Final Report

Management of Rotations, Soil Structure and Water

(Rotations Research Partnership)

Work package 4 (WP4):

Linking Soils, Water and Roots to Productivity

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Overall co-ordination	Marc Allison NIAB
Development of DNA root assays	Marc Allison (NIAB)
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2. REPORTING OF WORK PACKAGE 4

The AHDB Project Management of Rotations, Soil Structure and Water (Project 91140001) comprised four interlinked work packages (WPs) designed to achieve the project's objectives. WP1 included project management and knowledge transfer but also the gathering and analysis of survey data from collaborating growers; the reinstatement of a long-term, rotational experiment at Broom's Barn, Suffolk and conducting some replicated experiments investigating composts and cover crops. The main objective of WP2 was to investigate the use of spatial information (e.g. maps of cereal and potato yields or of soil properties) to define higher and lower yield zones within fields which may then be used to improve crop management practices. In addition, this WP investigates novel scanning technologies to better understand the dynamics of soil organic matter. Much of the experimental work with cover crops and soil amendments were done in WP3 and a further output from this WP was decision support tools to aid management of both soil structure and organic matter content. WP4 will investigate novel method to quantify root distribution and the effects of soil conditions and crop management on root function and crop productivity.

For simplicity, the key finding of WP4 will be discussed in this report. Similarly, background literature, conclusions, appendices will also be reported here. However, practical recommendations from the whole project will be synthesised and reported in the project summary report.

2.1. Areas of work

The work package comprised three main areas of work:

1. Using DNA technology to quantify potato root density (NIAB).
2. Development of model of root penetration resistance (The James Hutton Institute).

3. DEVELOPMENT OF A DNA BASED ASSAY FOR QUANTIFYING ROOT DISTRIBUTION IN POTATO AND OTHER ROOT CROP

3.1. Materials and Methods

3.1.1. Root sampling and sub-sampling

On 17 August 2016, soil samples were taken from potato, carrot and parsnip fields farmed by WO & PO Jolly, Roudham, Norfolk. In the potato field the soil was sampled at 10 representative locations whereas in the carrot and parsnip fields a single location was sampled. At each sample location a rectangular ('brick') steel corer (10 × 10 × 20 cm) was inserted into the soil to take intact cores from 10-20, 20-30 and 30-40 cm in the centre of planted rows and a single sample (0-10 cm) was taken from the adjacent tractor wheeling. In the potato field, four "control" samples (10-20 cm) were taken from the field margins in areas assumed to contain no potato root. Each sample was then split in half and weighed. One sub-sample was then allocated at random to have its root content measured by washing and root counting using the method of Tennant (1975). The other sample was sent to NIAB to have root length determined using an experimental method using DNA probes. In total there were 63 paired samples.

3.1.2. Quantification of roots using DNA based technology

3.1.2.1. DNA extraction from soil samples

The fresh field soil crores were stored were stored frozen (-18°C) before analysis. Prior to DNA extraction the soil was first dried at 30°C in a re-circulating oven for a minimum of 72 hours. The dried soils were then milled to a fine powder using a Humboldt H4199.5F soil mill fitted with a 2mm screen. DNA was extracted from each sample in duplicate using a PowerSoil kit (MO BIO Laboratories, Inc., Carlsbad, USA.) in accordance with the manufacturer's protocols; thus two technical replicates were obtained for each milled sample. This kit has been shown to achieve equivalent DNA yields from soil as commercial soil extraction methods (Haling et al., 2011).

3.1.2.2. Preparation of calibration materials

DNA was extracted from samples of 100 mg dried potato haulm in accordance with Tanksely's method and the extracted DNA re-suspended in 100 µl TBE buffer. Calibration standards were prepared by a series of ten-fold dilutions from this primary reference.

3.1.2.3. Quantification of potato root DNA

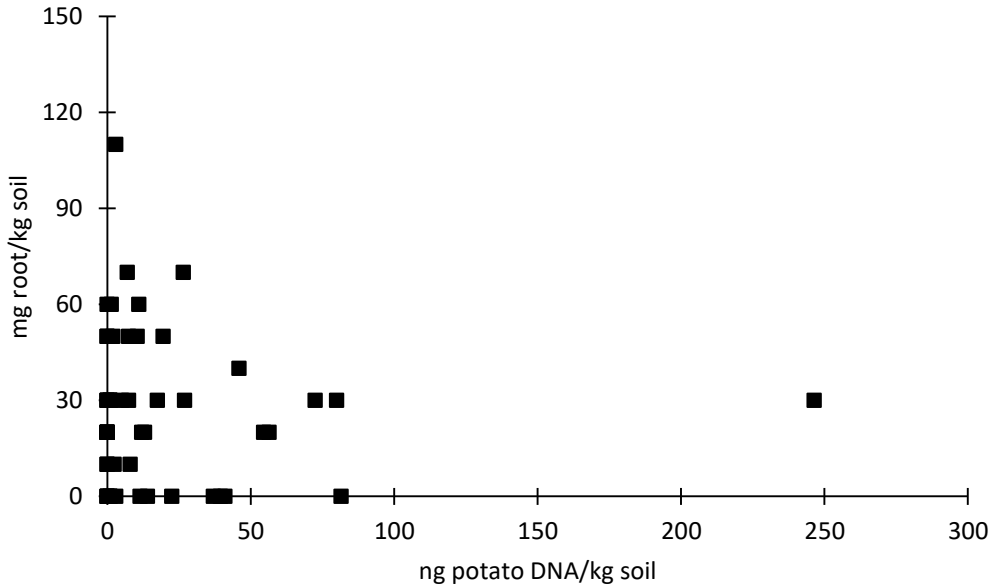
Potato DNA in the soil extracts was quantified by real time PCR using an ABI 7900 with triplicate 6 µl reactions comprising 1.0 µl template from soil extract, 0.5 µl primers with primers and probes at 5 mM, 2.5 µl Thermo Fisher Scientific ABsolute Blue qPCR ROX Mix and 2.0 µl water. Amplification was conducted using 10-minute activation at 95°C followed by 40 cycles of 15s at 95°C then 60s at 60°C, monitoring fluorescence at each cycle. The primers and probes targeted the potato internal transcribed spacer region within the 5.8S ribosomal RNA gene. The primers and probes were designed using Primer3 (Untergrasser et al., 2012). All qPCR data were processed using Applied BioSytems SDS 2.2 and the results collated and analysed in Microsoft Excel. All statistical analyses were conducted on raw data without prior averaging of technical replicates.

Root data derived from the DNA method and the traditional washing and counting method were both converted to amounts per kg dry soil and the comparison between the two methods is shown in Figure 1.

3.2. Results and Discussion

The correlation between the two methods was poor and not statistically significant (**Figure 1**). It was particularly concerning that when no potato root DNA was detected in the sample, root weight could vary from 0 to *c*. 110 mg/kg. At present it is unknown why the DNA method was such a poor predictor of potato root mass but after consultation it was felt that this problem was unlikely to be resolved within the life-time of this project.

Figure 1. Relation between weights of dried root obtained from washing and the amount of potato root DNA in paired soil samples



4. MODELS OF ROOT PENETRATION RESISTANCE

4.1. Introduction

Multispectral imaging has previously mainly been used in terms of remote sensing using satellite or drone imagery. However, this project proposed to evaluate soils at a scale relevant to the interaction of plant roots and soil. Root elongation at the early stages of crop development can be significantly impeded in soil with high bulk density, but differences in pore structure between soils of the same density can alleviate or worsen the effects of soil strength (Valentine *et al*., 2012). Once a crop is established, unless roots continue to elongate, the size of the root system will affect access to water depending on the relationship between soil water pools and root soil contact. The exact relationship between ability to elongate in drying hard soils and the root soil contact, will thus depend on differential root traits (e.g. root diameter) that vary significantly within and between cereals and root crops such as potato and carrot/parsnips but also on the pore structure of the soil developed due to underlying soil properties and the management used. Previously work with barley, has shown the influences of soil strength and pores size distribution on root elongation and had shown significant reduction in root elongation in soils both across the landscape scale in Scotland, and in soil cores taken from different tillage systems, within field trials across the UK (McKenzie *et al.*, 2017; Valentine *et al.*, 2012), This project therefore aimed at assessing the initial root growth characteristics of carrot and parsnip roots, in structured soil. This required the initial assessment of root growth under controlled mono conditions, the development of a soil-based root elongation assay, followed by testing of root elongations under heterogenous structured soil conditions, all under controlled water conditions.

4.2. Material and Methods

4.2.1. Soil preparation

Soil samples were prepared as above for soil core imaging, after imaging, the soil halves were realigned to re-instate the 10cm high core status. Water content was then adjusted to the required level for root elongation assays. Unless stated otherwise, cores were adjusted to a matric potential of -50kPa.

4.2.2. Germplasm

Carrot and Parsnip seeds were obtained from NIAB, or from suppliers (e.g Elsoms seeds). Obtained seeds included some with or without seed coatings. For root growth experiments, seeds were initially soaked to remove seed coatings, then were pregerminated after treating with gibberellic acid (GA). After soaking for 6-8 hours, seeds were transferred to damp filter paper in petri-dishes and incubated for 3 days prior to transfer to soil cores/ or blue germination paper. Only seeds that had visibly germinated were transferred.

4.2.3. Root elongation experiments

Two root growth assessment methods were used. Firstly, seedlings were grown on blue germination paper. The moisture content of the filter paper was adjusted to the required Matric potential by saturated the sheets of paper and placing them on a tension table to reduce the water content to the required level. Sheets were placed in Perspex imaging container, with 10 pre-germinated seeds aligned approximately 2cm from the top ridge of the container (Bengough

et al., 2004). These chambers have previously been used with gel as the growth medium, however here they were used with filter paper filling. Seedling growth chambers were sealed and incubated at 15^{o}C. Seedlings were imaged using a flatbed scanner at 7, 14 & in some cases 21 days. For soil grown plants, soil was sampled from mid Pilmore, at the James Hutton site Invergowrie, Dundee, Grieves House Tillage Trial (GHTT) & Centre for integrated cropping (CSC), Balruddery farm, Dundee. For the initial soil base experiment Pilmore soil was mixed 50:50 with sand and packed at specific dry bulk densities and gMC in 5cm diameter x 10 cm high cores. For soil sampled from Tillage / management plots at the GHTT & CSC) repacked soil cores were packed at specific dry bulk densities (DBD) and, where necessary for validation or comparison, soil cores, were saturated and taken through partial water release curves. Finally, the matric potential of cores was adjusted to -50kPa. If cores were double height cores, the duct-tape was removed from the outside of the cores, and the cores were broken by pulling the core apart allowing the soil to fracture according to the soil structure, without sheer movement. After imaging cores, if the soil cores were part of a 10 cm high core pair cores were "re-joined" to re-create 10cm tall cores, and water adjusted to required MP, prior to addition of pre-germinated carrot or parsnip seeds. Five pregerminated seeds were sown into the tops of each core, by making five small holes one cm from the edge of the core evenly spread around the circumference. Soil was used to cover the seedlings, then cores were placed back into plastic bags, and transferred to a growth room at 15 °C, with 16hours light / 8 hours dark. Plants were grown for 14 days, after which, the cores were gently broken apart and roots extracted. Root lengths were measured manually with a ruler, and were also imaged, using a flatbed scanner.

Images were analysed using imageJ, using the segmented line to assess the root length, or using RootNAv (https://www.nottingham.ac.uk/research/groups/cvl/software/rootnav.aspx).

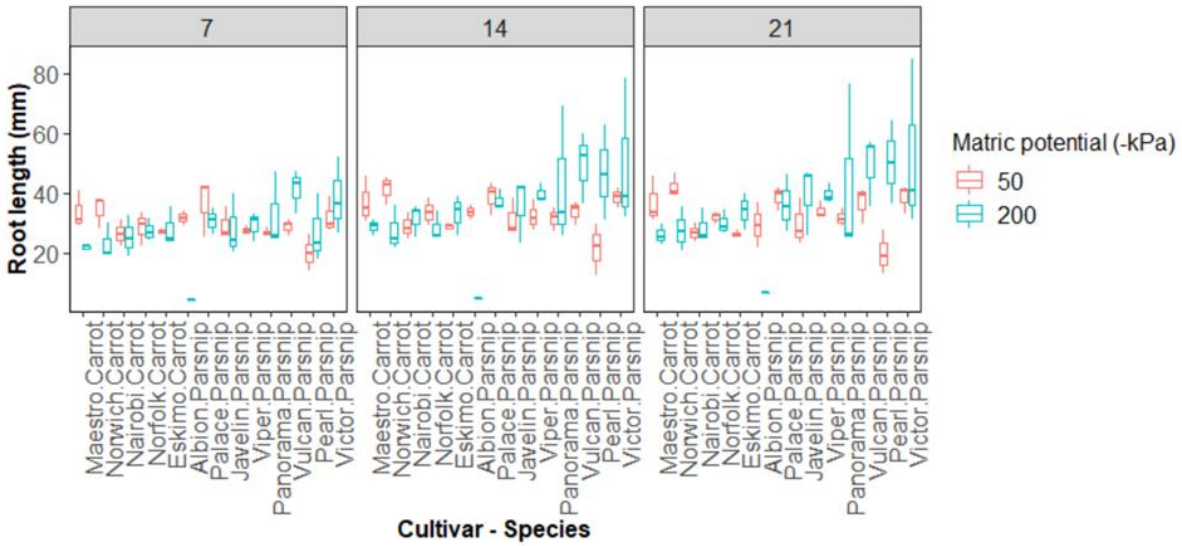
4.2.4. Statistical Analysis

All statistical analysis was performed in Rstudio running R.

4.3. Results

The root growth of four carrot and eight parsnip species was compared when grown on blue filter papers. Figure 2 shows the variation in root growth on 7, 14, and 21 days. Root tended to grow to a maximum length at 14days in these conditions. A model was fitted to assess the different behaviours of Carrots vs Parsnips and cultivar levels effects in response to differing water availability. Anova assessment of the final fitted model suggested effects of time, matric potential, variability in cultivars within species, variation in species responses to matric potential and variation in cultivars response to matric potential within species.

Figure 2. Analysis of root growth of Carrot and Parsnip species on germination paper equilibrated at two matric potentials over 21 days

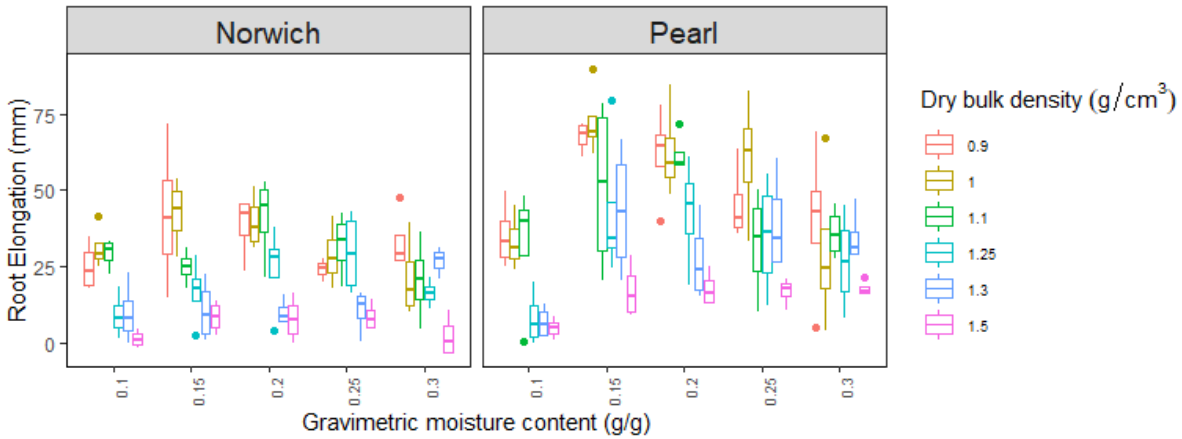


At the species level, Parsnips grew faster under conditions of (slight) water deficit (-200kPa) to compared to the Carrots. There were no differences at the -50Pa. (Table 1). While there were differences in the overall growth root length measured between cultivars within the root crop types, there was no evidence of differences in response to water availability (with respect to cultivar differences).

4.3.1. Cultivar response to soil physical conditions in reduced structure soil (Repacked – single field origin)

One variety each of Carrots and Parsnips with seeds sourced from two different suppliers were grown in repacked soil (mixed with sand) at six different DBD levels and 5 different gMC. Figure 3 shows the overall effect on the root length after 14 days growth. There were significant effects of Cultivar, dry bulk density, gravimetric water content, an interaction between the two soil properties (Table 2).

Figure 3. Root elongation over 14 days of Carrot (Norwich) and Parsnip (Pearl) in repacked soil at a range of Dry bulk density and gravimetric water content.



There was also a significant interaction between cultivar and moisture content, but not cultivar with dry bulk density. The highest DBD almost 100% inhibited the root growth of both varieties in the most inhibiting water content. Both varieties were significantly inhibited by the extremes of water content. However, the Parsnip cultivar overall had a high growth rate, growing at almost double the rate of the carrot variety Norwich in some treatments. Significant differences in root growth rate were found at 0.2,0.25 & 0.30 gMC averaged across all DBD treatment (Contrasts p =0.022, p=0.019, p=0.038 respectively).

Table 1. Anova (type 3) evaluation of root length growth data from filter paper grown Carrots and Parsnip species (multiple cultivars). Bold = p<=0.05

Parameter	Model_type	X.Intercept.	Species	Day	KPa	Specie Cultivar	Species Day	Species KPa	Day KPa	Species Cultivar Day	Species Cultivar KPa	Species Day KPa	Species Cultivar Day KPa
yeo.johnson("RootNav_Length_mm", 0.13)	Full	**0.000**	0.542	0.798	0.184	0.780	0.949	0.212	0.738	0.931	0.693	0.857	0.861
yeo.johnson("RootNav_Length_mm", 0.13)	Reduced	**0.000**	0.277	**0.000**	**0.013**	**0.001**		**0.003**					

Table 2. Model evaluation of the impact of gravimetric moisture content and dry bulk density on Carrot (Norwich) and Parsnip (Pearl) root elongation over 14 days in repacked (unstructured) soil.

Parameter	Model_type	X.Intercept.	Cultivar	DBD	poly(gMC,2)	Cultivar DBD	Cultivar poly(gMC,2)	DBD poly(gMC,2)	Cultivar DBD_poly. gMC_num..2.
Seedling_ELA_Ave	Full	**0.000**	**0.002**	**0.000**	0.129	0.085	0.113	0.195	0.302
Seedling_ELA_Ave	Reduced	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**	**0.000**	

4.3.2. Response of Parsnip to structured vs unstructured soil from Rotational trials

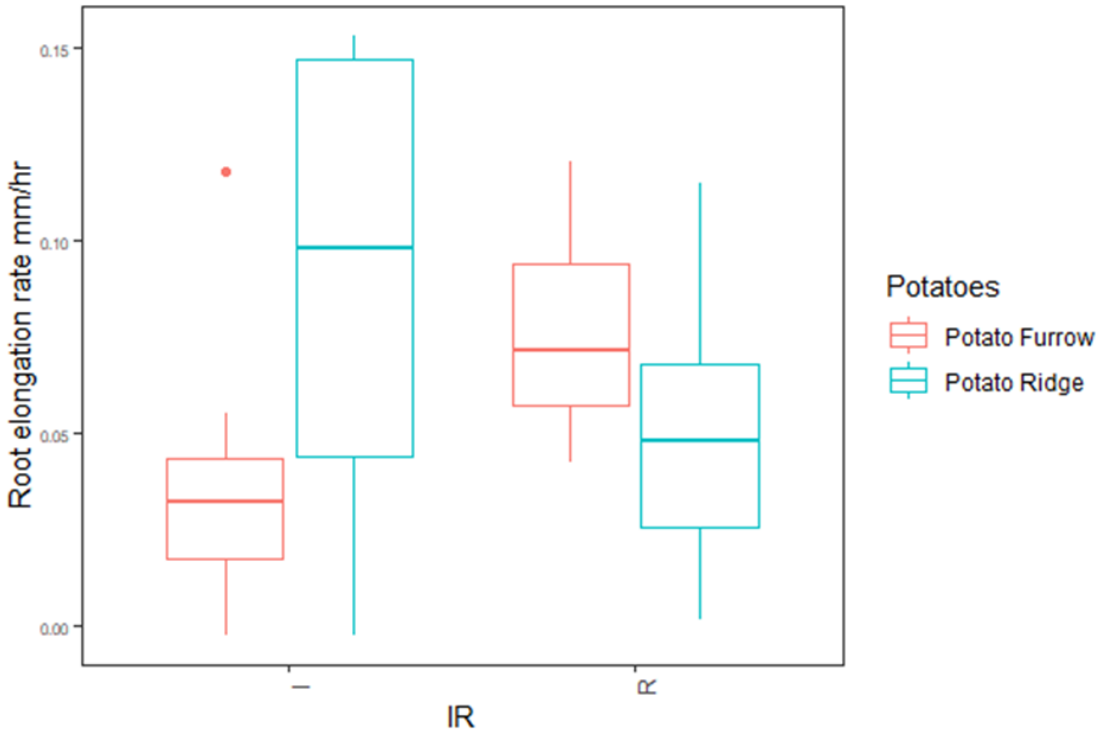
A comparison was made of the growth rate of a single parsnip cultivar in soil from two trials (Grieves House & CSC) under rotational management. The soil was adjusted to -50kPa prior to the seeds being transferred to the soil.

Baseline soil physical measurement showed impacts from Rotation, Tillage, soil either alone or as interactions (Appendix 1 Table 1). The soil management impacted the different soil parameter in different combinations. Based on reduced models only gMC, DBD and VWC was only impacted by repacking the cores ($p<0.001$, $p<0.001$, $p = 0.014$ respectively), DBD was impacted by interactions of repacking and Tillage management ($p = 0.021$), AFP and VWC were impacted by the repacking, crop within the rotation, Rotation and tillage as four-way interactions ($p = 0.006$, $p = 0.038$ respectively).

Root growth was linked to the Rotation ($P = 0.003$, contrast $p=0.010$ Spring - Winter) but not the Crops with the rotation or the Tillage. Averaged across the Tillage and crops with the rotation, root growing in the soil from the Spring Rotation grew 0.019 ± 0.007 mm/hr faster than those growing in soil form the winter rotation ($p = 0.010$). Interestingly there was no effect of the repacking of the cores on the root elongation rates.

At the CSC root growth differences were linked to effects of repacking the soil from the different fields and effects of the potato cultivation in the intact cores (contrast between Ridges & Furrows $p = 0.013$ Intact cores, Figure 4). There was no difference in root growth in the repacked cores between the ridges and furrow.

Figure 4. Figure CP_3 Root elongation rates in soil sample from the ridges and furrows of the potato field form the CSC. I = Intact soil cores, R = repacked soil cores.



The relationship between baseline soil physical conditions and root elongation rates were explored. The only significant relationship found was to the polynomial relationship with gMC ($p <= 0.001$, with a starting model including DBD, poly(gMC,2) & AFP, Table 3 and Table 4 for both Grieves House soils and the CSC soils.

The root elongation rate was also linked to the parameters extracted using image analysis. In an additive model, significant effects for Grieves House evaluation were found for the number of features ($p<0.001$), the total area of features ($p = 0.044$), the total perimeter of features ($p <=0.001$) and the kurtosis of the size of features ($p= 0.002$) (Table 5). For CSC significant effects were found for Total Area ($p<0.001$), Total Perimeter ($p<0.001$), Skew of the Perimeter histogram ($p<0.001$) and Kurtosis of the perimeter histogram ($p<0.001$) (Table 5). Overall, the reduced models accounted for (5.7 / 33), (29 / 41) & (9.5 / 48) % of the variation in the root growth rates (fixed effects only / full model) for the field cropping systems, soil physical baseline measurements, and structural variation models respectively for the GH samples, and (36 / 54), (5.8 / 23) & (19 / 37) % respectively for the CSC samples.

Table 3. Analysis of the relationship between soil physical baseline measurements and root elongation rates in structured and unstructured soil. (GH)

Parameter	Model	X.Intercept.	DBD	poly(gMC, 2)	AFP	DBD 2)	DBD	poly(gMC, 2)	DBD 2)
yeo.johnson("Ave_ER", -9.48)	Full	0.336	0.310	0.655	0.274	0.703	0.505	0.777	0.703
yeo.johnson("Ave_ER", -9.48)	Reduced	**0.000**		**0.000**					

Table 4. Analysis of the relationship between soil physical baseline measurements and root elongation rates in structured and unstructured soil. (CSC)

Parameter	Model type	X.Intercept.	DBD	poly(gMC, 2)	AFP	DBD poly(gMC, 2)	DBD AFP	poly(gMC, 2) AFP	DBD poly(gMC, 2) AFP
yeo.johnson("Ave_ER", -4.8)	Full	0.629	0.618	0.513	0.606	0.997	0.948	0.995	0.717
yeo.johnson("Ave_ER", -4.8)	Reduced	**0.000**		**0.016**					

Table 5. Analysis of the relationship between structural features obtained from image analysis and root elongation rates GH & CSC

	Parameter	Model_type	X.Intercept.	Features	Total_Area	Total_Perim	Mean_size	Mean_Perim	SD_size	SD_Perim	Skew_size	Skew_Perim	Kurt_size	Kurt_Perim
GH	yeo.johnson("Ave_ER", -3)	Full	0.222	**0.021**	**0.020**	**0.026**	0.329	0.531	0.893	0.805	0.281	0.207	0.055	0.099
	yeo.johnson("Ave_ER", -3)	Reduced	**0.008**	**0.000**	**0.044**	**0.001**							**0.002**	
CSC	yeo.johnson("Ave_ER", -3)	Full	0.436	0.235	**0.040**	0.094	0.956	0.689	0.856	0.595	0.937	0.453	0.869	0.209
	yeo.johnson("Ave_ER", -3)	Reduced	0.622		**0.001**	**0.001**						**0.000**		**0.001**

4.3.3. Cultivar responses to structured soils (including multiple water content)

4.3.3.1. Soil properties of the soils used

To assess the variation in responses of the cultivars to soil conditions in structured and unstructured soils models were fitted to data obtained from growing Carrot and Parsnip varieties in structured soil from rotational trials, at different water contents. Due to the number of cores and trial design it was not always appropriate to fit full interaction models as a starting point. The starting point models for each section are therefore shown as the full models in each of the tables below.

The variation in the soil physical properties for the soil cores used to assess root elongation across a range of Carrot and Parsnip varieties are shown in Figure 5. The soil physical indicators for the GH samples were affected by difference aspects of the soil management, including Rotation and Tillage, and as expected the post sampling treatment of repacking the soil and adjustments to water content (Table 6). Soil physical properties similarly varied significantly across the Field/Crops of the CSC (with the caveat of this not being true randomisation replication). There was also evidence of differences in the soil physical properties relating to the soil management in field and post sampling repacking (Table 7).

Figure 5. Soil physical status of soils used for root elongation experiments to evaluate differences in root growth responses. (A) Grieve's house, (B) CSC. (i) Dry bulk density, (ii) Penetrometer resistance (iii) Gravimetric water content, (iv) Air filled porosity.

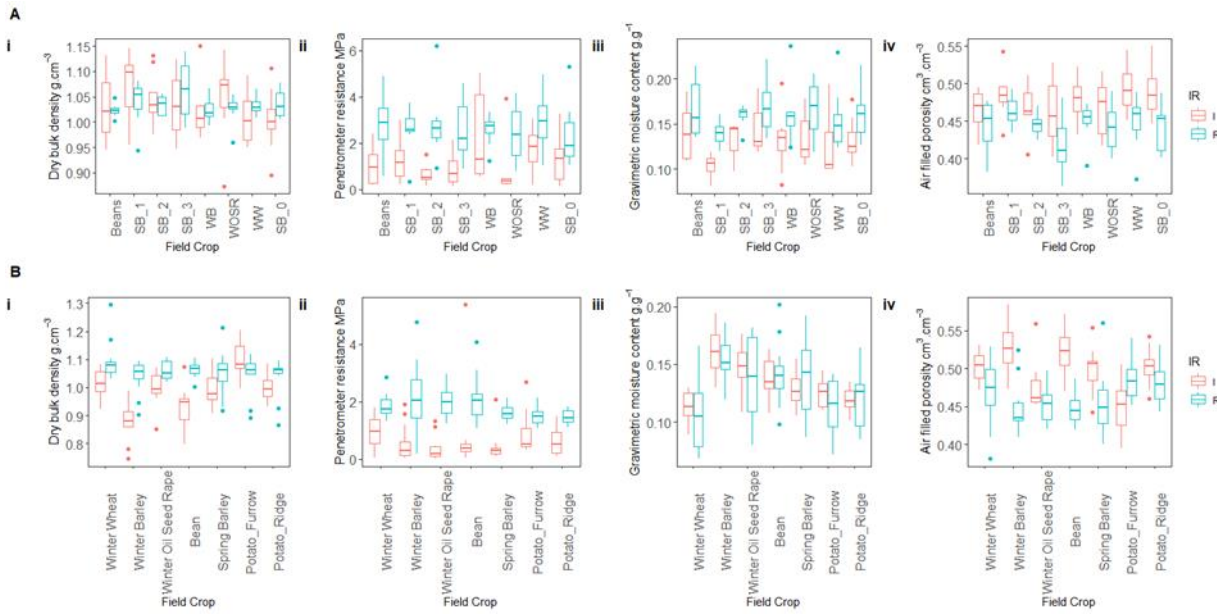


Table 6. Variation in the soil physical properties of the soil core used for evaluating differences in Carrot and Parsnip cultivar responses to structured soil (Bold = p<=0.05, R2m = marginal R^2 - (Fixed effects), R2c = conditional R^2 (Complete model), obtained from Grieves House Tillage Platform

Parameter	Model_type	X-Intercept	MP Cat	Tillage	IR	Rotation	MP_Cat...Tillage	MP_Cat...IR	Tillage...IR	MP_Cat...Rotation	Tillage...Rotation	IR...Rotation	MP_Cat.Tillage.IR	MP_Cat.Tillage.Rotation	MP_Cat...IR.Rotation	Tillage...IR...Rotation	MP_Cat...Tillage...IR...Rotation	R2m	R2c
DBD	Full	**0.000**	0.512	0.292	0.385	0.102	0.163	0.544	0.513	0.535	0.944	0.705	0.151	0.404	0.823	0.718	0.478	0.166	0.390
DBD	Reduced	**0.000**																**0.000**	0.257
yeo.johnson("gMC", -3)	Full	**0.000**	**0.000**	0.125	0.224	**0.044**	0.217	**0.015**	0.343	0.118	**0.024**	0.149	0.097	0.094	0.507	0.334	0.696	0.752	0.867
yeo.johnson("gMC", -3)	Reduced	**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.025**	**0.008**		**0.001**	**0.000**			**0.003**				0.741	0.848
yeo.johnson("PR", 0.2)	Full	0.418	0.235	0.113	**0.000**	**0.008**	0.412	0.920	**0.046**	**0.032**	0.078	**0.006**	0.545	0.219	**0.031**	**0.046**	0.058	0.598	0.625
yeo.johnson("PR", 0.2)	Reduced	**0.001**	**0.016**	0.194	**0.000**		**0.013**		**0.001**									0.481	0.481
AFP	Full	**0.000**	**0.004**	**0.042**	0.097	0.615	0.207	0.268	0.995	0.910	0.204	0.165	0.222	0.409	0.410	0.733	0.317	0.615	0.676
AFP	Reduced	**0.000**	**0.000**		**0.000**													0.536	0.618

Table 7. Variation in the soil physical properties of the soil core used for evaluating differences in Carrot and Parsnip cultivar responses to structured soil (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), obtained from Centre for Sustainable Cropping (CSC)

Parameter	Model_type	X.Intercept.	Field_Crop_type_Inc_Pots_Short	Tillage	IR	Field_Crop_type_Inc_Pots_Short...Tillage	Field_Crop_type_Inc_Pots_Short...IR	Tillage...IR	Field_Crop_type_Inc_Pots_Short...Tillage...IR	R2m	R2c
linkfun(DBD)	Full	**0.000**	**0.000**	**0.005**	**0.000**	0.298	**0.000**	0.848	0.092	0.563	0.571
linkfun(DBD)	Reduced	**0.000**	**0.000**	**0.008**	**0.000**		**0.000**			0.516	0.522
gMC	Full	**0.000**	**0.000**	**0.000**	0.700	0.096	0.294	**0.003**	0.269	0.503	0.513
gMC	Reduced	**0.000**	**0.000**	**0.000**	0.690			**0.006**		0.428	0.436
yeo.johnson("PR", -0.27)	Full	**0.000**	0.124	0.702	**0.000**	0.704	**0.008**	0.626	**0.022**	0.625	0.625
yeo.johnson("PR", -0.27)	Reduced	**0.000**	0.124	0.702	**0.000**	0.704	**0.008**	0.626	**0.022**	0.625	0.625
AFP	Full	**0.000**	**0.001**	0.158	**0.000**	0.471	**0.000**	**0.008**	**0.029**	0.536	0.542
AFP	Reduced	**0.000**	**0.001**	0.158	**0.000**	0.471	**0.000**	**0.008**	**0.029**	0.536	0.542

4.3.3.2. Effect of soil properties and cultivar on root elongation rates

The links between the root elongation rates in the soil samples were assessed over 14 days. Root elongation rates across the Trials and soil Field/Crop source of the soils are illustrated in Figure 6. Root elongation rates in the intact and repacked soils, were influenced by the cultivar and the gMC (Category) rather than the Tillage, Rotation or soil repacking process (IR) in the reduced models for the Grieves house soil cores (Table 8). In the CSC where all soils were adjusted to the same MP, root elongation rates were linked to cultivar, field/crop and soil repacking, again with no evidence of an effects of Tillage (Table 9).

Figure 6. Root elongation rates of the carrot and parsnip cultivars in soil cores from (A) GH, (B) CSC either intact or after repacking at constant DBD. GH samples include measurement across MP categories.

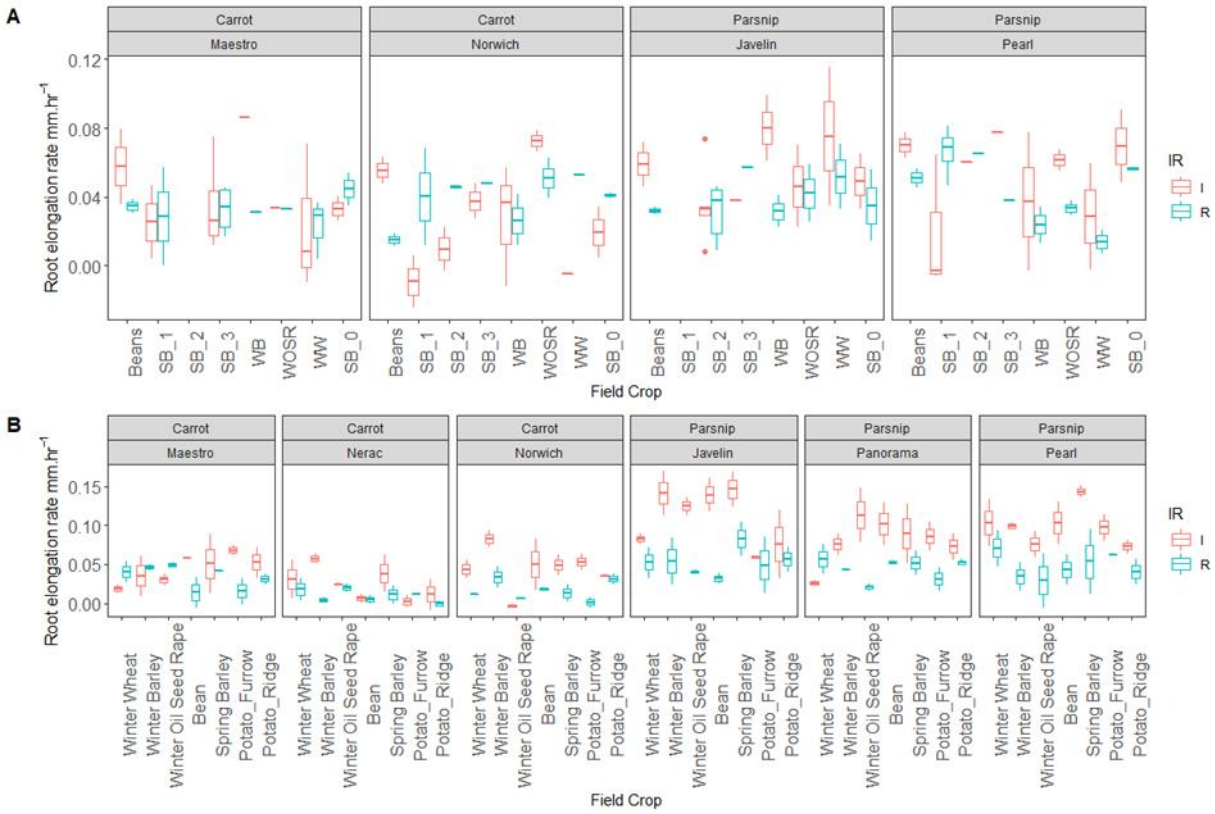


Table 8. Models of linkages between soil management and the root elongation rates of the Carrot and Parsnip (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), using soil obtained from GH

Parameter	Model_type	X.Intercept.	Apiaceae_Cultivar	MP_Cat	Tillage	IR	Rotation	Apiaceae_Cultivar...MP_Cat	Apiaceae_Cultivar...Tillage	Apiaceae_Cultivar...IR	Apiaceae_Cultivar...Rotation	R2m	R2c
Ave_ER	Full	**0.000**	0.133	0.575	0.709	**0.030**	0.055	0.055	0.699	0.072	**0.041**	0.430	0.430
Ave_ER	Reduced	**0.000**	**0.022**	**0.000**								0.269	0.269

Table 9. Models of linkages between soil management and the root elongation rates of the Carrot and Parsnip (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), using soil obtained from CSC

Parameter	Model_type	X.Intercept.	Apiaceae_Cultivar	Field_Crop_type_Inc_Pots_Short	Tillage	IR	Apiaceae_Cultivar...Field_Crop_type_Inc_P...	Apiaceae_Cultivar...Tillage	Apiaceae_Cultivar...IR	R2m	R2c
Ave_ER	Full	**0.000**	**0.000**	**0.003**	0.235	**0.000**	0.059	0.476	**0.000**	0.692	0.692
Ave_ER	Reduced	**0.000**	**0.000**	**0.022**		**0.000**			**0.001**	0.601	0.601

The root elongation rates were also explored in terms of interactions between cultivar and soil physical properties of the soil cores from GH and CSC (Table 10). Interactions / effects were found with all the soil physical properties measured in both trials either as main effects and as parts of interaction terms.

The interaction of the soil physical properties and the impact of cultivar on the root elongation rates were investigated for GH (Table 11). While in the GH soil cores, there was a strong interaction between the cultivar and gMC, for the CSC there were interaction between the cultivar and all the soil properties measured.

Table 10. Models of linkages between soil management and the root elongation rates of the Carrot and Parsnip (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), using soil obtained from GH & CSC

	Parameter	Model_type	X.Intercept.	DBD	poly.gMC..2..raw...TRUE.	AFP	PR	DBD...poly.gMC..2..raw...TRUE.	DBD...AFP	poly.gMC..2..raw...TRUE....AFP	DBD...PR	poly.gMC..2..raw...TRUE....PR	AFP...PR	DBD...poly.gMC..2..raw...TRUE....AFP	DBD...poly.gMC..2..raw...TRUE....PR	DBD...AFP...PR	poly.gMC..2..raw...TRUE....AFP...PR	DBD...poly.gMC..2..raw...TRUE....AFP...PR	R2m	R2c
GH	Ave_ER	Full	0.267	0.233	0.066	0.233	0.156	0.198	0.153	0.189	0.126	**0.025**	0.121	0.341	0.080	0.064	0.078	0.145	0.423	0.469
	Ave_ER	Reduced	0.789	0.778	**0.010**	0.871	**0.028**	**0.048**		**0.009**			**0.043**						0.351	0.373
CSC	yeo.johnson("Ave_ER", -7.33)	Full	0.568	0.460	0.100	0.435	0.395	0.193	0.169	0.144	0.318	0.221	0.298	0.396	0.126	0.147	0.098	0.243	0.275	0.275
	yeo.johnson("Ave_ER", -7.33)	Reduced	**0.006**	**0.006**	**0.009**	**0.006**	**0.007**	0.175		**0.023**	**0.007**	**0.029**	**0.007**		**0.008**				0.236	0.236

Table 11. Models of linkages between soil physical properties & cultivar and the root elongation rates of the Carrot and Parsnip (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), using soil obtained from GH

	Parameter	Model_type	X.Intercept.	Apiaceae_Cultivar	DBD	PR	poly.gMC..2..raw...TRUE.	AFP	Apiaceae_Cultivar...DBD	Apiaceae_Cultivar...PR	Apiaceae_Cultivar...poly.gMC..2..raw...TRUE.	Apiaceae_Cultivar...AFP	R2m	R2c
GH	yeo.johnson("Ave_ER", -9.31)	Full	0.399	0.947	0.412	0.213	0.172	0.400	0.930	0.344	0.725	0.941	0.534	0.574
	yeo.johnson("Ave_ER", -9.31)	Reduced	**0.007**	**0.038**	**0.007**	**0.001**	**0.005**	**0.009**			**0.001**		0.514	0.553
CSC	yeo.johnson("Ave_ER", -5.03)	Full	0.466	**0.028**	0.543	**0.005**	0.101	0.445	**0.022**	**0.028**	**0.008**	**0.038**	0.698	0.698
	yeo.johnson("Ave_ER", -5.03)	Reduced	0.466	**0.028**	0.543	**0.005**	0.101	0.445	**0.022**	**0.028**	**0.008**	**0.038**	0.698	0.698

4.3.3.3. Assessment of the interaction of cultivar image analysis obtained soil properties on root elongation rates

Finally, we explored whether the soil structural information obtained from the RGB image analysis of the soil cores obtained information that could predict root elongation rates (Table 12). It should be noted that this does not consider the gMC treatment over laid onto the GH samples. Evidence was found for difference linked to Cultivar and structural feature parameters including the Total perimeter, and mean perimeter. Interactions included those with the number of features, total area, total perimeter (in the CSC dataset), interactions between cultivar and mean perimeter and SD feature size in the Grieves House dataset. Across both trials interactions were found between Cultivar and Skew/Kurtosis of the size and perimeter parameters, that were significant across both sites.

Table 12. Table GH / CSC_RG_7 - Models of linkages between RGB image analysis extracted features & cultivars with relation to the root elongation rates of the Carrot and Parsnip (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), using soil obtained from GH

	Parameter	Model_type	X.Intercept.	Apiaceae_Cultivar	Features	Total_Area	Total_Perim	Mean_size	Mean_Perim	SD_size	SD_Perim	Skew_size	Skew_Perim	Kurt_size	Kurt_Perim
GH	yeo.johnson("Ave_ER" , 0.55)	Full	0.257	0.649	0.262	0.527	0.232	0.806	0.444	0.269	0.109	0.453	0.254	0.330	0.333
	yeo.johnson("Ave_ER" , 0.55)	Red.	0.463	0.927					**0.044**	0.334	0.075	0.421	0.189	0.368	0.334
CSC	yeo.johnson("Ave_ER" , -3)	Full	0.501	**0.000**	**0.006**	0.119	**0.001**	0.132	**0.020**	0.181	0.132	0.278	0.315	0.407	0.704
	yeo.johnson("Ave_ER" , -3)	Red.	**0.000**	**0.000**	0.202	0.306	**0.002**					1.000	0.926	0.700	0.365

Table 13. Table GH / CSC_RG_7 - Models of linkages between RGB image analysis extracted features & cultivars with relation to the root elongation rates of the Carrot and Parsnip (Bold = p<=0.05, R2m = marginal R2 - (Fixed effects), R2c = conditional R2 (Complete model), using soil obtained from GH (continued)

	Parameter	Model_type	Apiaceae_Cultivar...Features	Apiaceae_Cultivar...Total_Area	Apiaceae_Cultivar...Total_Perim	Apiaceae_Cultivar...Mean_size	Apiaceae_Cultivar...Mean_Perim	Apiaceae_Cultivar...SD_size	Apiaceae_Cultivar...SD_Perim	Apiaceae_Cultivar...Skew_size	Apiaceae_Cultivar...Skew_Perim	Apiaceae_Cultivar...Kurt_size	Apiaceae_Cultivar...Kurt_Perim	R2m	R2c
GH	yeo.johnson("Ave_ER", 0.55)	Full	0.348	0.884	0.679	0.954	0.853	0.291	**0.018**	**0.039**	**0.001**	**0.041**	**0.004**	0.386	0.386
	yeo.johnson("Ave_ER", 0.55)	Red.					**0.010**	**0.027**	**0.000**	**0.021**	**0.001**	**0.038**	**0.007**	0.343	0.343
CSC	yeo.johnson("Ave_ER", -3)	Full	**0.002**	**0.000**	**0.000**	0.405	0.158	0.123	0.204	**0.001**	**0.003**	**0.002**	**0.003**	0.735	0.735
	yeo.johnson("Ave_ER", -3)	Red.	**0.008**	**0.000**	**0.000**					**0.007**	**0.002**	**0.011**	**0.002**	0.671	0.671

5. DISCUSSION

Evaluation of the response of Carrot and Parsnip seedlings was undertaken initially on filter paper in ex-situ tests to establish a suitable seed germination protocol and the growing time that would be needed to assess differences in growth responses in soils. Differences in species level responses to water availability were found, with differences in overall growth rate of cultivars within species. Analysis of growth rates over a range of soil compaction levels and moisture contents also indicated an interaction at the Species level with gMC, but no interaction between the DBD and the Species. In this experiment there was only one representative of each cultivar per species, but each was represented by two lots of seeds from different sources. There was significant variation between packet source (data not shown), so further work is needed to assess the impact of seed quality and / or age on seedling establishment. Both these experiments however strongly suggested variation in the response to water availability between Carrot and Parsnips, with parsnips increasing their root growth at a faster rate than Carrots in optimal growth conditions.

Parsnips and Carrots were also sown into soil cores from two rotational trials (GH& CSC), both of which are under rotational management and with two types of soil management (Conventional plough vs Direct drill and Conventional vs Integrated management respectively). GH also has two types of rotation (Spring (with the soil exposed over winter) & Winter (with the soil covered for as much of the year as possible)). GH is fully replicated whereas CSC has a six-year rotation across six field such that in a single year crops and field function as confounding effects in models. When roots were assessed under a single water treatment root growth was associated with Rotation at GH, and Field/Crop x repacking in the CSC. It was also possible to show a link between root elongation rates and the potato furrow verse rows in the CSC samples. We also evaluated the relationship root growth, the soil physical measurements and some of the descriptors of soil properties obtained from the image analysis. Significant relationships were found between the root growth and the descriptors, with the overall percentage of variation accounted for varying between the two trials. There was also evidence of the direct effect of cultivar on the root elongation rates as well as interactions with the soil descriptors (particularly gMC), although there were interactions with other descriptors. It does however suggest that the image analysis extracted information from the RGB image analysis process can be as effective in predicting root elongation rates as information on the field trial design or basic soil physical measures. Further work is required to investigate if other combination of feature information may prove useful in following changes in rotations / management, across multiple years and their impact on root growth and yield. While there were some direct effects on the root elongation of the soil parameters, often interactions with cultivar were found (both within and between the Carrot and Parsnip cultivar grouping), the impact and extent of cultivar effects will also need further investigation.

6. CONCLUSIONS

- Variation in root responses to soil physical properties including DBD and water availability have been shown at the species and cultivar level for Carrot and Parsnips.
- In intact field soils root elongation was associated most strongly with the gravimetric water content of the soils, suggesting these small seedlings are more vulnerable to changes in water than to the variation in DBD.
- Features obtained from the image analysis of the soil cores using the RGB method were linked with the root elongation rates of the cultivars, including interactions between cultivars and the parameters, suggesting cultivars are highly sensitive to changes in soil structure.

7. REFERENCES

BENGOUGH, A.G., GORDON, D.C., AL-MENAIE, H., ELLIS, R.P., ALLAN, D., KEITH, R., THOMAS, W.T.B. & FORSTER, B.P. (2004). Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant and Soil* **262**, 63–70.

MCKENZIE, B.M., STOBART, R., BROWN, J.L., GEORGE, T.S., MORRIS, N., NEWTON, A.C., VALENTINE, T.A. & HALLETT P.D. (2017). Platforms to test and demonstrate sustainable soil management: integration of major UK field experiments. *AHDB Final Report RD-2012-3786*. AHDB, Stoneleigh.

TENNANT, D. (1975). A test of a modified line intersect method of estimating root length. *Journal of Ecology*, **63**, 995-1001.

VALENTINE, T.A., HALLETT, P.D., BINNIE, K., YOUNG, M.W., SQUIRE, G.R., HAWES, C. & BENGOUGH, A.G. (2012). Soil strength and macropore volume limit root elongation rates in many UK agricultural soils. *Annals of Botany* **110**, 259–270.

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