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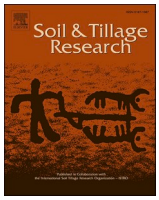

Effects of soil structure complexity to root growth of plants with contrasting root architecture

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ABSTRACT

Soil structure has a huge impact on plant root growth, but it is difficult to isolate from other soil properties in field studies, and generally overlooked in laboratory studies that use sieved and homogenised repacked soil. This study aimed to compare root and shoot growth under controlled soil conditions where only soil structure varied. Soil treatments used soil sieved to < 2 mm, packed in uniform layers to create a homogenous structure. A heterogeneous structure was packed from artificially formed aggregates created by breaking apart the homogeneous soil after intense compaction. Barley, peas and Arabidopsis, selected for contrasting root sizes, were grown under three levels of compaction (1.25 g cm^{-3}, 1.40 g cm^{-3}, 1.55 g cm^{-3}) in both homogeneous and heterogeneous structured soils for 10 days. Penetration resistance increased from about 0.4 MPa at 1.25 g cm^{-3} to 1.3 MPa at 1.55 g cm^{-3} for either soil structure. Soil structure was quantified from water retention characteristics and X-ray Computed Tomography (CT) as complementary methods to assess the soil's pore size distribution and properties. Heterogenous soil had 50% more macropores at 1.55 g cm^{-3} when compared to homogenous soils. Pore structure complexity in the heterogeneous structure was found to be beneficial for root growth of peas and barley but not Arabidopsis. Shoot biomass of peas grown in heterogeneous soil at 1.55 g cm^{-3} increased by 65% when compared to homogenous soil, whereas barley and Arabidopsis shoot biomass did not differ significantly between any treatments. Chlorophyll, flavonoid, and nitrogen content could only be measured on barley or peas due to shoot size, but only minor differences were observed between soil structures. Soil structural heterogeneity influenced many root properties and above-ground biomass, with impacts found to be species-dependent and likely caused by the interaction between root size and preferential growth in macropores.

1. Introduction

In agricultural fields, soil strength often increases with depth, restricting rooting depth, but roots may find paths with less mechanical resistance through the exploitation of macropores (Gao et al., 2016; Bai et al., 2019). In compacted soil, deep roots are found to be almost exclusively growing in pre-existing pore spaces, highlighting the importance of macropores for root growth (White and Kirkegaard, 2010; Valentine et al., 2012; Islam et al., 2021). Although field studies of root growth maintain an undisturbed soil structure, root analysis in field grown plants is limited to destructive sampling or the use of mini rizhotrons, which have less control of environmental variables. Moreover, roots can form extensive networks, so sampling may miss parts of the root system or be affected by interacting plants (Zhu et al., 2011; McMichael and Taylor, 2015).

Many root growth studies exploring the impacts of soil physical constraints are therefore conducted under controlled conditions using repacked cores so that properties are more reproducible between replicates (Bai et al., 2019) and to allow for control of abiotic stresses (Mittler, 2006; Chapman et al., 2012). Laboratory grown plants provide easier analysis of the root system, either through using transparent soil, sand, or sieved soil and through the use of imaging technologies, but whilst laboratory methods provide a controlled environment and require fewer resources, they simplify field conditions and may miss important variables that affect root growth (Zhu et al., 2011; Chapman et al., 2012).

Given the difference in soil structure complexity between the laboratory and field, it is not surprising that disparities between experimental systems have been reported (Chapman et al., 2012; Bai et al., 2019). Most studies in laboratory conditions use sieved soil that is

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packed to form uniformly hard or uniformly soft levels of mechanical impedance (Stirzaker et al., 1996). In the field, however, mechanical impedance of the soil is spatially and temporally variable due to changes in water content and the interactions between soil particles, biology and weather (Tardieu, 1988; Kirkegaard et al., 1992). For instance, weather interacting with shrinking clays may form cracks through wetting-drying cycles and biopores may be formed by soil fauna or previous crops (Stirzaker and White 1995; de Oliveira et al., 2021). The macropores that are formed by these processes provide paths of least resistance to root growth (McKenzie et al., 2009).

By omitting soil structure in laboratory studies, root plasticity to soil compaction tests only for mechanical impedance. Rice cultivars screened by Clark et al. (2002) that could penetrate a strong wax-layer buried in sand did not exhibit good hardpan penetration in the field (Clark et al., 2002). Bai et al. (2019) also found a similar wax layer screen did not transfer to wheat rooting depth in the field. The same inconsistency was encountered in dwarf wheat, screened using gels and sieved soil, at a range of mechanical impedances, and in the field (Wojciechowski et al., 2009). At similar levels of mechanical impedance, this experiment found that total root length was greater in sieved soil columns, with plants grown in controlled conditions, than root length in structured soil in the field. Despite evidence mounting that "A lack of consideration of the physical limitations of experimental systems could lead to the failure to identify desirable root traits in laboratory-based root screening methods" (Chapman et al., 2012) sieved, homogeneous soil, remains a popular choice to study physical constraints to root growth (Colombi and Walter, 2015; Rivera et al., 2019; Xiong et al., 2020). This is not surprising given that sieved homogenous soil removes spatial complexity within samples and between replicates that confounds understanding.

Here we provide a bridge between laboratory control studies and field complexity to study the impact of soil structure on plant root growth. Homogeneity of the soil between replicates and treatments was retained by using the same sieved and mixed soil. Soil structure complexity was simplified to a comparison between commonly used repacked sieved soil and repacked beds of soil 'aggregates'. These 'aggregates' are formed by breaking apart a homogeneous soil that had been severely compacted in the laboratory. When repacked, the soil structure consists of interaggregate macropores and intraggregate micropores, producing a more complex pore structure than homogeneous soil at the same bulk density. Three bulk densities, ranging from loose to compacted soil, were investigated. Three plant species were selected for different root architectures: barley (*H. vulgare* L. cv Optic) was used as a model crop of the cereal species with fibrous roots; pea (*P. sativum* L. cv Kelvedon wonder) as a dicotyledon species with thicker roots; and Arabidopsis (*A. thaliana* ecotype Col-0) with a fine and small root system. Root diameter differed considerably between species, with pea roots being on average 7 times thicker than Arabidopsis roots and 2 times thicker than barley roots. The aim was to recreate a heterogeneous soil environment that was less artificial than typical laboratory tests that use uniformly hard, or conversely, uniformly soft, soil. This development of a new methodology will allow the application of a range of stresses to plant growth whilst considering soil structure, a key natural feature, typically overlooked in controlled environment studies. Several parameters were measured to describe the physical condition of the soil. Despite bulk density being one of the most prominent indicators of soil structure (Rabot et al., 2018) it is a parameter that may not be suitable to describe soil physical condition on its own (Dexter, 1997). Therefore, while we controlled bulk density, we also measured penetrometer resistance and macroporosity to measure differences between the homogenous and heterogeneous soil structures. X-ray Computed Tomography provided a detailed analysis of macropore structure and pore size distribution. The following study tests the hypothesis that macropores allow roots to overcome stresses from mechanical impedance, with impacts greatest for plants with fine root systems and in compacted soils.

2. Material and methods

To understand the importance of soil structure in compacted soil, plants were grown in six soil treatments consisting of different levels of compaction and contrasting soil structures. Three levels of compaction were chosen with soil cores packed to bulk densities of either 1.25 g cm^{-3}, 1.40 g cm^{-3}, or 1.55 g cm^{-3} (Table 1). To create different soil structures at each bulk density, cores were packed with either sieved homogeneous soil or pre-made soil aggregates as illustrated in Fig. 1. Artificial aggregates with a density of 1.70 g cm^{-3} were formed by packing the soil in 2 cm layers with a 2.5 kg proctor hammer falling twenty times from a height of 0.3 m. The compacted soil was then broken down into aggregates < 15 mm in size to resemble field conditions of a tilled seedbed, with aggregates > 16 mm considered not desirable, and aggregates between 8–16 mm providing the greatest inter-aggregate aeration (Braunack & Dexter, 1989). Such an approach ensured that chemical and biological differences between the structured treatments was minimised, but allowed for differences in pore structure at controlled bulk densities to be explored.

Soil used in this study was a Eutric Cambisol with a sandy loam texture sampled from Bullion Field (Lat 56°27′36.44″N; Long 3°4′21.74″W) at the James Hutton Institute, Dundee, UK. The soil was comprised of 71% sand, 19% silt, and 10% clay with a pH of 6.2 (Loades et al., 2015; Liang et al., 2017).

2.1. Soil cores

Packing of the soil for plant growth was done in PVC cores of 5 cm diameter and 8 cm height. Prior to packing, cores were lined with a 0.5 mm thick acetate sheet to aid the removal of the intact soil at harvest. A mesh fabric was placed at the bottom of each core to retain the soil and allow for drainage. The soil was air-dried and sieved to < 2 mm. Following sieving a sub-sample was collected and dried at 105 °C for 48 h until water loss ceased to quantify soil moisture. The moisture content was increased to ~ 0.20 g g^{-1}, the optimal water content for soil packing as determined by a proctor compaction test (Liang et al., 2017). Water was added with a spray bottle in layers to avoid aggregates forming and then left for 48–72 h at 4 °C to equilibrate before packing. Following equilibration soil moisture content was measured again through drying at 105 °C. All the cores were packed at a moisture content of ~20% with four replicates per treatment. Cores were packed in 2 cm layers with each layer roughened before adding the subsequent layer to ensure a homogeneous core of soil and to eliminate potential localised changes in soil density at the interface between layers. Cores were packed to a defined bulk density using a proctor hammer by adding a specific amount of soil to each layer and applying pressure to pack soil to the desired volume.

Three unstructured soil density treatments of 1.25, 1.40 and 1.55 g cm^{-3} were packed using the < 2 mm sieved soil, providing an unstructured soil that is typical in laboratory conditions. Structured soil cores were packed to achieve the same bulk density, however with

Table 1
Six different soil treatments used during the experiment.

Treatment	Bulk Density (g cm^{-3})	Aggregate size	Soil Structure
Loose unstructured	1.25	< 2 mm	Homogeneous
Loose structured	1.25	varying in size up to 15 mm	Heterogenous
Lightly compacted unstructured	1.4	< 2 mm	Homogeneous
Lightly compacted structured	1.4	varying in size up to 15 mm	Heterogenous
Highly compacted unstructured	1.55	< 2 mm	Homogeneous
Highly compacted structured	1.55	varying in size up to 15 mm	Heterogenous

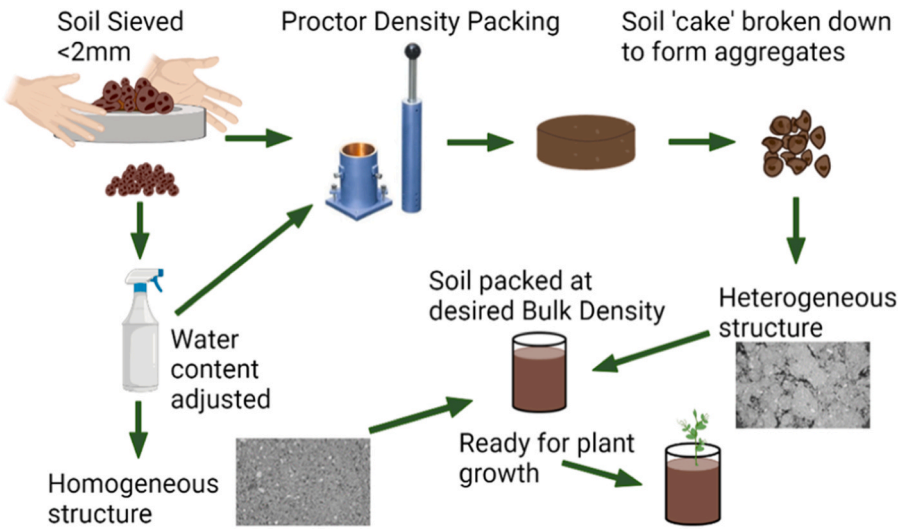

Fig. 1. Schematic representation of the methodology used to pack the soil cores used in the experiment.

altered pore size distribution as described above in Section 2. The structured treatment provided a less artificial, more heterogeneous media, with conditions similar to a well-structured tilled seedbed in the field (Braunack & Dexter, 1989). The six treatments are illustrated in Table 1.

2.1.1. Soil measurements

To ensure the penetrometer resistance measurements aligned with the resistance experienced by the roots during their growth, soil penetrometer resistance was assessed on the soil samples after they reached equilibrium at − 20 kPa water potential. This water potential was selected to not restrict water availability but to induce mechanical impedance at the greatest bulk density, as chosen by other researchers (Valentine et al., 2012). Measurements were taken with a miniature cone penetrometer (1 mm diameter and cone opening angle of 30° with rebated shaft behind the cone) attached to a 5 kN load cell using a mechanical test frame (Zwick All Round Z5, Zwick-Roell, Ulm, Germany) controlled by TestXpert III software (Zwick GmbH, Ulm, Germany). The penetrometer cone was inserted into the cores three times per soil core in different locations, to a depth of 5 mm at a speed of 2 mm min^{-1}, with the average penetration resistance between 3 and 4 mm depth used to assess mechanical impedance roots will encounter. The penetrometer resistance was calculated by dividing the force required for penetration by the area of the base of the cone.

Total porosity was calculated from bulk density assuming a particle density of 2.65 g cm^{-3}. Macroporosity was calculated by subtracting the volumetric water content at – 5kPa water potential from the total porosity. To estimate macroporosity the weight of the cores at 0.20 g g^{-1} water content was recorded prior to full saturation of the cores. After saturation, the cores were placed on a tension table (Eco-Tech Suction Plate, Bonn, Germany) to adjust the matric potential to − 5 kPa, equivalent to the draining of 60 μm pores. Following macroporosity measurements, cores were placed again on a tension table to adjust the matric potential to − 20 kPa for the growth of the plants.

2.2. Growth conditions and species

Three plants with contrasting root structures were grown in the soil cores previously described within a controlled environment. *Hordeum vulgare* L. cv Optic (barley) has fibrous roots, *A. thaliana* ecotype Col-0 (Arabidopsis) has a small and simple root system with a single primary root that produces smaller lateral roots, and *P. sativum* cv Kelvedon (pea) with a tap root and larger diameter roots than other plants used.

Before sowing, barley was pregerminated following surface sterilisation with 2% sodium hypochlorite for 10 min, rinsed in sterile deionized water three times, and soaked for 4 h in water. Seeds were germinated in trays between three layers of damp filter paper. The plates were covered in aluminium foil to keep the seeds in the dark (Naveed et al., 2018). Plants were grown in a growth cabinet (Fitotron PG660, Gallenkamp) under controlled conditions with temperature maintained at 18 °C day time and 14 °C night time, relative humidity was kept at 60–70% with light supplied at a minimum of 200 μmol/m^2sec during a 14 h period. Once germinated, one plantlet with similar root length was placed in each soil core within a pre-drilled 4 mm diameter hole at a depth of 4 mm. Following transfer, plants were allowed to grow for a further 10d in the growth chamber with the above conditions.

Arabidopsis seeds were surface sterilized with 50% sodium hypochlorite prior to washing five times with distilled sterilized water. Seeds were placed in distilled sterilized water in Eppendorf tubes to stratify for 3–4 days at 4 °C in the dark. After the stratification period, three seeds per core were sown and left to germinate under controlled conditions in the above-mentioned growth chamber to ensure at least one plant established within the cores. Once 3 d after planting, plants were thinned to one plant per core of a similar size to grow for a further 10 d with light supplied at 100–130 μmol/m^2sec) during a 16 h period. Temperature was maintained between 21–22 °C and relative humidity between 40–50%.

Pea seeds were surface sterilized with 2% sodium hypochlorite for 10 min prior to rinsing in sterile deionized water three times. Seeds were germinated in trays between three layers of damp filter paper. The plates were covered in aluminium foil to eliminate light. Seeds were germinated under controlled conditions of 22 °C during the day and 16 °C at night, and relative humidity at 40%. Light was supplied at 330 μmol m^{-2} s^{-1} during a 12-hr photoperiod. Plantlets of similar root length were placed in the soil cores within a pre-drilled 4 mm hole at 10 mm depth before growing for a further 10d in the growth chamber with the above conditions.

All plants were grown at approximately − 20 kPa water potential, with cores watered to weight daily by gently spraying from above. As matric potential was maintained at –20 kPa, water availability was maintained at a level not to induce water stress throughout the experimental period. Different plant species were grown at different times due to the large amount of time required to harvest samples with four replicates per plant species.

2.3. CT scanning and image analysis

CT scanning was used to quantify and visualise differences in pore

size distribution between heterogeneous and homogeneous soil cores. Prior to scanning, all above-ground biomass was removed with cores stored at 4 °C if not scanned immediately. At harvest, soil cores were scanned using an XT H 225 ST CT Scanner (NIKON Metrology, Tring, UK) with settings of 120 kV, 120 μA, 0.12° steps with 500 ms exposure time, 2 mm Al filter and pixel size at 40.056 μm. Three-dimensional reconstruction was performed on the original images using the software VG Studio Max (Version 3.2) (Volume Graphics). The digital image processing and analysis were conducted with ImageJ (Version 1.52 u) (Schindelin et al., 2012). The bleach correction macro for Image J was used to correct fluctuations in the intensity of the brightness, normalizing the images of a stack to the same mean intensity the plugin version of Jens Rietdorf's macro was used (Miura, 2020). After adjusting brightness and contrast, the images were cropped to a region of interest with 500 slices used for analysis. Reducing the stack, and cropping the images, was done to avoid ring artefacts caused by edge effects and beam hardening (Mooney et al., 2006; Deurer et al., 2009). Images were denoised using the non-local means denoising plugin with the sigma value autoestimated by the plugin (Buades et al., 2011). To separate the soil matrix from the pores, images were segmented using the maximum entropy thresholding method using the plugin developed by Jerek Sacha in 2004 (Tracy et al., 2012). Pore size distribution was determined using the 'thickness' plugin of BoneJ (Doube et al., 2010), a method that measures the diameter of the largest sphere that fits inside the 3D pore space that touches the bordering soil matrix.

2.4. Root morphology

Total root length, root volume, number of tips and average root diameter were measured after 10 days of growth for the three species. To separate out roots, the soil cores were gently emptied, and the soil was carefully rinsed away from the roots using tap water over a 500 μm sieve. Once harvested roots were immersed in a 50% ethanol solution and kept at 4 °C until scanned. The roots were placed on a plexiglass tray with a layer of water around 5 mm deep and spread to minimize overlapping. Greyscale images (600 DPI) were taken using an Expression 10000XL Scanner (Epson, Suwa, Japan). The total root length, root volume, number of tips and average root diameter were obtained using WinRhizo (Version 2017a) (Regent Instrument Canada Inc). The number of lateral roots per cm of root length measurements were taken at 2–3 cm from the start of the root using Image J (Version 1.52 u). 2.5 Plant vigour.

Prior to harvest, leaf 'greenness', or chlorophyll content, and nitrogen content were measured to assess plant stress in barley and pea. This step was not performed in Arabidopsis as the leaves were too small to measure them. Various studies have shown that changes in chlorophyll content provide an evident indicator of plant stress (Lichtenthaler et al., 1996; Lichtenthaler and Babani, 2007; Pavlović et al., 2014). Three measurements per plant were taken 10d after germination at the youngest fully developed leaf with Dualex meter (Force A) in three different positions. The Dualex provided measurements for chlorophyll content, nitrogen balance index, and epidermal flavonoid content. The three measurements were then averaged.

Plant height was measured by straightening the plant tissue and using a ruler. The number of leaves was recorded and the shoots were then cut at the soil surface before root washing. Fresh weight of the roots and the shoots was recorded before oven drying at 60°C for 3–4 days before weighing.

2.5. Statistical analysis

All statistical analyses were conducted with R version 4.0.3. A two-way ANOVA was used to determine whether there were significant differences between bulk density and soil structure for each plant species, with data checked to make sure all the assumptions were met. The two-way ANOVAs were conducted for each species separately for the root morphology and plant vigour measurements. Normality of residuals was assessed with the use of probability plots and the Shapiro-Wilk test ($P > 0.05$). Homogeneity of variances was checked using Bartlett's test ($P > 0.05$). Post Hoc analysis was carried out by the Tukey's HSD test for significant differences between treatments at $P < 0.05$ level. Where the homogeneity of variances was violated, as assessed by Bartlett's test of Homogeneity of Variance, data were transformed and if the assumption was not met the test was carried on as per Lindquist (1953) who showed that ANOVA is robust to violations of its assumptions if there is an equal sample size or something close to equal sample size between the groups being compared (Lindquist, 1953). Soil physical properties were analysed as a block design with each species treated as a different block. To determine significant statistical differences in pore size distribution between unstructured and structured soil packed at the same bulk density the Students t-test was performed for different pore size ranges. There were 6 plants that developed very poorly and were identified as outliers and excluded from the analysis.

3. Results

3.1. Physical properties of the soil

The compacted soil used to form the structured treatment had a porosity of 0.17 $m^3\ m^{-3}$ and a penetration resistance of 3.23 MPa before it was broken apart. Packing to form unstructured and structured cores allowed for control of bulk density, but other physical properties varied between these treatments (Table 2). From the loose 1.25 g cm^{-3} to the compacted 1.55 g cm^{-3} treatment, total porosity declined by 18% ($P < 0.001$), with macroporosity measured from water content at − 5 kPa water potential decreasing by 70% ($P < 0.01$).

When comparing structured to unstructured soil at a bulk density of 1.25 g cm^{-3} there was a small 0.03 $m^3\ m^{-3}$ decrease in total porosity in the structured soil ($P < 0.001$), but no significant differences in total porosity at higher bulk densities of 1.40 g cm^{-3} and at 1.55 g cm^{-3} ($P > 0.05$). Compaction preferentially removed some macropores from the soil as estimated from water retention characteristics, with 1.55 g cm^{-3} structured soil retaining 50% greater macroporosity than the unstructured soil ($P < 0.001$), despite the total porosity remaining the same in both soils (Table 2). In the loose treatments, packed at 1.25 g cm^{-3} macroporosity, estimated from water retention was 17.4% lower in structured than unstructured soil ($P < 0.001$) (Fig. 2, Table 2). On lightly compacted soil at 1.40 g cm^{-3} the macroporosity did not differ between structured and unstructured soil (Table 2).

As would be expected air-filled porosity, determined from water retention measurements at − 5 kPa water potential, decreased with increasing compaction. In soil packed at 1.25 g cm^{-3} and 1.40 g cm^{-3}, unstructured soil had significantly greater air-filled porosity ($P > 0.001$) when compared to structured soil (Table 2). Volumetric water content at − 20 kPa in soil packed at 1.25 g cm^{-3} and 1.40 g cm^{-3} was significantly higher in structured soil compared to unstructured soil and in soil packed at 1.55 g cm^{-3} with volumetric water content the same (Table 2).

Different levels of compaction were reflected in the penetration resistance of the soil cores, with no significant differences observed between soil structure treatments at the same bulk densities ($P > 0.05$). However, penetration resistance was observed to fluctuate more within structured soil cores when compared to unstructured cores. The coefficient of variation (CV) was 18.2% for structured soils versus 14% for unstructured soils. Mean penetration resistance in compacted cores at 1.55 g cm^{-3} density was significantly greater than the loose treatments with a bulk density of 1.25 g cm^{-3} in both structured and unstructured soils ($P < 0.001$) (Table 2). There were no significant differences in penetrometer resistance of the cores used to test the different species, suggesting there was consistency.

As the soil cores were packed in batches for each plant species, consistency of soil pore structure properties was checked. There was no

Table 2
Selected physical properties of soils of six treatments. Water potential during barley growth was kept at − 20kPa, all measurements were done at − 20kPa with exception of macroporosity which was done at − 5kPa. Numbers in brackets are standard deviation of the mean. Different letters indicate that the means are significantly different ($P < 0.05$) n = 4.

Variable or Parameter	Unstructured Soil			Structured Soil		
	L (Loose)	LC (Lightly compacted)	HC (Highly Compacted)	L (Loose)	LC (Lightly Compacted)	HC (Highly Compacted)
Bulk Density (g cm^{-3})	1.25	1.40	1.55	1.25	1.40	1.55
Penetrometer Resistance (Mpa)	0.40(0.114)a	0.67(0.208)c	1.08(0.160)d	0.46(0.287)b	0.87(0.384)cd	1.25(0.500)d
Total Porosity (m^3m^{-3})	0.53(0.002)a	0.47(0.002)c	0.41(0.002)d	0.50(0.007)b	0.47(0.002)c	0.41(0.002)d
Macroporosity (m^3m^{-3})	0.23(0.01)a	0.14(0.008)bc	0.04(0.016)e	0.19(0.015)b	0.13(0.019)d	0.06(0.022)e
Macroporosity (CT Scan m^3m^{-3})	0.21(0.012)a	0.11(0.008)b	0.04(0.001)c	0.22(0.003)a	0.14(0.012)b	0.06(0.003)c
Air Filled Porosity (m^3m^{-3})	0.26(0.013)a	0.18(0.009)c	0.08(0.010)d	0.22(0.016)b	0.16(0.010)c	0.08(0.005)d
Volumetric Water Content during growth (cm^3cm^{-3})	0.27(0.011)a	0.30(0.009)bc	0.33(0.011)e	0.29(0.010)b	0.31(0.010)d	0.33(0.006)e

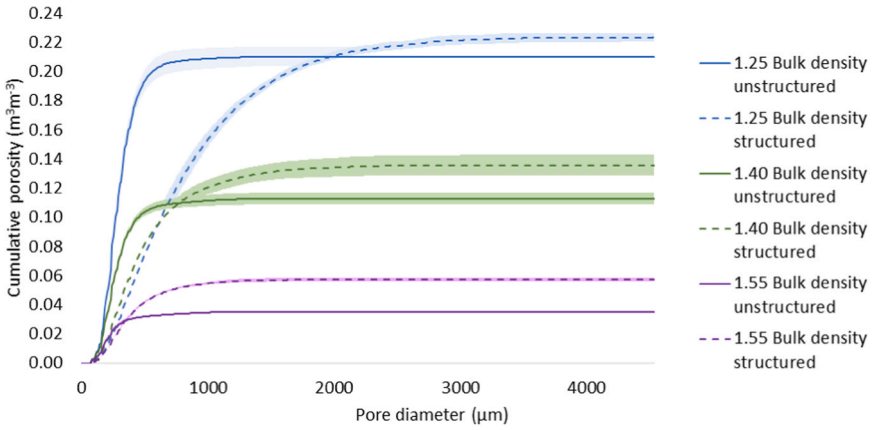

Fig. 2. Cumulative detectable porosity of the soil after harvest from CT scan with pores > 60 μm. The shaded area represents the standard error of the mean.

significant difference in macroporosity measured by water retention between treatments apart from macroporosity in unstructured soil at a density of 1.40 g cm^{-3} for Arabidopsis compared to barley and peas. No other properties differed for the same soil treatment between plants.

3.2. CT scan macroporosity and pore size distribution

X-Ray CT scanning revealed differences in pore structure between structured and unstructured soils, and also within the different bulk density treatments (Table 2; Fig. 2 and 3). Imaging resolution with this approach was limited to > 60 μm, equivalent to pore sizes drained at − 5 kPa used to measure macroporosity from water retention. Total macroporosity from X-Ray CT varied between compaction levels ($P < 0.001$) (Table 2). CT results of macroporosity in highly compacted treatments packed at 1.55 g cm^{-3} were the same as the water retention estimate, and 50% greater in structured soil compared to unstructured soil ($P > 0.05$). The macroporosity of Arabidopsis grown cores measured by CT scanning was 12.1% less than peas and 11.2% less than barley ($P < 0.001$).

Pore size distribution between structured and unstructured soils at the same bulk densities showed significant differences between treatments ($P < 0.001$) (Fig. 2). In soils packed at 1.25 g cm^{-3}, unstructured soil had over double the pore volume in the size range 200 μm − 500 μm, 0.145 m m^{-3} pore volume compared to 0.064 m m^{-3} in the unstructured treatment, with this size range accounting for ~68% of all the pores in unstructured soil when compared to ~28% in structured soil. At 1.25 g cm^{-3} structured soil had around four times more pores in the size range of 500 μm − 1000 μm compared to the unstructured soil which had a pore volume of 0.016 m m^{-3}, this size range accounted for ~35% of all the pores in the structured soil and only ~7% of all the pores in the unstructured soil. Within unstructured soil packed to 1.25 g cm^{-3}, no pores larger than 1500 μm were present, whereas structured soil had pores of up to 4500 μm. In soils packed at 1.40 g cm^{-3}, unstructured soil and structured soil had the same pore volume in the 200 μm - 500 μm range. However, in the size range of 501 μm − 1000 μm, structured soil had increased pore volume, with pores in this range accounting for ~29% of all the pores in structured soil versus only ~7% of pores in the unstructured soil. The pore size range of 1001 μm – 1500 μm accounted for only ~1% of the total pore volume in unstructured soil and ~7% of the pore volume in the structured soil. Moreover, at 1.40 g cm^{-3}, unstructured soil had no pores bigger than 1500 μm but structured soil had pores of up to 3000 μm. Unstructured soils packed at 1.55 g cm^{-3} had nearly double the number of pores in the size range of 60 μm − 200 μm when compared to structured soil, with this size range accounting for ~47% of all the pores in unstructured soil when compared to ~17% in structured soil. The unstructured soil also had a very similar pore volume between size ranges of 60 μm-200 μm and 201 μm- 500 μm, with the latter range accounting for ~44% of all the pores in the unstructured soil, whilst the structured soil had most of its pores ranging in size from 201 μm-500 μm with this size range accounting for ~56% of all the pores in structured soil, followed by the size range of 501 μm-1000 μm which accounted for ~22% of all the pores in the structured soil. Unstructured soil did not have pores bigger than the size range 1001 μm − 1500 μm, whilst structured soil had pores of up to 3000 μm diameter. In summary, at the same bulk density, the pore structure differed markedly between the structured and unstructured treatments with a greater number of large pores present in the structured soil.

3.3. Root growth response to treatments and changes in root morphology

Root growth of the three plant species was significantly affected by increased bulk density (Table 2, Figs. 3 and 4). However, the impacts on root growth at 1.55 g cm^{-3} were less in structured soil for barley, with its fibrous root system, and peas with its larger root system, but not Arabidopsis with its fine roots.

In peas, the presence of soil structure significantly increased root length in soil packed at 1.25 g cm^{-3} when compared to unstructured soil ($P < 0.05$). Peas exhibited an increase in total root length of 46.8% in structured soil when compared to unstructured soil at 1.25 g cm^{-3} bulk density, whereas barley roots only increased by 13.2% and Arabidopsis root length decreased by 26.8% (Table 3, Fig. 4). The structured soil at 1.25 g cm^{-3} had the greatest total root length for peas and barley of all treatments and in peas it was significantly different from all the other structured and unstructured treatments ($P < 0.01$). In soil packed at 1.55 g cm^{-3} bulk density the structured soil provided a positive effect on the total root length for peas and barley, in which peas recorded a ~100% increase in total root length compared to the unstructured soil and barley root length saw an increase of 58.6% ($P > 0.05$) (Fig. 4a). Soil structure had a negative effect on root length of Arabidopsis with root length significantly reduced in structured soil packed at 1.55 g cm^{-3} when compared to root length in unstructured soil packed at 1.25 g cm^{-3} ($P < 0.05$), but this decrease was not significant when comparing the root length of Arabidopsis grown in unstructured soil at 1.55 g cm^{-3} ($P > 0.05$) (Fig. 4c). However, barley total root length decreased markedly from 1.25 g cm^{-3} to 1.55 g cm^{-3} bulk densities in unstructured soil ($P < 0.05$) with no significant differences in structured soil ($P > 0.05$) (Fig. 4b).

In lightly compacted unstructured soil packed at 1.40 g cm^{-3}Arabidopsis total root length performed better than in any other soil physical treatments (Fig. 4c). At the same level of compaction in peas, total root length remained the same for both structured and unstructured soil, whereas in barley total root length decreased 23.7% in structured soil compared to unstructured soil ($P > 0.05$) (Fig. 4a and b).

Soil structure did not affect root volume of any of the plant species. Barley and Arabidopsis root tip number was not affected by soil structure, whereas pea root tip number increased due to structure ($P < 0.05$). The total number of root tips in peas was significantly different ($P < 0.001$) in unstructured soil packed at 1.55 g cm^{-3} (64 tips) when compared to structured soil at 1.25 g cm^{-3} (1376 tips) (Table 3). When comparing the number of root tips in peas in the structured soil at 1.55 g cm^{-3} with the structured soil at 1.25 g cm^{-3} no significant differences were found (Table 3). Arabidopsis presented no significant differences in lateral root number in any of the treatments. In soils packed at 1.25 g cm^{-3}, barley and peas presented no significant differences in lateral root number (Table 3). Barley lateral root numbers increased by 280% between structured and unstructured soil at 1.55 g cm^{-3}bulk density, compared to only a 40% increase for peas (Table 3). Barley lateral root number in structured soil at 1.55 g $cm^{-3}$was not different from structured soil packed at 1.25 g cm^{-3}, whilst lateral root number in unstructured soil at

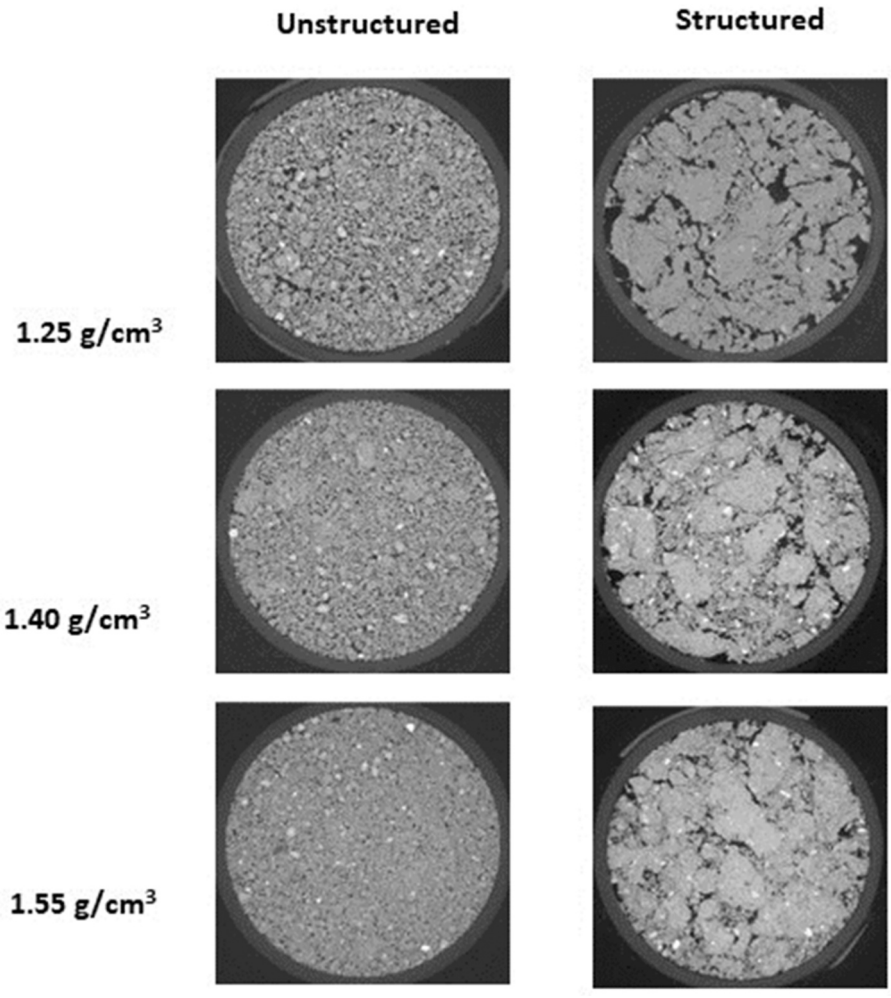

Fig. 3. 2D greyscale images of soil cores showing the difference in soil structure between the different treatments. Images from X-ray CT the day of harvesting the barley plants.

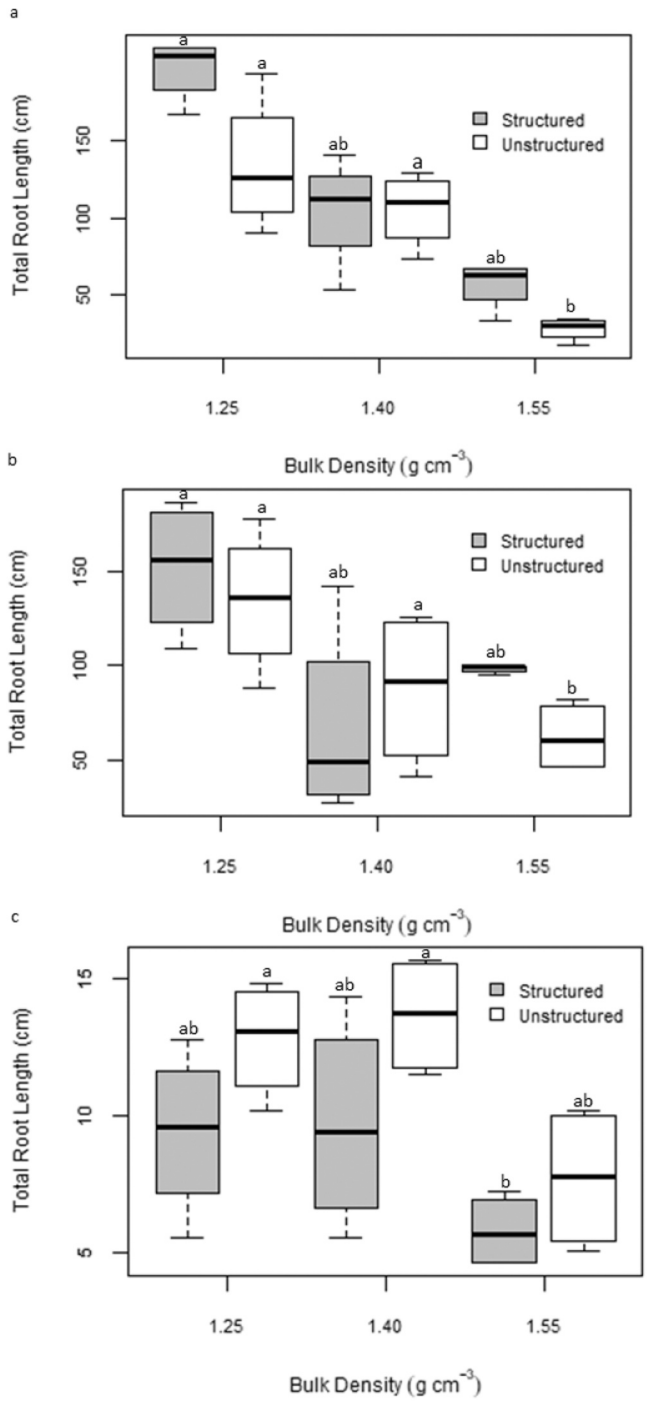

Fig. 4. Box plot of total root length of the different species 10d after germination under different soil treatments. a.Pea; b. Barley; c. Arabidopsis. U= unstructured soil, S= structured soil. Each box plot denotes the interquartile range (IQR), the lower and upper box boundaries 25th and 75th percentiles, respectively. The line inside the box represents the median or 50th percentile. The length of whiskers below and above the box represents lower and upper values, respectively, outside the interquartile range but within 1.5 × IQR n = 4.

1.55 g cm^{-3} was significantly different from structured soil packed at 1.25 g cm^{-3} ($P < 0.05$) (Table 3). Curiously in the lightly compacted treatments packed at 1.40 g cm^{-3}, barley lateral root number was significantly smaller in structured soil ($P < 0.05$).

The average root diameter increased with increasing bulk density in the three species, but only barley had significant differences in root diameter between structured and unstructured soil ($P < 0.05$). In highly compacted soils with a bulk density of 1.55 g cm^{-3} the unstructured soil had significantly thicker roots than the structured soil packed at 1.25 g cm^{-3} ($P < 0.05$), whilst the structured soil at 1.55 g cm^{-3} was not significantly different to structured soil packed at 1.25 g cm^{-3} (Table 3). Root dry weight decreased with compaction in all species, however, no significant differences between the structured and unstructured soil treatments were observed. Root dry weight was greater in structured soil for both peas and barley. Arabidopsis had a great variability in fresh root weight within treatments (Table 3). The root:shoot ratio was not influenced by structure in any of the three species with no significant differences found between treatments.

3.4. Plant vigour

The presence of soil structure did not influence plant height significantly in barley and Arabidopsis, but differences were observed within peas. Whereas pea shoot height was unaffected by bulk density in structured soil, it was reduced in unstructured soil packed to 1.55 g cm^{-3} when compared to either 1.25 g cm^{-3} or 1.40 g cm^{-3} ($P < 0.001$) (Table 4).

It was not possible to measure shoot biomass in Arabidopsis plants due to its small size. In barley, shoot biomass was unaffected by either bulk density or soil structure ($P > 0.05$). However, shoot biomass of peas decreased between 1.40 g cm^{-3} and 1.55 g cm^{-3} but was affected much less in structured soil (Table 4).

The number of leaves remained the same in all treatments in barley and Arabidopsis. In peas the number of leaves was significantly greater in structured soil packed at 1.25 g cm^{-3}when compared to unstructured soil packed at 1.55 g cm^{-3} ($P < 0.05$), but no significant differences were observed when compared to 1.55 g cm^{-3} structured soil (Table 4).

The presence of soil structure did not influence chlorophyll, flavonoid, and nitrogen content in barley or peas. Chlorophyll content, flavonoid content, and nitrogen content were not measured in any of Arabidopsis plants and peas in the unstructured soil packed at 1.55 g cm^{-3} due to the small size of the leaves (Table 4).

4. Discussion

Root growth was affected by both soil bulk density and pore structure heterogeneity, with impacts varying between species. This suggests that simple measurements of soil structure based on bulk porosity or penetrometer resistance are not sufficient when describing physical conditions for root growth. Although these terms are valuable for helping describe structure they do not provide a complete picture of the physical environment experienced by plant roots in field soils (Stirzaker et al., 1996). In the field, soil is heterogeneous and aggregated with roots growing preferentially through macropores to avoid compacted zones (Colombi et al., 2017). These compacted zones may differ spatially, particularly in agricultural soils with fragments produced through tillage actions (Or et al., 2021). Roots will not encounter evenly compacted soil but more likely encounter a range of different zones within a spatially diverse soil environment, variable with impacts from other factors such as water content, oxygen availability, and porosity (Tardieu, 1988; Passioura, 2002). Across two crop species, pea and barley, clear evidence was provided of the importance of macropores and soil structure to preferential root growth. The model plant, Arabidopsis, differed from the other species by having a less extensive root system in structured soil. This discrepancy likely resulted because of the difference in root morphology, as Arabidopsis roots are considerably smaller so there was likely poorer root-soil contact in structured soils.

4.1. Physical properties of the soil

Most previous studies on the impacts of soil physical condition on root growth focus on altering the density of sieved soil (Engelaar et al.,

Table 3
Root traits of the six treatments measured at 10d after germination. Numbers in brackets are standard deviation of the mean. Different letters indicate that the means are significantly different ($P < 0.05$) n = 4.

Variable or Parameter	Unstructured Soil			Structured Soil		
	L (Loose)	LC (Lightly compacted)	HC (Highly Compacted)	L (Loose)	LC (Lightly Compacted)	HC (Highly Compacted)
H. vulgare						
Bulk Density (g cm^{-3})	**1.25**	**1.40**	**1.55**	**1.25**	**1.40**	**1.55**
Lateral Root Number	6.00(1.63)a	6.00(2.70)ab	1.25(1.50)c	6.25(2.50)a	2.25(2.06)b	4.75(0.50)ac
Number of Tips	1165.50(316.25)a	728.75(409.71)abc	502.00(181.40)bc	1393.00(299.41)a	554.25(360.87)b	867.25(224.07)ac
Root Volume (cm^3)	0.183(0.053)a	0.162(0.058)a	0.156(0.024)a	0.214(0.081)a	0.152(.086)a	0.184(0.024)a
Average Root Diameter (mm)	0.418(0.314)a	0.500(0.0164)ab	0.577(0.0416)c	0.443(0.0694)a	0.570(0.0928)b	0.487(0.0273)ac
Dry Weight (g)	0.023(0.01)a	0.024(0.01)a	0.018(0.01)a	0.025(0.00)a	0.021(0.00)a	0.074(0.10)a
Root:Shoot ratio	0.147(0.619)a	0.199(0.094)a	0.148(0.042)a	0.115(0.012)a	0.192(0.073)a	0.472(0.585)a
P. sativum						
Lateral Root Number	6.75(2.17)a	5.25(1.09)ab	1.50(0.87)d	6.00(1.58)a	5.00(0.71)abc	2.50(1.12)cd
Number of Tips	668.25(433.65)ac	273.75(131.11)b	64.50(34.26)d	1376.00(566.95)a	396.75(284.79)bc	344.25(213.76)cd
Root Volume (cm^3)	0.615(0.12)a	0.584(0.08)abc	0.354(0.11)cd	0.792(0.08)a	0.532(0.08)c	0.483(0.10)d
Average Root Diameter (mm)	0.775(0.07)a	0.844(0.04)abc	1.264(0.20)c	0.714(0.04)a	0.826(0.10)abc	1.050(0.07)c
Dry Weight(g)	0.095(0.04)a	0.076(0.02)a	0.078(0.03)a	0.081(0.01)a	0.081(0.01)a	0.074(0.04)a
Root:Shoot ratio	1.663(0.498)a	1.235(0.327)a	2.901(1.006)a	1.149(0.169)a	1.722(0.703)a	1.768(0.717)a
A. thaliana						
Lateral Root Number	5.00(1.41)a	3.25(1.70)a	2.75(1.50)a	4.00(1.41)a	3.50(0.58)a	3.00(0.82)a
Number of Tips	196.30(103.83)a	222.00(22.31)a	86.50(19.43)a	146.30(71.22)a	214.00(165.52)a	79.50(39.95)a
Root Volume (cm^3)	0.0018(0.0005)a	0.0018(0.0005)a	0.0015(0.0006)a	0.0013(0.0005)a	0.0015(0.0006)a	0.001(0.0000)a
Average Root Diameter (mm)	0.1256(0.012)a	0.1323(0.009)a	0.1444(0.13)a	0.1418(.013)a	0.1356(0.013)a	0.1491(0.012)a

Table 4
Shoot traits of the six treatments 10d after germination. Numbers in brackets are standard deviation of the mean. Different letters indicate that the means are significantly different ($P < .05$). n = 4.

Variable or Parameter	Unstructured Soil			Structured Soil		
	L (Loose)	LC (Lightly compacted)	HC (Highly Compacted)	L (Loose)	LC (Lightly Compacted)	HC (Highly Compacted)
Bulk Density (g cm^{-3})	1.25	1.40	1.55	1.25	1.40	1.55
H. vulgare						
Shoot Height (cm)	11.10(0.983)a	9.15(1.678)a	9.38(1.153)a	11.98(1.209)a	7.83(4.020)a	10.38(1.43)a
Shoot Biomass (mg)	0.173(0.475)a	0.142(0.580)a	0.123(0.024)a	0.222(0.594)a	0.139(0.110)a	0.168(0.040)a
Chlorophyll Content (dualex)	20.413(4.556)a	20.975(2.751)a	18.862(2.257)a	23.738(3.434)a	20.975(4.681)a	18.800(2.052)a
Nitrogen Balance Index (dualex)	30.30(5.553)a	29.95(3.699)a	26.41(4.158)a	30.41(2.356)a	29.04(6.248)a	28.20(2.179)a
Number of Leaves	2(0.000)a	2(0.000)a	2(0.000)a	2(0.000)a	2(0.000)a	2(0.000)a
Flavonoid content (dualex)	0.665(0.036)a	0.718(.030)ab	0.708(.033)ab	0.776(.062)ab	0.717(.030)b	0.670(0.064)a
P. sativum						
Shoot Height (mm)	55.25(3.59)a	53.75(9.78)a	32.75(6.60)b	65.25(12.69)a	49.25(9.75)ab	46.75(5.85)ab
Shoot Biomass (mg)	0.469(105)ab	0.480(.137)ab	0.195(0.063)c	0.588(.029)a	0.401(0.134)abc	0.322(0.068)bc
Chlorophyll Content (dualex)	33.98(3.90)ab	35.64(3.05)ab	n/a	30.33(3.13)a	35.04(1.57)ab	39.19(3.18)b
Nitrogen Balance index (dualex)	37.26(2.10)a	41.13(3.45)ab	n/a	37.08(3.27)a	44.96(3.32)ab	48.27(7.29)b
Number of Leaves	6.50(1)a	6.50(1)a	4.25(3.1)a	8.50(1)a	7.00(2)a	5.50(2)a
Flavonoid content (dualex)	0.91(0.059)a	0.87(0.079)a	n/a	0.83(0.039)a	0.78(0.020)a	0.81(0.059)a
A. thaliana						
Shoot Height (mm)	2.00(0.000)a	2.25(0.500)a	1.63(0.479)a	2.00(0.816)a	2.00(0.816)a	1.75(0.500)a
Shoot Biomass (mg)	n/a	n/a	n/a	n/a	n/a	n/a
Chlorophyll Content (dualex)	n/a	n/a	n/a	n/a	n/a	n/a
Nitrogen Balance Index (dualex)	n/a	n/a	n/a	n/a	n/a	n/a
Number of Leaves	6.0(0.000)a	6.0(0.000)a	6.0(0.000)a	5.5(1.000)a	6.0(0.000)a	5.5(1.000)a
Flavonoid content (dualex)	n/a	n/a	n/a	n/a	n/a	n/a

2000; Valentine et al., 2012; Gao et al., 2012). As they use mostly uniform soft soil or uniform hard soil (Stirzaker et al., 1996) structured conditions of the soil in the field are overlooked. However, homogeneous soil tested in the laboratory has the advantage of good reproducibility of the growth environment and the removal of secondary artefacts that could affect differently structured or compacted soils collected from the field (e.g. microbial communities, nutrients). Artefacts were removed in this experiment by starting with homogeneous soil that was packed differently to create a more complex pore structure containing greater macropores. We have shown that at the same density and compaction, soil structure can create vastly different physical conditions for root growth, likely by providing preferential pathways for root growth (Stirzaker et al., 1996; Passioura, 2002; Islam et al., 2021) that are not present in structureless soil exerting the same mechanical impedance to the root systems in the bulk soil. It has been shown that roots are able to sense soil physical conditions and thus send inhibitory signals to the shoot to prepare the plant against a deteriorating environment, a behaviour known as feedforward. These inhibitory signals can affect stomatal conductance, cell division, cell expansion and the rate of leaf appearance above ground, and root growth below ground

(Aiken and Smucker, 1996; Passioura, 2002).

Although the average penetrometer resistance was similar between structured and unstructured soils at the same density, it ranged from 0.46–1.25 MPa in structured treatments compared to 0.40–1.08 MPa in unstructured treatments. For this experiment, greater variability in penetration resistance was expected in the structured treatments as the cores were compacted prior to the formation of aggregates and subsequent packing (Fang et al., 2018) to create a heterogeneous structure. Variability in observed penetrometer resistance within structured soils was due to localised areas of high-density soils caused by the aggregates, with the needle sometimes penetrating through aggregates and alternately macropores. The largest penetration resistance measurements indicate limiting mechanical conditions for root growth, which occur at $>$ 0.8–2 MPa, dependent on species, with root elongation typically halved beyond 2 MPa (Bengough et al., 2011).

A decrease in macroporosity was also expected with increasing bulk density (Colombi et al., 2017). At 1.25 g cm^{-3} bulk density, structured soil had a decrease in macroporosity because the aggregates were compacted before being repacked into structured soil creating aggregates devoid of macropores (Table 2). This decrease in macroporosity was not the case when bulk density increased to 1.55 g cm^{-3} because compaction created fewer macropores in the unstructured soil, whereas in structured soil the aggregates retained macropores between them.

Mechanical impedance was found to be one of the soil properties limiting root growth, with macropores appearing to alleviate the influence of increased soil bulk density by providing less resistant growth pathways. Another limiting factor to root growth was likely air-filled porosity. At the − 20 kPa water potential used for plant growth, the air-filled porosity for 1.55 g cm^{-3} bulk density in both structured and unstructured was 8%, which is below the 10% air-filled porosity threshold considered to be limiting to root elongation (Bengough et al., 2011a; Valentine et al., 2012). However, the 10% air-filled porosity is simplistic and does not factor in pore connectivity that affects aeration (Rabot et al., 2018). Air-filled porosity can be different to soil aeration. It has been proven that larger pore diameters with the same air-filled porosity will result in different air permeability, which is directly related to the diameter of the air-filled pores (Stepniewski et al., 1994; Lipiec and Hatano, 2003). At the same level of compaction, coarser structure increases air permeability (Lipiec and Hatano, 2003). The increase in pore size in the structured soil may have improved soil infiltration and oxygen exchange as previously shown by McCourty et al. (2018) and thus providing a more favourable condition for roots (Valentine et al., 2012; Cook et al., 2013; Colombi et al., 2017; McCourty et al., 2018).

4.2. Root growth response to treatments and changes in root morphology

Pea root growth benefited from a heterogenous soil structure at 1.25 g cm^{-3} and 1.55 g cm^{-3} bulk densities, but not at 1.40 g cm^{-3} bulk density. The significant increase in root growth in the structured soil at 1.25 g cm^{-3} compared to the unstructured soil at 1.25 g cm^{-3} can be explained by a better root-soil contact, improved by the slight increase in compaction (15% increase in penetrometer resistance) (Veen et al., 1992), but also by the improvement of the pore size distribution and the complexity of the heterogeneous media (Nimmo, 2004; Suzuki et al., 2007). It has been shown that some species, such as *N. tabacum*, can benefit from moderate compaction stress and improve plant growth at bulk densities of 1.40 g cm^{-3} due to better root-soil contact, with light compaction improving hydraulic conductivity (Alameda and Villar, 2009; Alameda et al., 2012; Rivera et al., 2019). The doubling of pea root length at a soil bulk density of 1.55 g cm^{-3} in structured compared to unstructured soil could be explained by the increase in macroporosity and beneficial changes in pore size distribution, since bulk density, total porosity, air-filled porosity, and volumetric water content were the same in both unstructured and structured soil (Table 2). Increased macroporosity has been shown to play an important role in increasing oxygen availability and soil infiltration with larger soil pores providing pathways that allow root, water and air penetration (Allaire-Leung et al., 2000; Lipiec and Hatano, 2003; Anderson et al., 2010; Rab et al., 2014). Passioura (2002) showed how plant roots can sense their environment and as such adapt their growth through the most favourable zones in the soil Fig. 5.

Arabidopsis roots grew better in the homogenous soil, particularly in soil packed at 1.40 g cm^{-3}. This treatment had a large number of pores between 60–200 μm, which was similar to the average Arabidopsis root diameter being ~130 μm. Homogeneous soils packed at 1.25 g cm^{-3} and 1.40 g cm^{-3} provided the plant with optimal water content, with very little compaction, good porosity, and a better root-soil contact due to the smaller nature of pores. A better root-soil contact improves water and nutrient uptake by plants (Rivera et al., 2019). Observed detrimental effects of a heterogenous soil structure in Arabidopsis root growth may be associated with poor root-soil contact as a consequence of the small size of the roots when compared to the other two species, typically observed to be 3 to 7 times thicker, and slightly increased penetrometer resistance in structured soil (Materechera et al., 1992; Place et al., 2008). Evidence suggests that poor root-soil contact in higher porosity soils can have an adverse effect on plant growth, with best results in lightly compacted soils with an intermediate porosity (Veen et al., 1992). Varieties like peas, lupin and safflower, with thicker roots, have been shown to grow better in compacted soils than varieties with thinner roots, like wheat, barley and ryegrass (Materechera et al., 1992).

Arabidopsis, barley and pea root morphology was not different in structured and unstructured treatments, but it did change with changes in soil compaction. At higher compaction levels, average root diameter increased (Materechera et al., 1991; Lipiec et al., 2012) and lateral roots decreased in both structured and unstructured soils as expected. In barley, structured soil provided an increase of 280% in the lateral root number when compared to unstructured soil at 1.55 g cm^{-3}, although the difference was not significant, this suggests structure did improve soil exploration by the roots. Lateral roots increase the volume of soil reached by the root, thus benefiting water and nutrient uptake (Dubrovsky and Laskowski, 2017). It has been shown that lateral roots in young barley can contribute between 25% and 60% of the total root water uptake this highlights the importance of lateral roots for maintaining plant water status (Schneider et al., 2020). At 1.55 g cm^{-3} unstructured soil barley lateral root number was highly variable. In peas, the structured soil had 66.6% more lateral roots than the unstructured soil at 1.55 g cm^{-3}. The increase of lateral roots in structured soil could be due to the bigger pore size in structured soils, as suggested by field studies that have found plant roots to be attracted by macropores in unfavourable plough pans (Stirzaker et al., 1996; White and Kirkegaard, 2010; Pfeifer et al., 2014). The increase of macropores in structured soil could have improved oxygen diffusion, microbial activities, and biochemical reactions, which could benefit lateral roots (Horn and Smucker, 2005; Bauke et al., 2017).

4.3. Plant vigour

Soil compaction has been shown to affect shoot growth, and parameters of plant vigour such as reduced plant height, leaf discolouration, reduced leaf gas exchange, and an increase in stomatal resistance, resulting in a decreased crop yield (Monteith et al., 1965; Lipiec and Hatano, 2003; Saud et al., 2017). The interaction of the root system with the soil structure was reflected positively in pea plant vigour of treatments with bulk density of 1.55 g cm^{-3}, pea shoot height observed a 42.7% increase in structured soil. Shoot biomass was also higher in structured soil packed at 1.55 g cm^{-3}, with an increase of 65.1% compared to unstructured soil. This increase in shoot height and shoot biomass due to a heterogeneous soil structure could be due to the increase in total root length resulting from paths of least resistance presented by macropores, but also due to more available water and oxygen

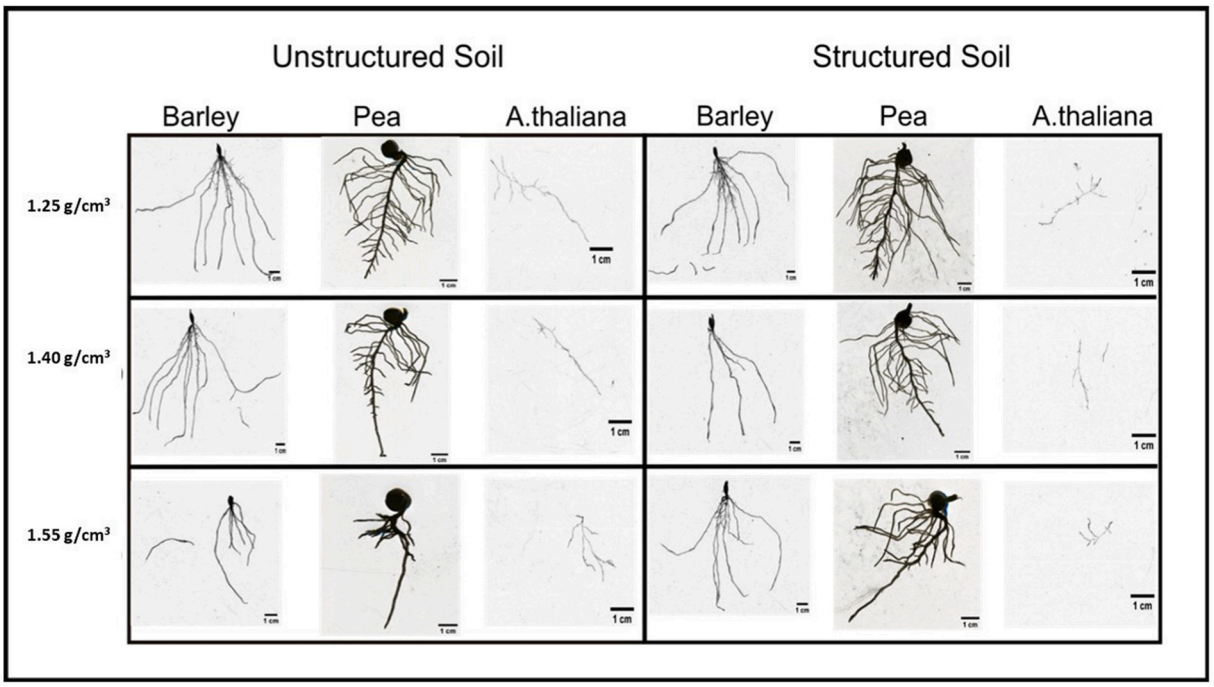

Fig. 5. Barley, Pea and Arabidopsis, 2D root images of the different treatments bulk density x soil structure.

to the plant through bigger soil pores, allowing the roots to elongate and explore the soil better. Colombi et al. (2017) found that the axial and lateral root number were highly correlated to plant height in compacted soils and when there is an increase in bulk density, more axial roots were needed to maintain shoot growth. In barley and Arabidopsis there was no apparent effect on above ground parameters due to soil structure, but this may be due to the plants being only 10d old. Plant vigour measurements should be further investigated in older plants. The increase in chlorophyll content at 1.55 g cm^{-3} structured soil in peas, when compared to 1.25 g cm^{-3}, could be explained by peas being able to adapt chlorophyll content under stressful conditions. It has been shown that within certain genotypes, peas are able to maintain, or even increase, their chlorophyll content under stress conditions (Sanwal et al., 2018) (Table 4). Plants have the ability to adapt to certain stresses and environments (Mareri et al., 2022) and it has been commonly considered that plants can adjust chlorophyll content in order to adapt and optimize photosynthesis (Li et al., 2018; Agathokleous et al., 2020).

5. Conclusions

Structured soil provided roots of peas and barley with a more favourable soil environment to grow in when compared to the typical uniformly hard soil used in laboratory studies of root growth. Peas benefitted the most from a heterogeneous soil structure when compared to barley. Peas exhibited increased shoot height and biomass in the most compacted structured soil with a bulk density of 1.55 g cm^{-3}, showcasing the positive correlation between root system interaction with soil structure, enhanced macroporosity, and improved plant performance. Conversely, soil structure had an adverse effect on Arabidopsis, likely due to the smaller diameter and length of the roots compared to peas and barley.

Our study demonstrates that root growth is influenced by both soil bulk density and pore structure heterogeneity, and these effects vary among plant species. The findings emphasize that relying solely on simple measurements such as bulk density or penetrometer resistance is inadequate for describing the complex physical conditions influencing root growth. In natural field soils, characterized by heterogeneity and aggregation, plant roots encounter a diverse spatial environment, influenced by factors like water content, oxygen availability, and porosity. Further research exploring these parameters, as well as more mature plants would be helpful to determine the effects on aboveground parameters. Furthermore, mature plants with a more developed root system will also make it possible to quantify the root-soil-contact by X-Ray CT, better highlighting differences between structured and unstructured soil for each plant species. Further studies should also consider the analysis of pore connectivity and tortuosity which highly influence oxygen and water content in the soil.

Key takeaways include the species-specific response of roots to soil structure, the significance of macropores in enhancing root growth, and the need for a comprehensive understanding of soil dynamics. Future studies should make use of laboratory manipulated structured soil to provide a more realistic and holistic approach when studying soil compaction in laboratory settings and a greater emphasis on pore size distribution, which can influence oxygen and water availability, but also macropores which can provide roots with paths of least resistance.

CRediT authorship contribution statement

Hallett Paul D.: Resources, Supervision, Writing – review & editing. **Giuliani Licida M.:** Data curation, Writing – original draft. **Loades Kenneth W.:** Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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