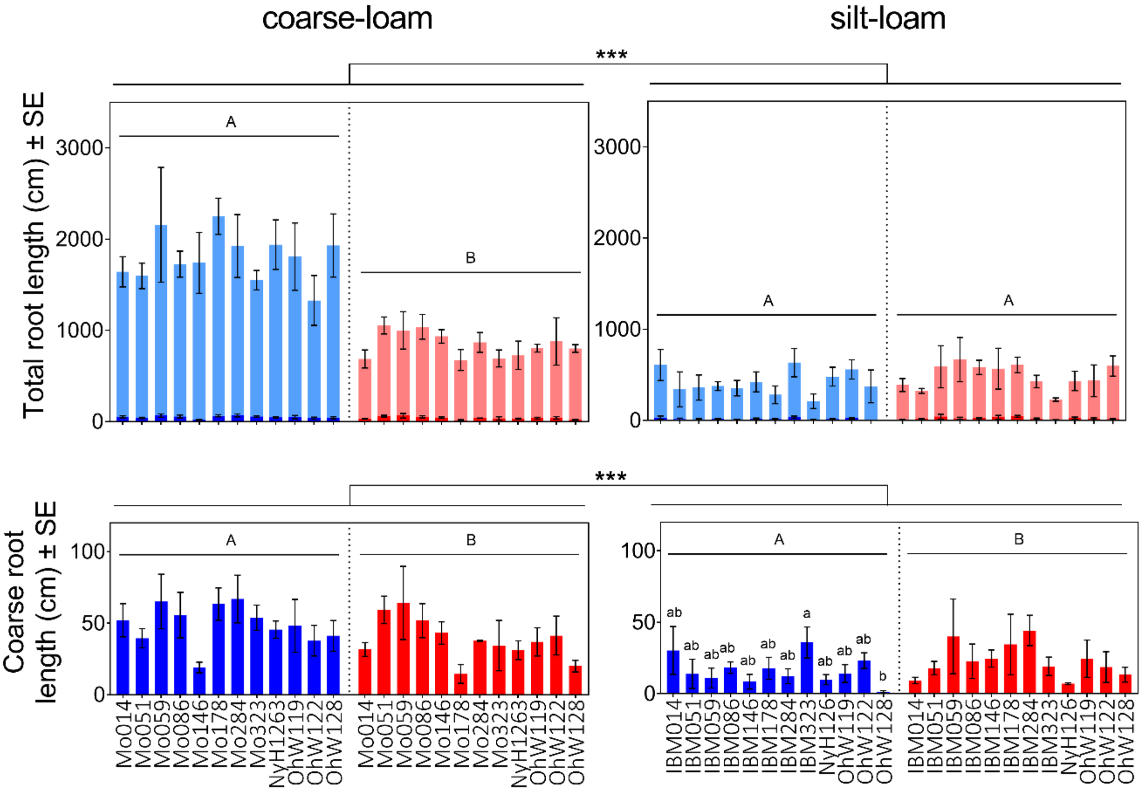

Figure 2 - Average total root length (cm) ± SE split into coarse (dark blue, dark red) and fine (light blue and light red) root length (cm) for maize genotypes tested at acoarse-loam (ARBC) versus silt-loam (PSU) field site. Coarse roots are defined as having diameters larger than 1 mm, while fine roots are those with diameter smaller than 1 mm. Compacted measurements in red, non-compacted measurement in blue. Error bars represent standard deviations. If differences between the field sites (***, $p \leq 0.001$), treatments (A/B, $p \leq 0.05$) and genotypes (a/ab/b, $p \leq 0.05$) were present.

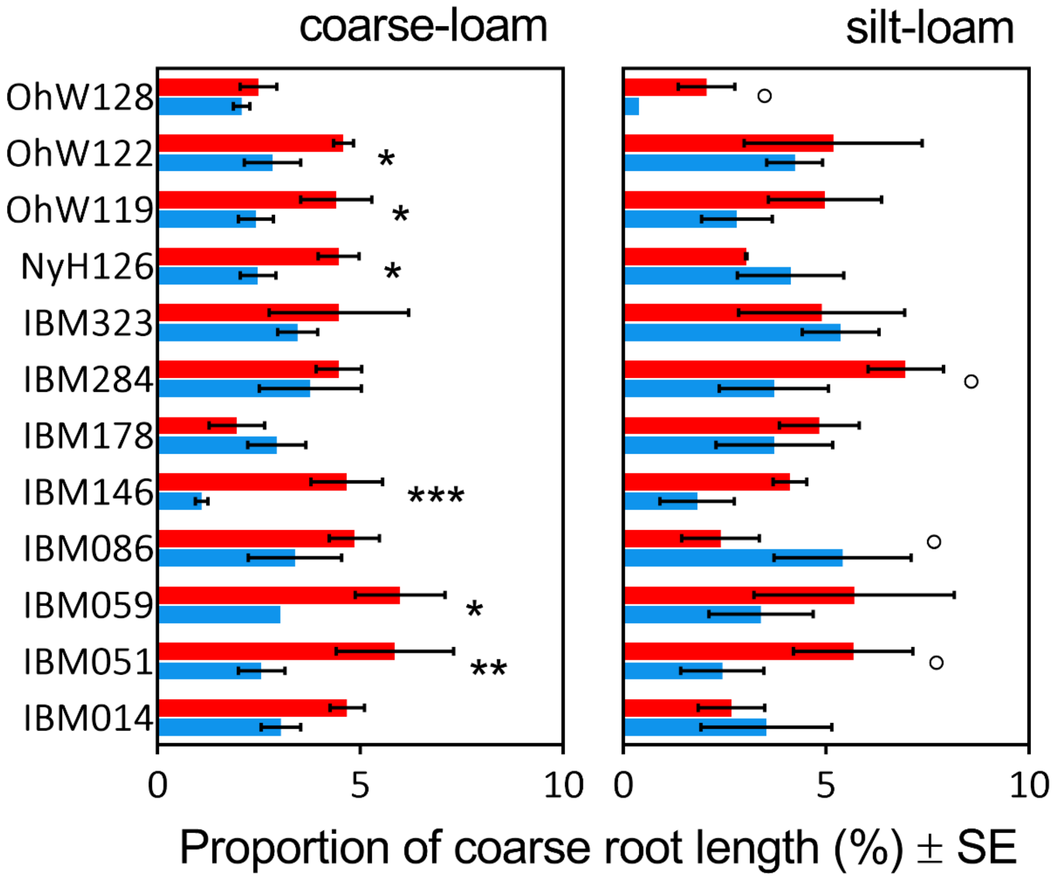

Figure 3 – Proportions of coarse (>1.0 mm diameter) root length (%) ± SE found in cores of different genotypes in two field sites. Non-compacted data in blue, compacted data in red. IBM059 (coarse-loam at ARBC) and OHW128 (silt loam at PSU) have such small standard errors they could not be visualised. Post-hoc Tukey comparisons within field site indicate when treatment effect was significant for each genotype at significance level ° P ≤ 0.10, * P ≤ 0.05-0.01, ** P ≤ 0.01-0.001, *** P ≤ 0.001.

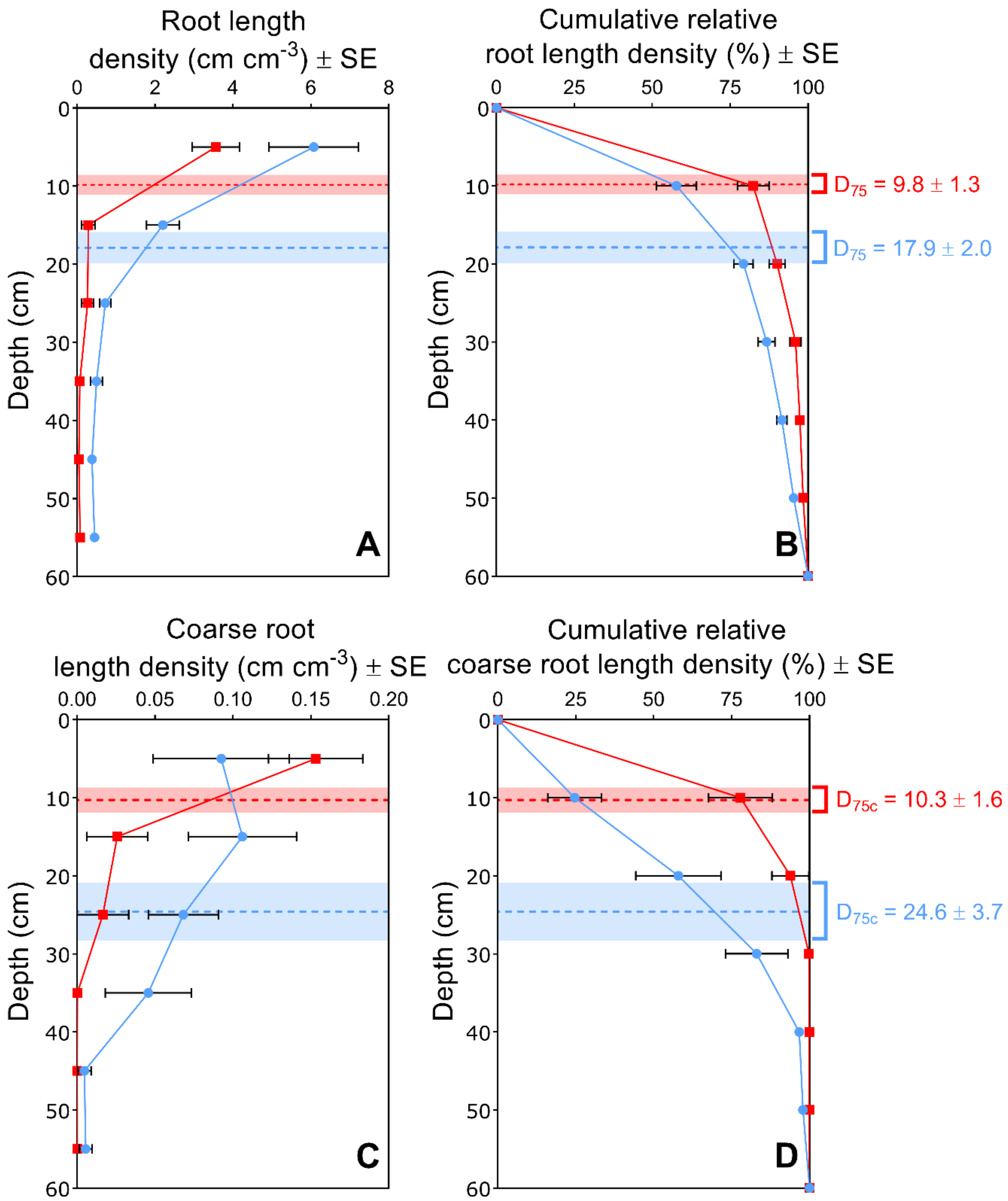

Figure 4 – Illustration of difference between absolute versus relative root length density distributions for genotype IBM014 considering total root length and coarse root length at the coarse-loam field site (ARBC). (A) + (C) Absolute distributions of root length densities, (B) + (D) Relative distributions of root length densities. Compacted data in red and non-compacted data in blue. Error bars represent standard errors. The rooting depth (cm) ± SE where 75% of the total root length (D_{75}) or coarse root length (D_{75c}) was visualised by the striped line, coloured region represents SE for the depth measurements. No error bars shown when standard error was too small to visualise.

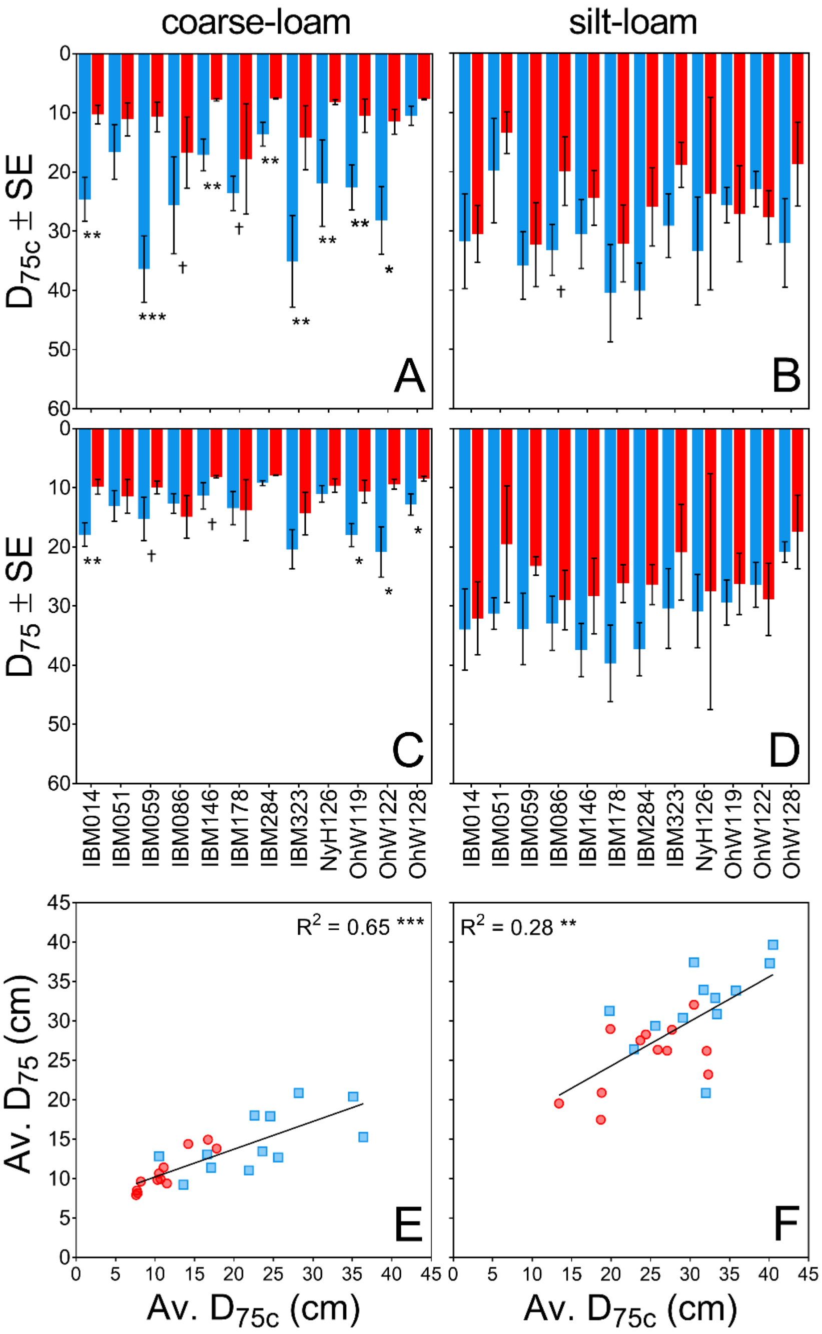

Figure 5 – Coarse and total rooting depth and their correlation for both field sites under compaction (red) and non-compacted (blue) conditions.. (A) + (B) Average coarse rooting depth (D_{75c}), (C) + (D) Average total rooting depth, (E) + (F) Correlation between D_{75} and D_{75c}. Error bars represent standard errors. (A) + (C) + (E): ARBC field site (coarse-loam) and (B) + (D) + (F): PSU field site (silt-loam). Post hoc Tukey comparisons between compaction and noncompaction within each field site for each genotype were carried out on rooting depth data (panels A-D). Coarse and total rooting depth are correlated (E-F) Levels of significance † $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

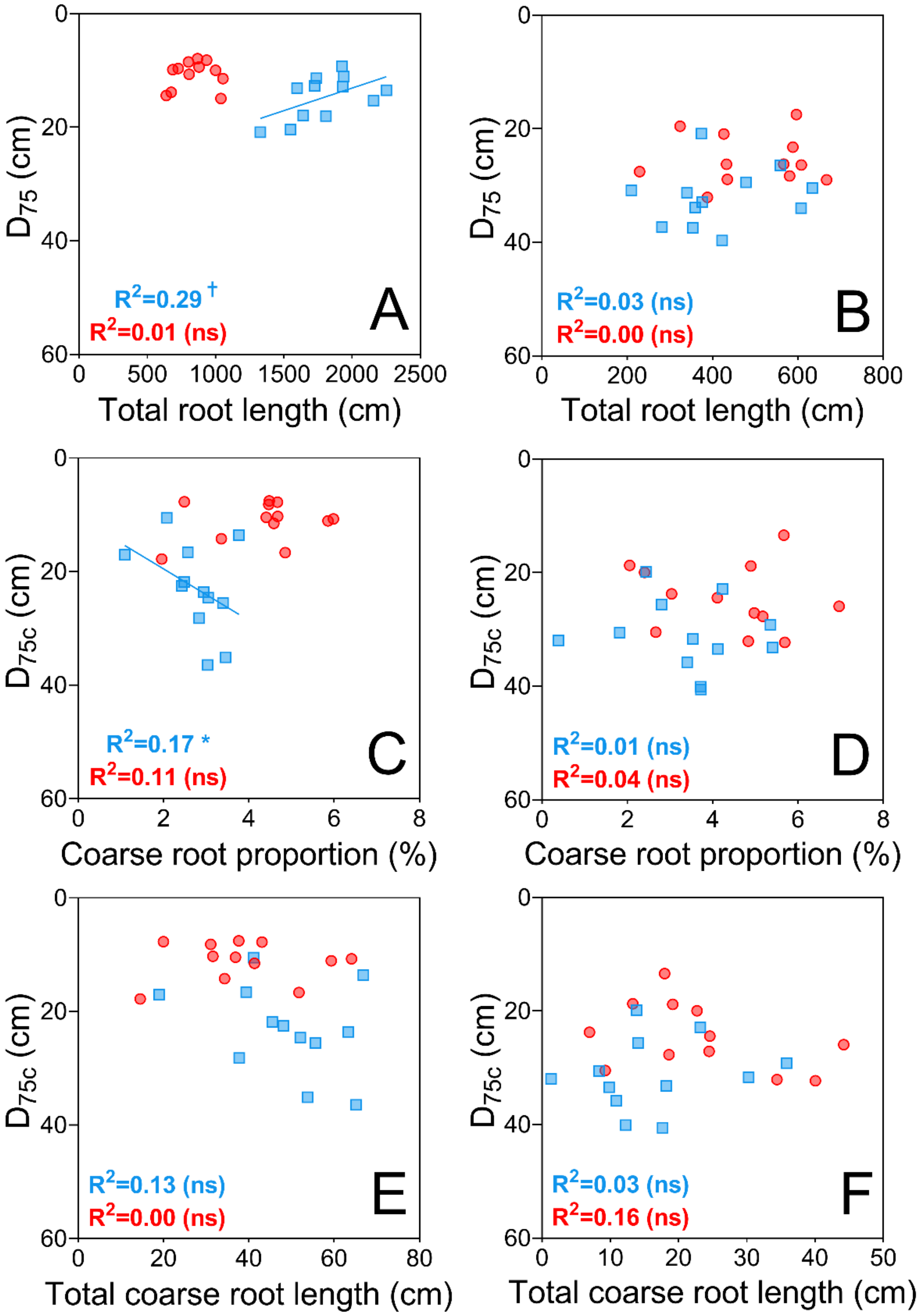

Figure 6 – Linear regressions between root distribution variables at the two different field sites. Field site ARBC (coarse-loam) visualised in A, C, E and field site PSU (silt-loam) visualised in B, D, F. Compacted data (red) and non-compacted data (blue). Each datapoint represents the averaged value across the replicates for each genotype tested. Normal linear regression was used for A–B and E-F, and a betaregression was used for C-D as data was proportional. When a significant relationship was found this was at a level of significance of † P ≤ 0.10 or * P ≤ 0.05.

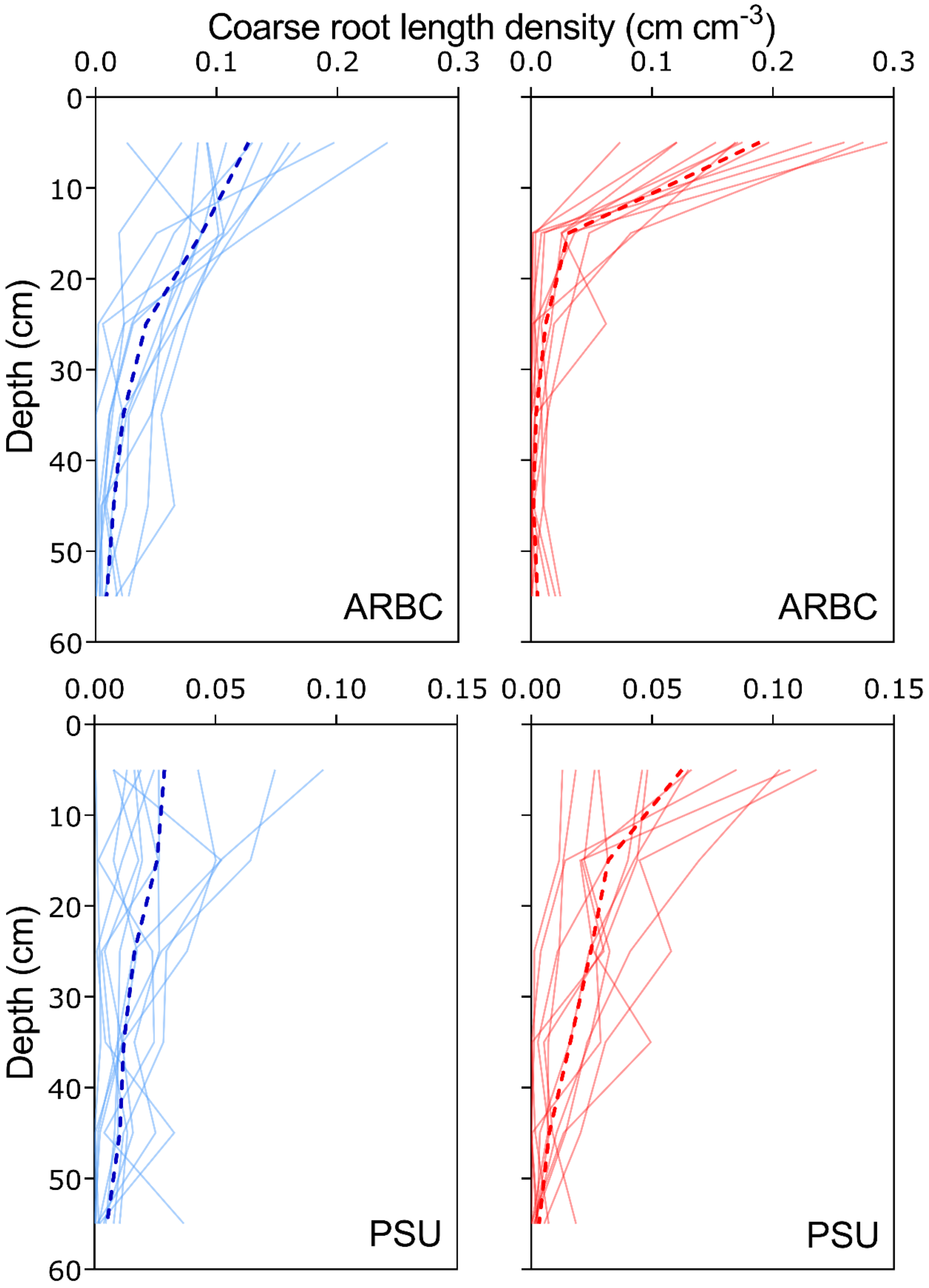

Figure 7 - Genotypic variation in the coarse root length density (cm cm^{-3}) per depth increment across two field sites and two compaction treatments. Non-compacted data in blue and compacted data in red. The ARBC field site (coarse-loam) and PSU field site (silt-loam) had different soil textures. The striped lines are the averages across all genotypes, lighter coloured lines are the average for individual genotypes tested. Similar plots for total root length density distributions can be found in Figure S9.

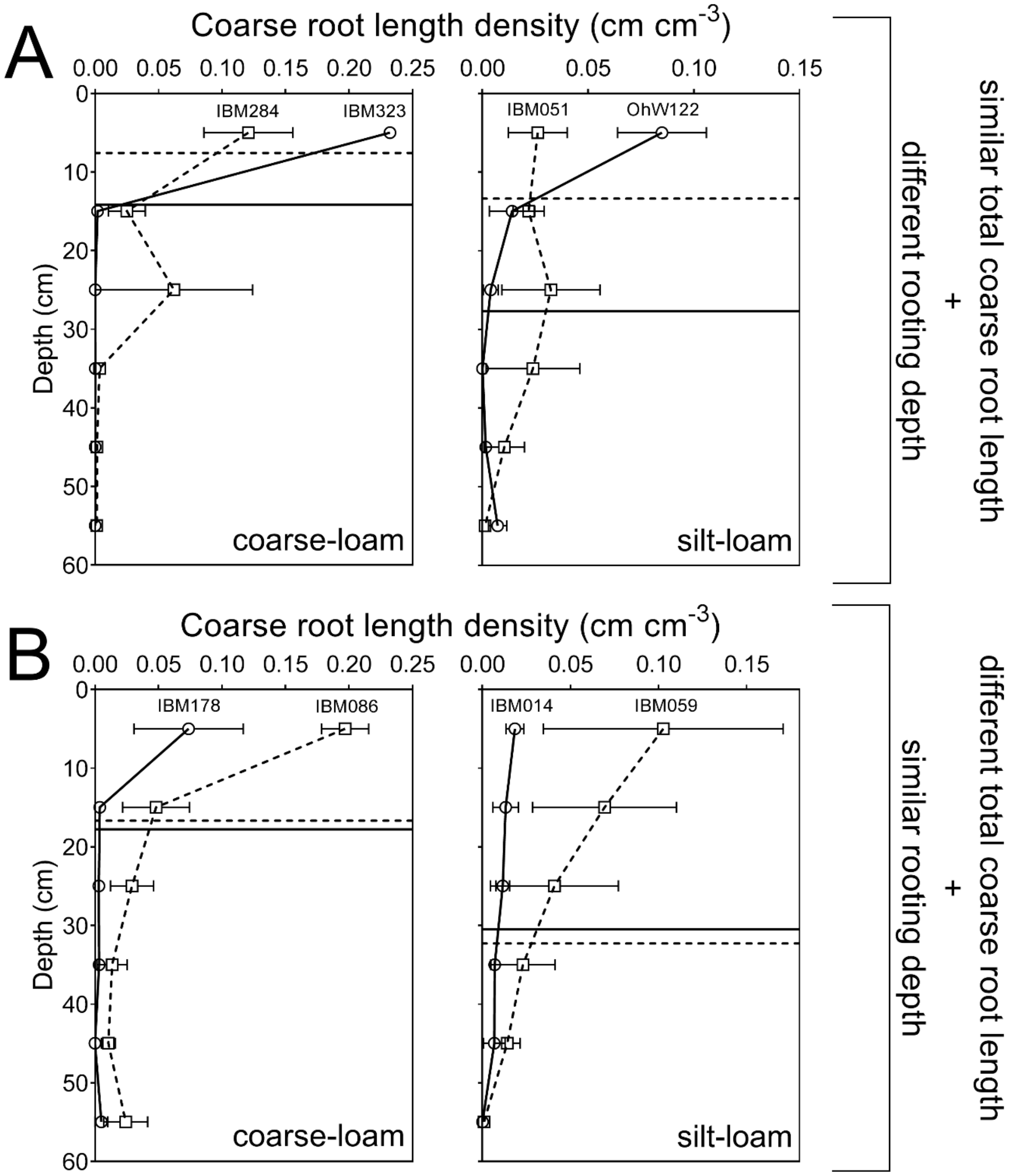

Figure 8 – Coarse root length densities (cm cm^{-3}) ± SE distributions with soil depth on compacted plots comparing (A) two genotypes per field site (coarse loam at ARBC and silt-loam at PSU) with similar total coarse root length but with different associated rooting depths and (B) two genotypes with similar rooting depths but with different total coarse root lengths. For (A) striped lines stands for the deeper rooting genotype and associated D_{75c}, while the solid line stands for the shallower rooting genotypes and associated D_{75c}. For (B), the solid line is used for the genotype that produces less roots but reaches equally deep as the genotype that produces more roots (striped lines). No error bars shown when standard error was too small to visualise. Selection comparison can be found in Figure S10. Similar plots for total root length density distributions can be found in Figure S12.

ARBC: Biomass ~ Genotype + $D75_c$ + D75 + TRL_f + TRL_c + Compaction		
	F-value	p-value
Genotype	1.144	ns
$D75_c$	140.93	***
D75	0.231	ns
TRL_f	37.629	***
TRL_c	2.287	ns
Compaction	5.83	*
PSU: Biomass ~ Genotype + $D75_c$ + D75 + TRL_f + TRL_c + Compaction		
	F-value	p-value
Genotype	0.649	ns
$D75_c$	14.491	**
D75	5.244	ns
TRL_f	0.083	ns
TRL_c	3.75	ns
Compaction	9.713	*

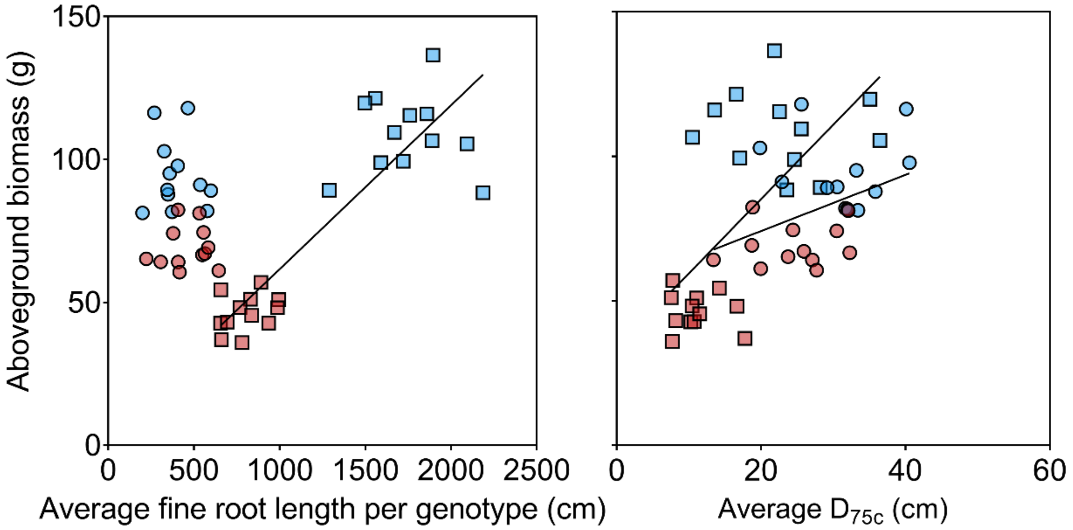

Figure 9 – Relationships between average biomass and root distribution values and are visualised in the accompanied plots where squares are data from the ARBC (coarse-loam) field site while circles are data from the PSU (silt-loam) field site. Compacted data in red, non-compacted data in blue. Level of significance in the ANOVA analysis are *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$.

Tables

Table 1 – Definitions of the different measurements obtained after WinRHIZO analysis of the soil cores.

Measurement	Abbreviation	Definition	Unit
Total root length	TRL	The summation of all individual root sections per 10 cm increment of the entire soil core	cm
Total coarse root length	TRL_c	The summation of all individual root sections per 10 cm increment with a diameter greater than 1 mm over the entire soil core	cm
Total fine root length	TRL_f	The summation of all individual root sections per 10 cm increment with a diameter smaller than 1 mm over the entire soil core	cm
Coarse root proportion	P_c	The ratio of total coarse root length versus total root length	%
Fine root proportion	P_f	The ratio of total fine root length versus total root length	%
Root length density	-	The root length found in the soil volume of a 10 cm increment of the soil core and this including all root diameter classes	cm cm^{-3}
Coarse root length density	-	The total coarse root length found in the soil volume of a 10 cm increment of the soil core	cm cm^{-3}
Relative root length density	-	The ratio of total root length density of a single 10 cm increment versus the sum of the total root length density found over the entire core	%
Relative coarse root length density	-	The ratio of coarse root length density of a single 10 cm increment versus the sum of the coarse root length density found over the entire core	%
Rooting depth	D_{95}	The rooting depth above which 95% of the total root length is located	cm
	D_{75}	The rooting depth above which 75% of the total root length is located	cm
Coarse rooting depth	D_{95c}	The rooting depth above which 95% of the total coarse root length is located	cm
	D_{75c}	The rooting depth above which 75% of the total coarse root length is located	cm

Table 2 F-values for split plot analysis results of the different root distribution variables at the two field sites. P-values tested at the following levels of significance: † $p \leq 0.10$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Subscript c stands for coarse and f stands for fine when measurements are made on a separate root class. TRL stands for total root length, P stands for proportion of coarse or fine roots. D75 and D95 stand for rooting depth at which 75 and 95 percent of the total root length can be found.

		ARBC (coarse-loam)		PSU (silt-loam)	
TRL	Compaction	77.12	***	1.37	
	Genotype	0.67		0.54	
	Compaction x Genotype	0.87		0.85	
TRL_c	Compaction	4.59	†	3.61	†
	Genotype	1.58		1.12	
	Compaction x Genotype	2.11	*	1.35	
TRL_f	Compaction	78.81	***	1.25	
	Genotype	0.67		0.56	
	Compaction x Genotype	0.81		0.83	
P_c	Compaction	18.29	**	3.43	
	Genotype	2.60	**	1.97	*
	Compaction x Genotype	2.12	*	1.34	
P_f	Compaction	18.62	**	2.63	
	Genotype	2.46	*	1.77	†
	Compaction x Genotype	1.93	†	1.30	
D_{75c}	Compaction	76.53	***	4.65	†
	Genotype	3.15	**	1.67	
	Compaction x Genotype	0.71		0.55	
D_{95c}	Compaction	42.29	***	0.78	
	Genotype	3.86	***	0.65	
	Compaction x Genotype	1.33		0.60	
D_{75}	Compaction	17.31	**	6.78	*
	Genotype	2.74	**	1.08	
	Compaction x Genotype	0.87		0.36	
D_{95}	Compaction	25.02	***	1.56	
	Genotype	2.70	**	1.11	
	Compaction x Genotype	1.33		0.33	

Table 3 – Summary of general linear model results for the linear regression of total or coarse rooting depth (D_{75} or D_{75c}) with total root length (TRL) or total coarse root length (TRL_c). P-values tested at the following levels of significance: † $p \leq 0.10$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

D_{75} ~ TRL + Field site + Compaction + Genotype

	F-value	p-value
Field site	57.36	***
Compaction	12.21	*
Genotype	1.22	
Total root length	3.09	†

D_{75} ~ TRL_c + Field site + Compaction + Genotype

	F-value	p-value
Field site	106.37	***
Compaction	10.2	*
Genotype	1.17	
Total root length	0.34	

D_{75c} ~ TRL + Field site + Compaction + Genotype

	F-value	p-value
Field site	35.83	***
Compaction	25.51	***
Genotype	2.12	*
Total root length	2.73	

D_{75c} ~ TRL_c + Field site + Compaction + Genotype

	F-value	p-value
Field site	41.39	***
Compaction	34.77	***
Genotype	1.99	*
Total root length	1.39	

Appendices

Supplementary data

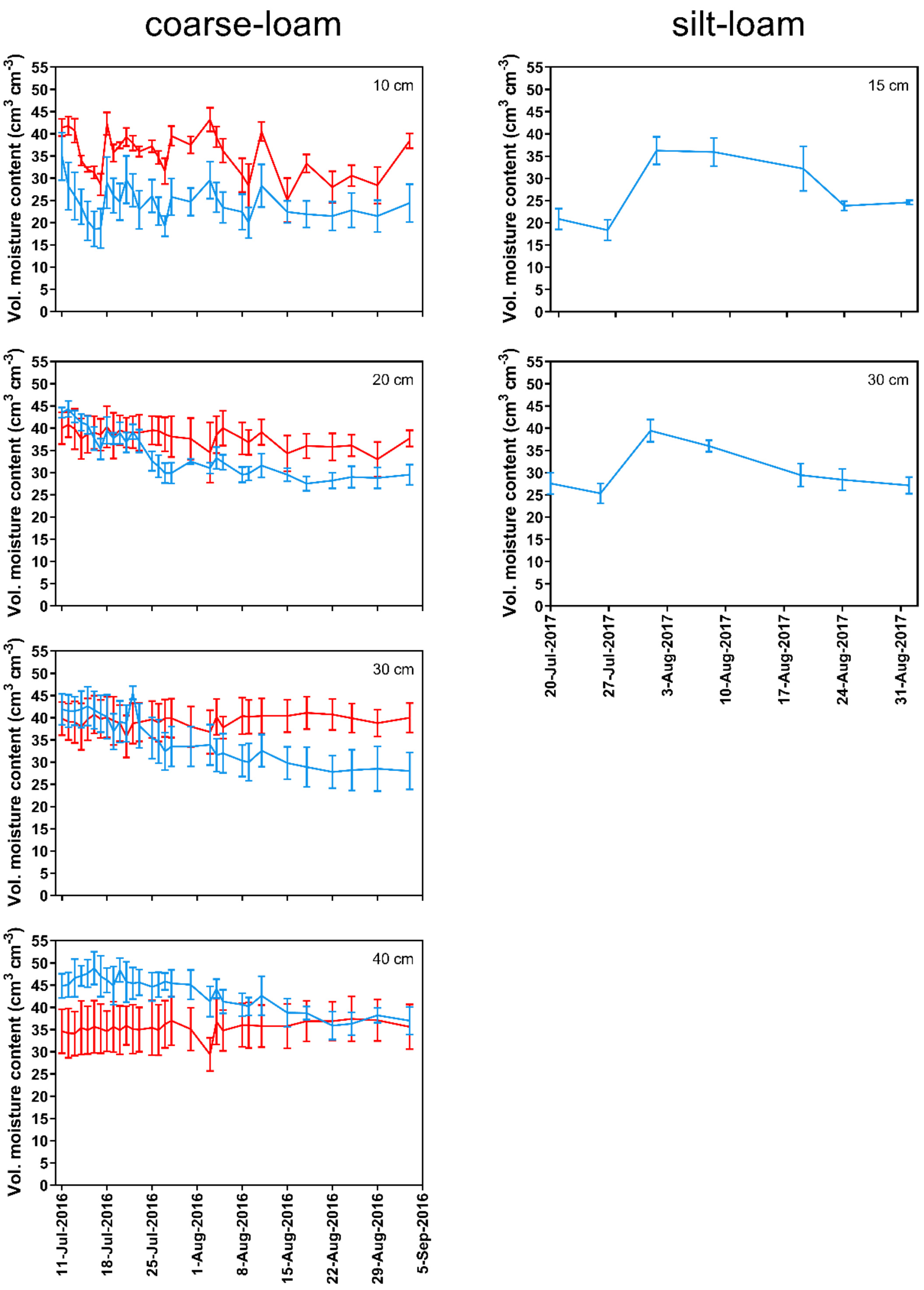

Figure S1 – Volumetric water content was used to steer irrigation (ARBC, coarse-loam field site) or to decide if irrigation was needed (PSU, silt-loam field site). On coarse-loam PR2-tubes were installed while on silt-loam TDR-probes were used to measure volumetric water content; gravimetric water content was calculated from this and the dry bulk density. Compacted site (red), non-compacted (blue).

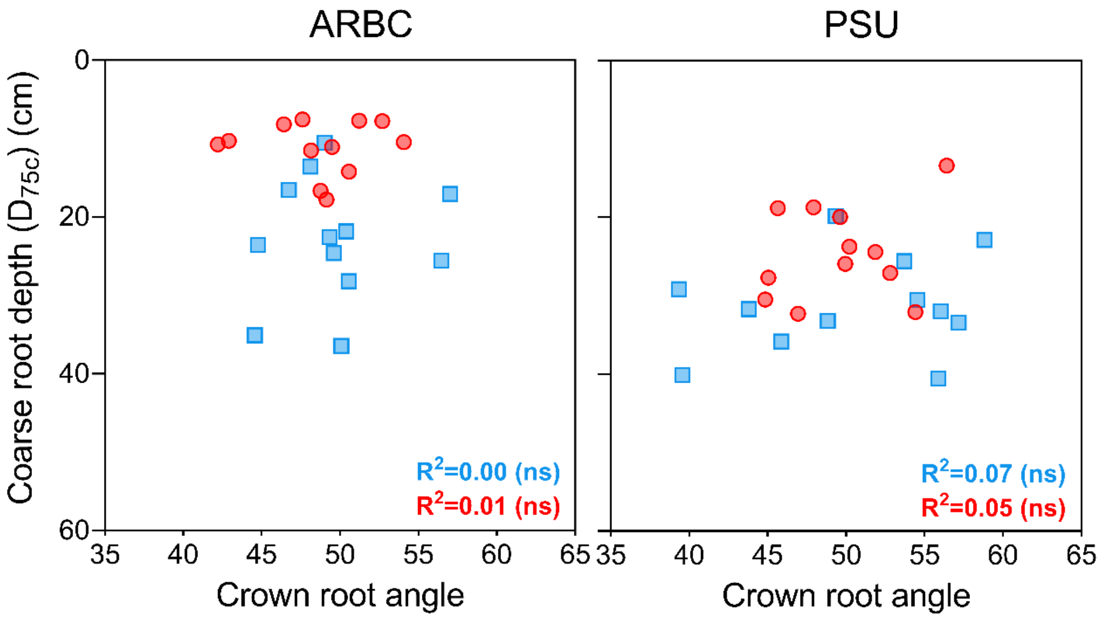

Figure S2 – Relationship between crown root angle and coarse rooting depth for ARBC (coarse-loam) and PSU (silt-loam) field sites. Figures adjusted from supplementary Figure 1 from Vanhees *et al.* (2020). Compacted site (red), non-compacted (blue).

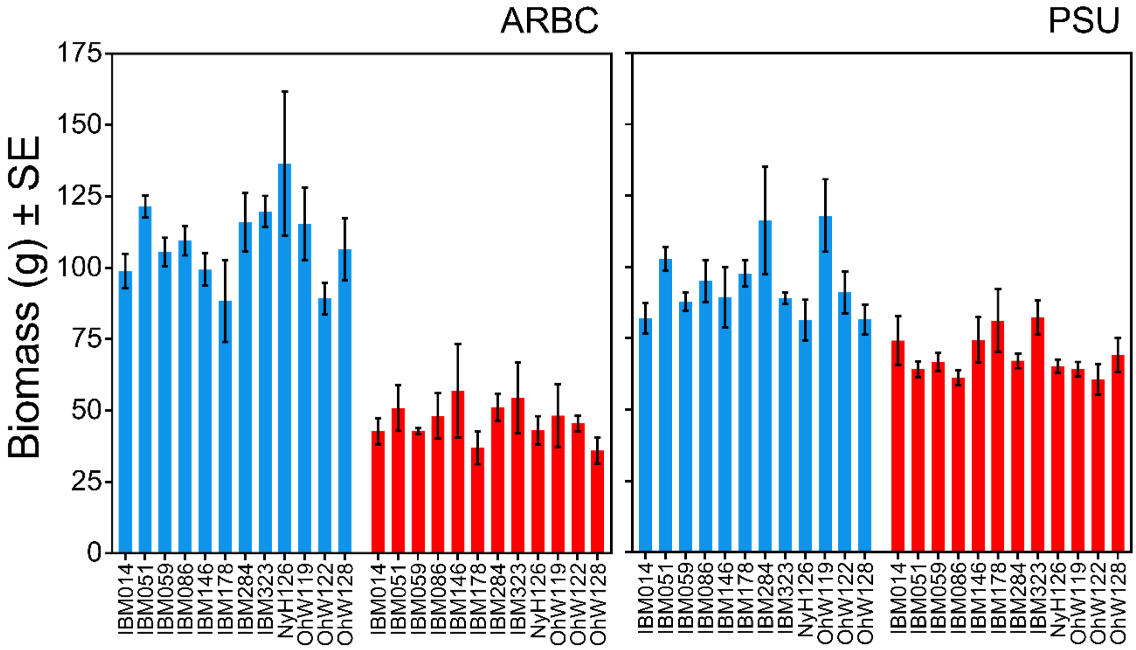

Figure S3 – Biomass ± SE at both field sites under compacted (red) and non-compacted (blue) conditions for each genotype. The ARBC field site has a coarse-loam soil while the PSU field site has a silt loam soil texture. Figure adjusted from supplementary Figure 3 from Vanhees *et al.* (2020).

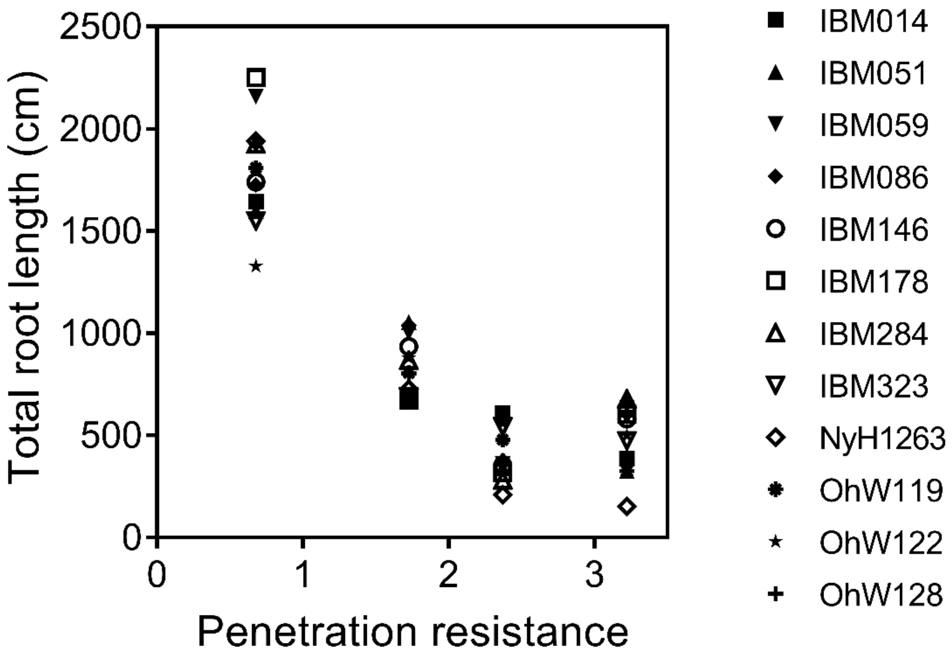

Figure S4 – Total root length of each genotype plotted at the averaged penetrometer resistance of the 2 field trial compaction treatment combinations.

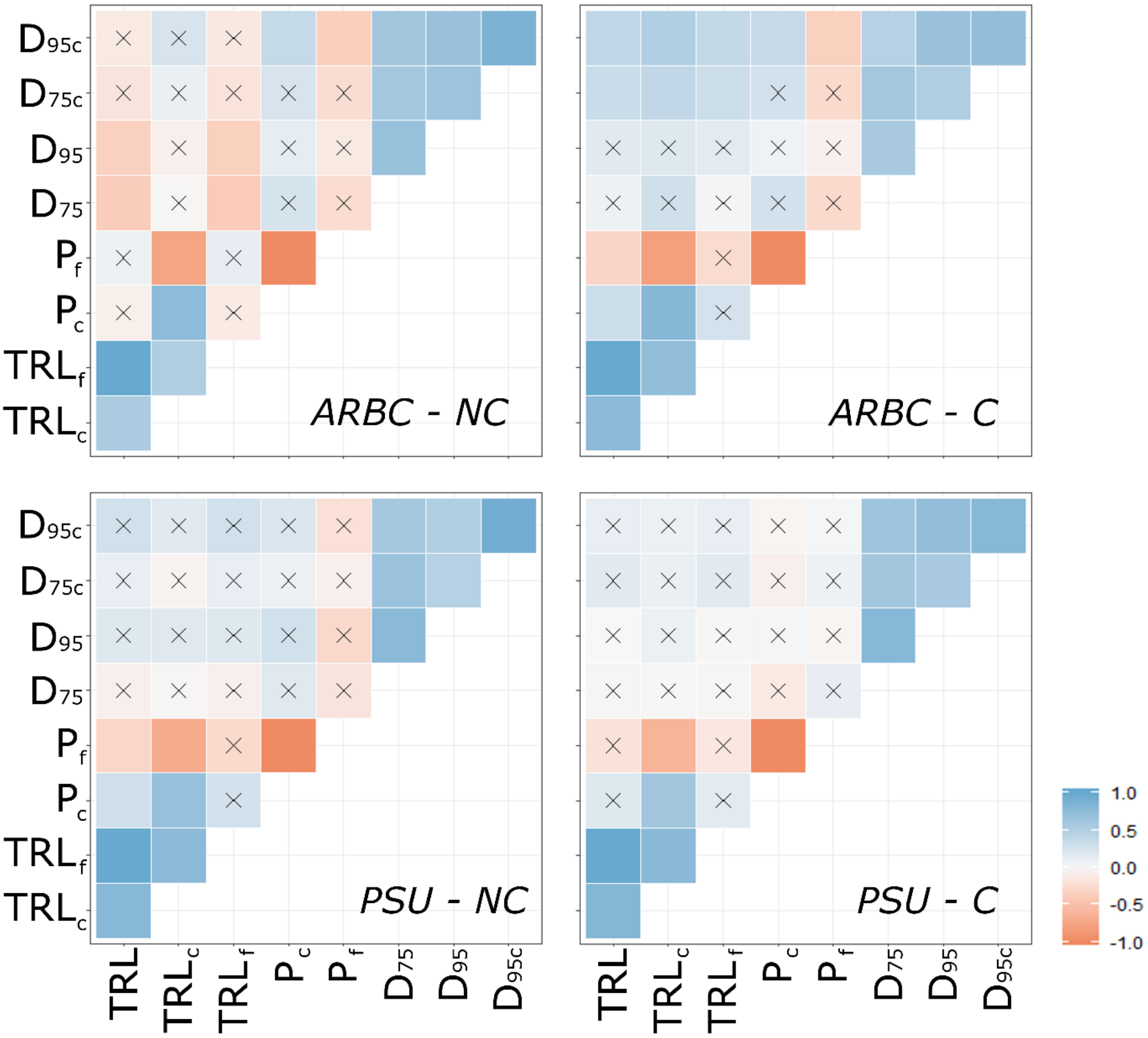

Figure S5 – Correlation plots between tested variables averaged over all genotypes across field sites (ARBC (coarse-loam) or PSU (silt-loam)) and compacted (C) or non-compacted plots (NC) combinations. The correlation coefficient is visualised by the scale bar, negative correlations are orange and positive correlations are blue. A cross represents a non-significant correlation at significance $p \leq 0.05$.

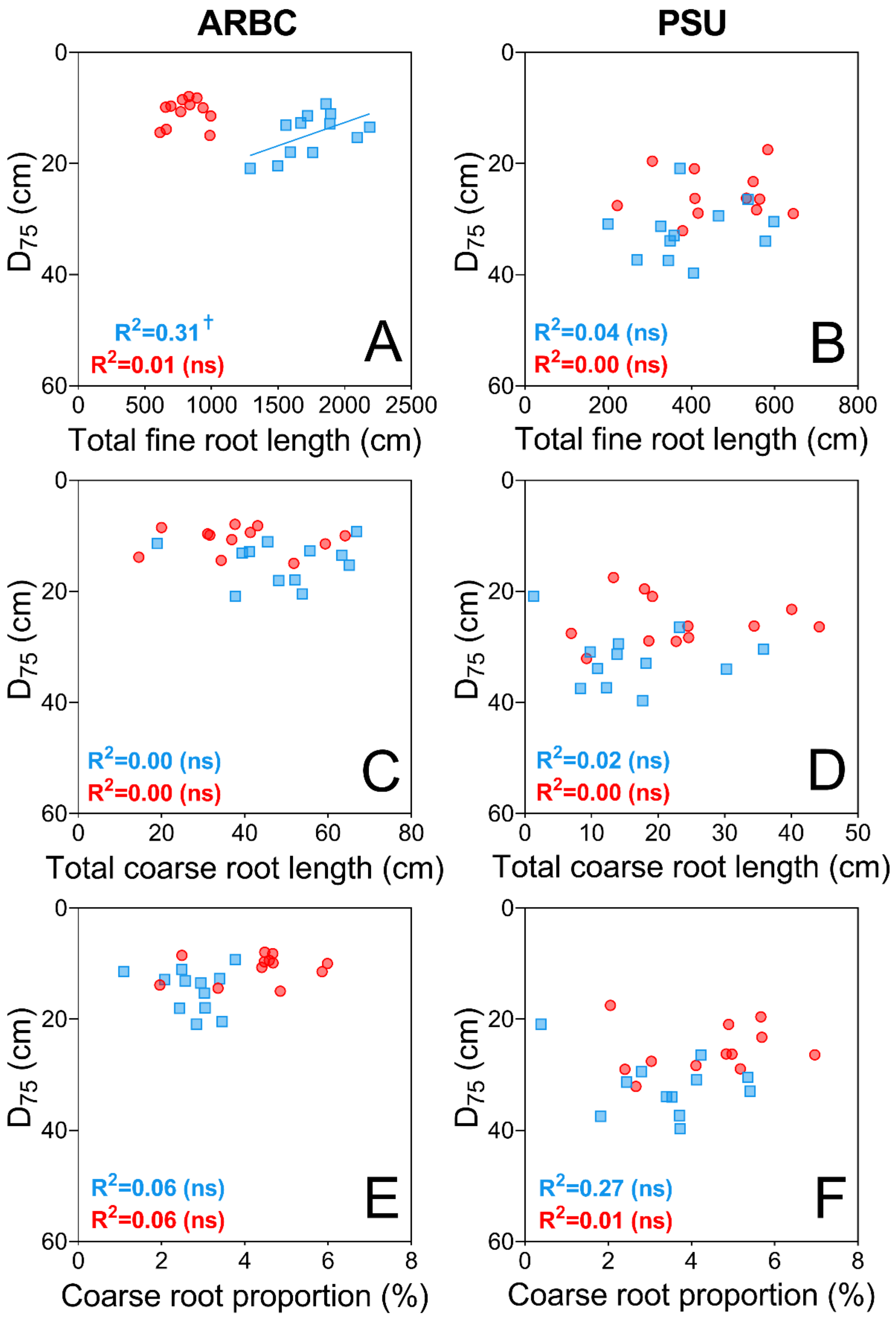

Figure S6 – Relationships between total rooting depth (D_{75}) and other root distribution variables across field sites and compaction treatments. Linear regression was used for A-D and beta-regression for E-F due to proportional data. Panels A,C and E represent field site ARBC (coarse-loam) and panels B, D and F represent field site PSU (silt-loam). Non-compacted data in blue, compacted data in red. One significant relationship was detected at significance level † $p \leq 0.10$, other relationships were non-significant (ns).

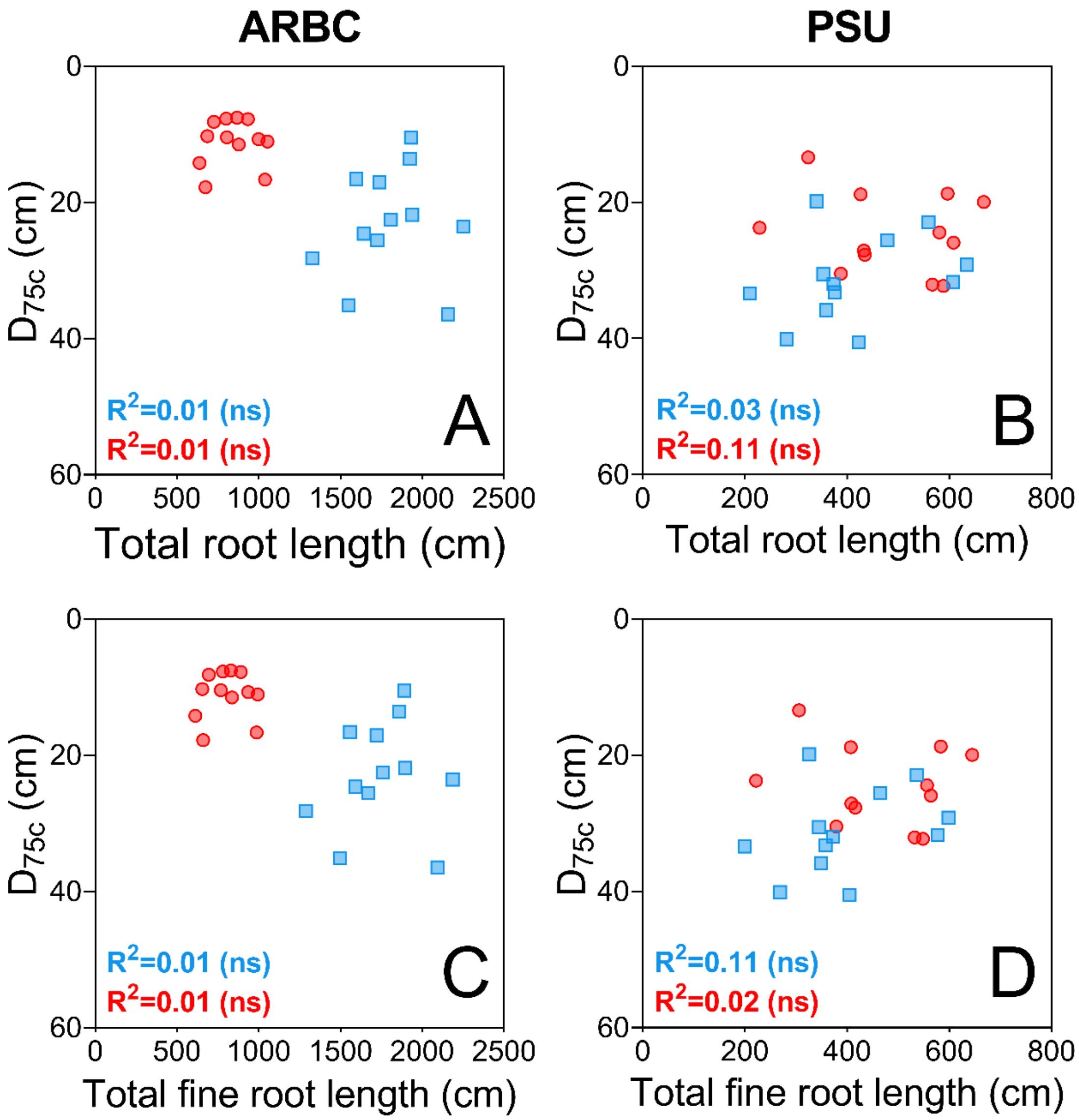

Figure S7 – Relationships between total rooting depth (D_{75c}) and other root distribution variables across field sites and compaction treatments. Panels A and C represent field site ARBC (coarse-loam) and panels B and D field site PSU (silt-loam). Non-compacted data in blue, compacted data in red. No significant (ns) linear relationships were detected.

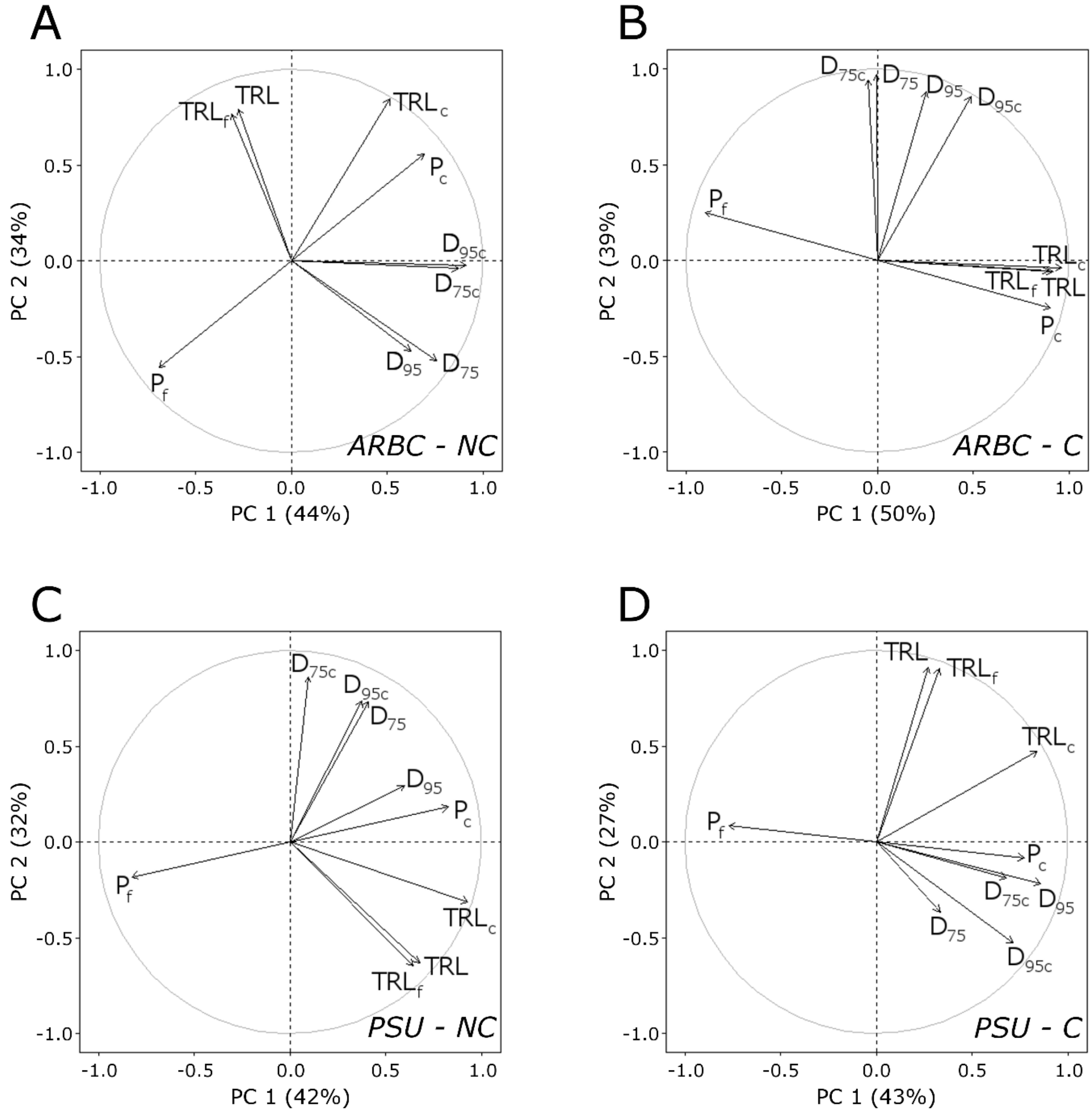

Figure S8 – Principle component analysis per field site (ARBC (coarse-loam) or PSU (silt-loam)) – compaction treatment (C – compacted; NC – non-compacted) combination illustrating relationships between root distribution variables within respective environmental conditions.

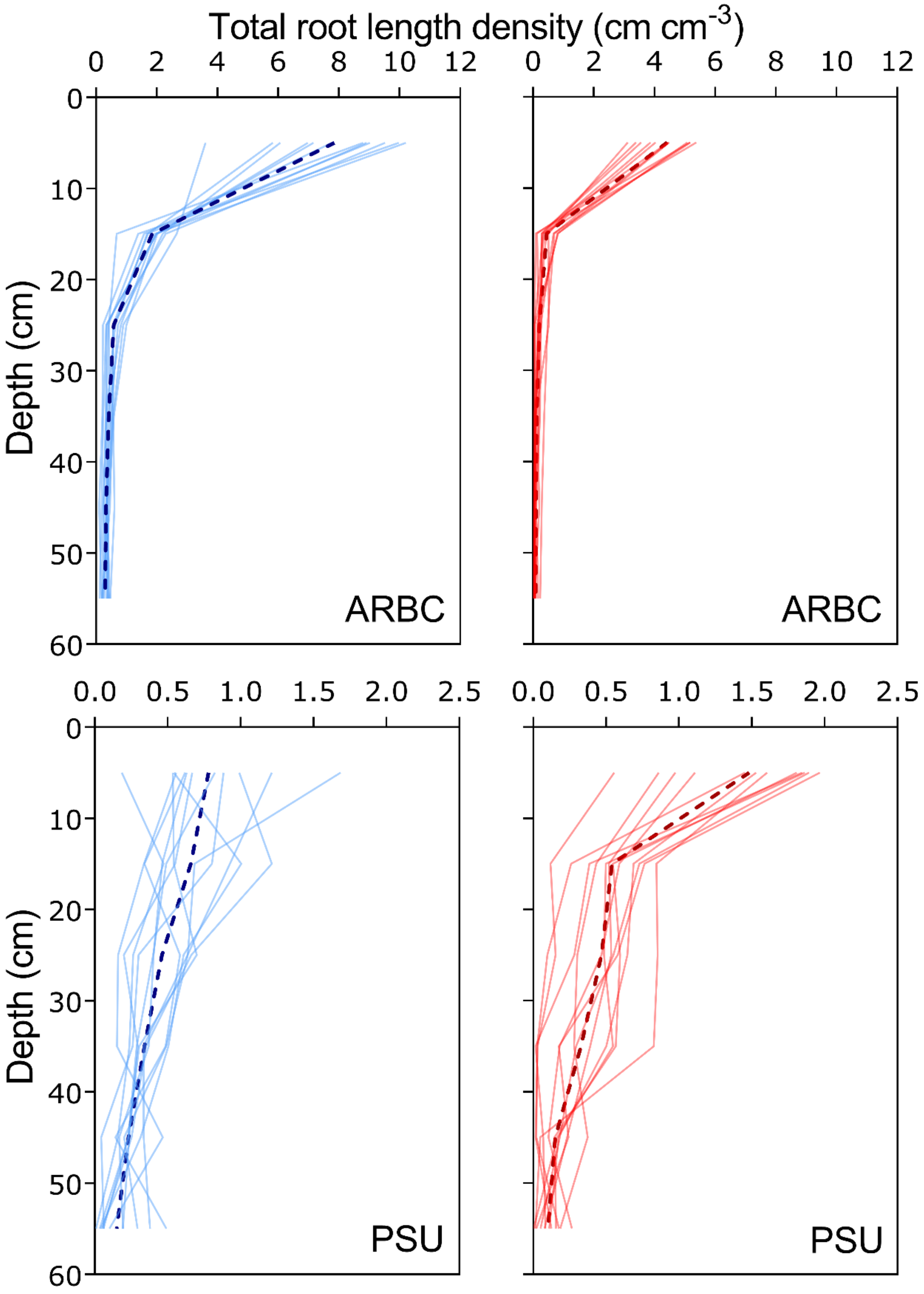

Figure S9 – Genotypic variation in total root length density (cm cm^{-3}) per depth increment across two field sites and two compaction treatments. The ARBC field site has a coarse-loam soil texture and the PSU field site has a silt-loam soil texture. Non-compacted data in blue and compacted data in red. The striped line are the averages across all genotypes, lighter coloured lines are the average for individual genotypes tested.

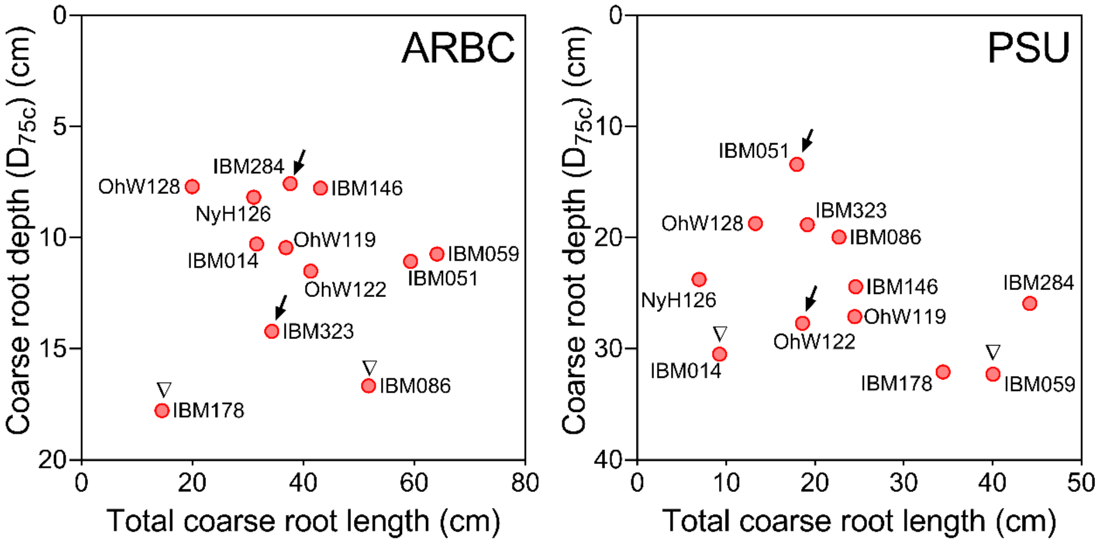

Figure S10 – Selection of genotypes to be compared based on their coarse rooting depth and coarse total root length. Genotypes indicated with an arrow were selected on the bases of similar coarse root length but different coarse rooting depths (shallow versus deep) and genotypes indicated with a triangle were selected on the basis of similar coarse rooting depth but are different according to total coarse root length (few versus many roots for deeper rooting genotypes). . The ARBC field site has a coarse-loam soil texture and the PSU field site has a silt-loam soil texture.

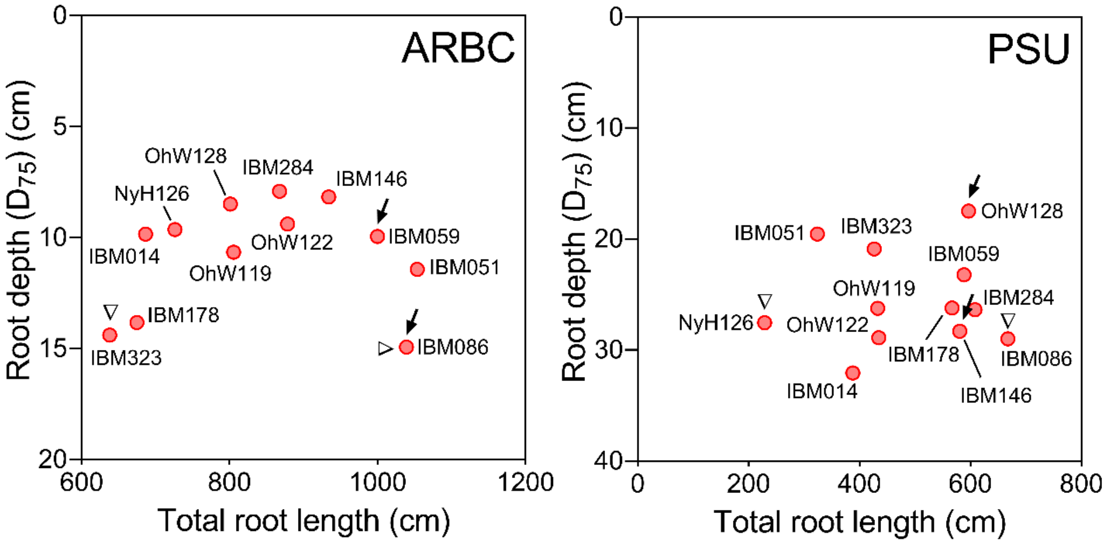

Figure S11 – Selection of genotypes to be compared based on their total rooting depth and total root length. Genotypes indicated with an arrow were selected on the bases of similar coarse root length but different coarse rooting depths (shallow versus deep) and genotypes indicated with a triangle were selected on the basis of similar coarse rooting depth but are different according to total coarse root length (few versus many roots for deeper rooting genotypes). . The ARBC field site has a coarse-loam soil texture and the PSU field site has a silt-loam soil texture.

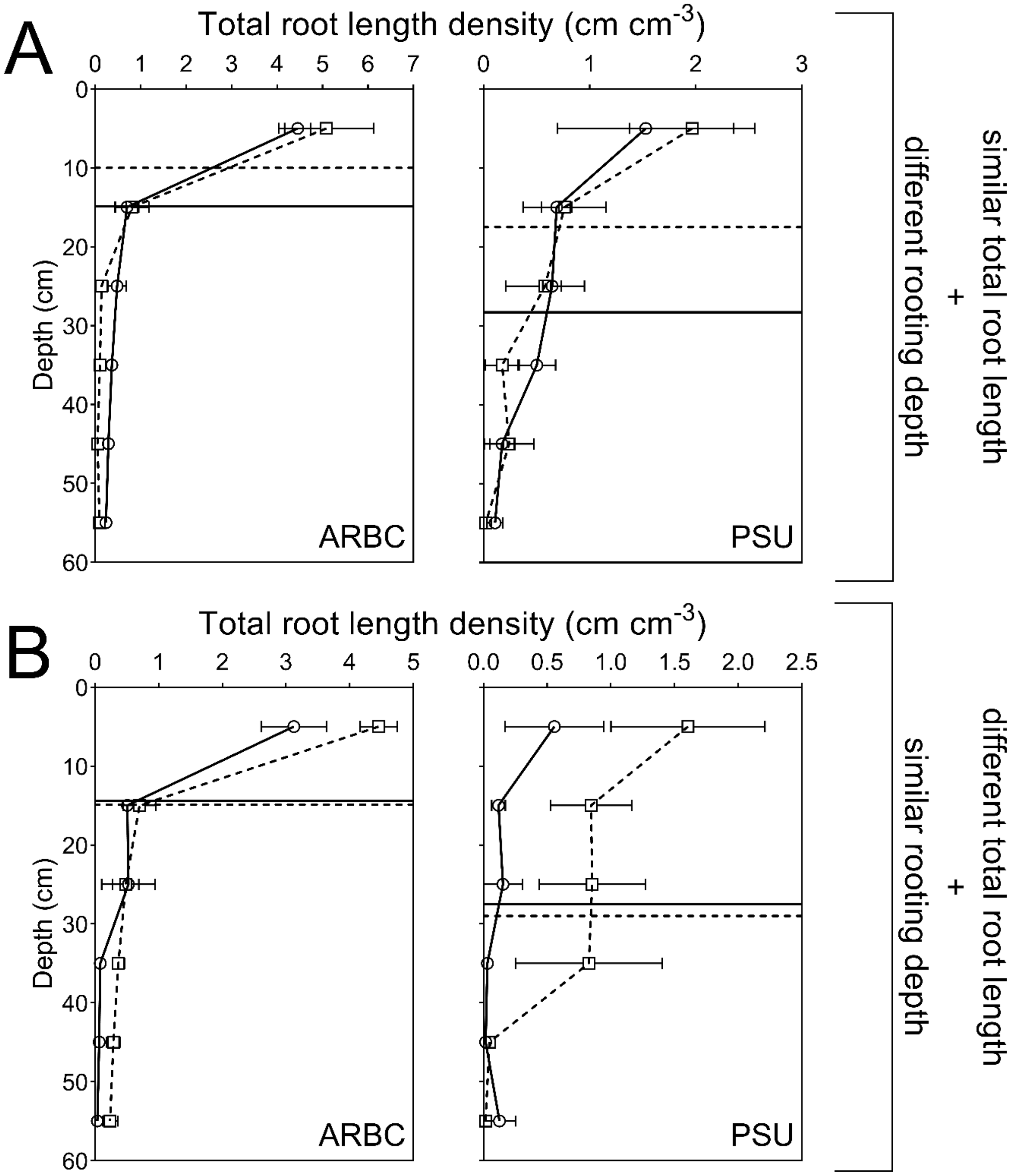

Figure S12 - Total root length densities (cm cm^{-3}) ± SE distributions with soil depth on compacted plots comparing (A) two genotypes per field sites with similar total coarse root length but with different associated rooting depths under compaction and (B) two genotypes with similar rooting depths but with different total coarse root lengths under compaction. For (A) solid lines stands for the deeper rooting genotype and associated D_{75}, while the striped line stands for the shallower rooting genotypes and associated D_{75}. For (B), the solid line is used for the genotype that produces less roots but reaches equally deep then the genotype that produces more roots (striped lines). . The ARBC field site has a coarse-loam soil texture and the PSU field site has a silt-loam soil texture.

Table S1 – Field applications during the field season. Table adjusted from supplementary Table 1 from Vanhees *et al.* (2020).

	Field applications					
	Irrigation		**Fertilizers**		**Pesticides**	
coarse-loam	18/06/2016	0.94 mm	04/06/2016	ProSol (15 gallons/acre)	17/06/2016	Atrazine and S-metolachlor
	21/06/2016	0.60 mm	14/06/2016	ProSol (12.5 gallons/acre)	14/06/2016	Copper, Azoxystrobin and Chlorantraniliprole
	22/06/2016	1.20 mm	15/06/2016	UAN (38.3 lbs/acre)	23/06/2016	Chlorantraniliprole
	23/06/2016	0.32 mm	16/06/2016	ProSol (12.5 gallons/acre)		
	25/06/2016	1.60 mm				
	28/06/2016	0.30 mm				
	04/07/2016	0.24 mm				
	06/07/2016	0.20 mm				
	08/07/2016	0.50 mm				
	10/07/2016	0.72 mm				
	17/07/2016	0.75 mm				
	20/07/2016	0.50 mm				
	24/07/2016	0.50 mm				
	27/07/2016	0.50 mm				
	09/08/2016	0.50 mm				
silt-loam	*No irrigation applied as moisture content remained high enough during growing season*		urea Nitrogen (200 lbs/acre) applied prior to planting		*No pesticides were applied*	

References in supplementary

Vanhees D.J., Loades, K.W., Bengough, A.G., Mooney, S.J., Lynch, J.P., 2020. Root anatomical traits contribute to deeper rooting of maize under compacted field conditions. J.Exp.Bot 71, 4243-4257. doi: 10.1093/jxb/eraa16