The effect of tillage depth and traffic management on soil properties and root development during two growth stages of winter wheat (*Triticum aestivum L.*)

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9 Abstract

10 The management of agricultural soils during crop establishment can affect root development by changes to soil 11 structure. This paper assesses the influence of tillage depth (250 mm, 100 mm & zero) and traffic management 12 (conventional type pressure, low type pressure & no traffic) on wheat root system architecture during winter wheat 13 (Triticum aestivum L.) tillering and flowering growth stages (GS) on a long-term tillage trial site. The study 14 revealed that zero-tillage systems increased crop yield through significantly greater root biomass ($P \le 0.001$), root length density and deeper seminal rooting analysed using X-ray Computed Tomography (CT) (P < 0.001) 15 16 compared to trafficked treatments. In general, conventional pressure trafficking had a significant negative 17 influence on crop yield (P < 0.01), root development (0.001), bulk density (P < 0.05) and total soil porosity (P < 0.05) 18 0.05) of deep and shallow tillage conventional pressure systems compared no traffic zero and deep tillage systems. 19 Visual improvements in soil structure under zero tillage may have improved crop rooting in zero tillage treatments 20 through vertical pore fissures (biopores), enhancing water uptake during the crop flowering period. This study 21 highlights the increasing implications of soil structural damage on root system architecture created by machinery 22 trafficking in crop production. Although tillage method was less important, the constricted root systems were 23 more pronounced in conventional pressure shallow tillage and deep tillage systems, emphasizing the importance 24 of using controlled traffic farming methods to improve soil management and reduce the trafficked areas of 25 agricultural fields.

26 1. Introduction

27 Soil resources are under significant pressure from anthropogenic activities especially conventional tillage. The 28 resulting soil degradation has significant implications for food security globally (Lal, 2010). Changing weather 29 patterns from prolonged rain to drought periods are being experienced on a global scale, substantiating the 30 challenges faced by food producers. In 2018, worldwide wheat production fell by 34.5 million ton due to 31 prolonged droughts across Europe, Australia, and Canada. Soil compaction from field traffic is a well-recognized 32 problem in many parts of the world (Chan et al., 2006; Arvidsson and Keller, 2007; Naderi-Boldaji et al., 2018 33 (Chan et al., 2006; Arvidsson and Keller, 2007; Naderi-Boldaji et al., 2018) affecting 33 million hectares in 34 Europe alone (Akker and Canarache, 2001). Soil compaction is a form of physical degradation caused by short 35 crop rotations and heavy farm machinery working on low organic matter soils in wet conditions resulting in the

36 loss of pore space due to an externally applied load, forcing soil aggregates together (Defossez and Richard, 2002).

The resulting anaerobic high density soils have significantly reduced capacity to store water and nutrients required
by growing crops (Hamza and Anderson, 2005) and severely compacted soils prevent soil exploration from root

39 growth (Tracy et al., 2012a).

40 Soil compaction is due in part to the pressure to complete field operations such as harvesting or drilling 41 often in short windows of good weather, which is exacerbated by the increasing use of larger machinery with 42 increasing axle loads designed to improve operational efficiencies. Common agricultural operations are conducted 43 using wheeled farm machinery which has tripled in weight and power since 1966 with wheel loads rising by a 44 factor of six (Chamen, 2006). When soils are cultivated in moist or wet conditions, soils can not withstand the 45 compressive forces applied post cultivation by heavy farm machinery traffic during operations such as seeding 46 (Raper, 2005), resulting in soil degradation (Batey, 2009). When soil is wet, tyre stress can propagate a greater 47 distance down through the soil profile. The depth and severity of soil stress is related to soil moisture, traction 48 device applied (track or tyre), track size, tyre inflation pressure and wheel load (Naderi-Boldaji et al., 2018).

49 Reforming the approach to soil management to mitigate challenges such as soil compaction and soil 50 erosion offer significant financial and environmental benefits compared to conventional agriculture. Cultivation 51 practice using minimal, or zero tillage techniques are widespread across many climatic conditions from semi-arid 52 Canadian plains to the temperate climates of Western Europe. In conventional tillage, the soil is either inverted 53 >200 mm using a mouldboard plough or deeply ripped using tines. The soil is then cultivated again to break down 54 soil aggregates to a crumb structure or fine tilth that is suitable to plant seeds (Morris et al., 2010). Conservation 55 tillage, also known as non-inversion tillage or reduced tillage, has been used for decades to improve soil structure 56 and health (Skaalsveen, Ingram and Clarke, 2019). Under conservation tillage, soil is disturbed to a lesser extent 57 (<100 mm using tines or discs) or not disturbed at all such as under zero tillage which involves the direct placement 58 of seed into undisturbed crop residues (Soane et al., 2012).

59 The successful adaption of reduced tillage systems is not universally guaranteed with factors such as soil 60 texture and drainage, crop type and weather influencing successful implementation (Soane et al., 2012). In 61 northern Europe, crop yields under reduced cultivation systems rarely exceed those achieved by ploughing 62 (Arvidsson, 2010). The exception under drier arid climates such as Spain, no tillage improved crop yields by 63 moisture retention in below average rainfall years (Muñoz-Romero et al., 2010). Higher bulk density and 64 penetration resistance are typically found throughout the formerly tilled or "plough pan" layer in no tillage soils within the first two years of adoption, resulting in root mechanical impedance (V. Boguzas et al., 2006). Yet, over 65 66 time, long term zero tillage has shown to attribute improvements in soil pore architecture and continuity 67 throughout the soil profile by bioturbation, suggesting roots could penetrate to lower soil horizons (Cooper et al., 68 2021).

To date, studies have focused on how tillage influences physical soil properties (bulk density, cone penetrometer, soil aeration) with root and crop yield responses (Whalley et al., 2008; Pires et al., 2017; Czyż, 2004). Soil types and tillage systems have a considerable influence on the structural integrity of soil which controls rooting potential (Morris *et al.*, 2017a). Studies have shown that low pressure tyres can reduce surface compaction compared to high tyre pressure (Soane et al., 1980; Boguzas and Hakansson, 2001). As trafficking increases soil strength and reduces a plant root's ability to penetrate soil layers, it is important to understand the relationship between tillage depth and root system architecture during the growing season in response to trafficking. A dearth of information exists on how tillage depth and tyre pressure affect rooting properties and crop yield on longer term field sites. Yield reduction by soil surface compaction can increase abiotic stress in plants in three ways. It reduces soil aeration, increases mechanical impedance of roots which in turn reduces root exploration of soil thus, mitigating the extraction of water and nutrients from the soil resource (Chamen, 2011).

80 Quantitative measurement of root system architecture in three dimensions (3D) has become tractable 81 using X-ray CT in pot experiments (Mairhofer et al., 2017). Few examples of root studies using high resolution 82 X-ray computed tomography have been successfully conducted in field trials using undisturbed soil cores. Many 83 studies have focused on measuring soil structural properties such as porosity, soil pore size and distribution and 84 the influence of tillage method and trafficking (Millington et al., 2017; Rab et al., 2014). However, studying root 85 development and architecture in three-dimensional field structured soils remains challenging with X-ray CT due 86 to a bottleneck of rapid and standardized root extraction methods available, insufficient resolution and inability to 87 segment similarities in grey scale values between root and organic materials (Zhou et al., 2021; Mooney et al., 88 2012; Pfeifer et al., 2015).

89 The purpose of this paper was to identify the effect and interaction of machinery traffic and tillage depth 90 using commercial crop establishment methods. Root architecture, soil physical structure and crop yield were 91 studied during two key growth stages of winter wheat. X-ray CT was deployed to show if root architecture 92 behaviors could be captured in-situ to the soil structural environment created by the tillage method. Three 93 cultivation practices and traffic management systems were studied: Deep tillage (250 mm), shallow tillage (100 94 mm) and zero tillage, under no traffic, low tyre pressure and conventional tyre pressure. The objectives of this 95 study were to (i) assess the relationship between of traffic management and three tillage depths and its effects on 96 root system architecture and soil physical properties (ii) Utilise 3D image analysis along with 2D destructive 97 methods to verify rooting properties responsible for crop yield.

98 2. Materials and Methods

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100 2.1 Site and soils

The study took place during the 2018/19 growing season. The experimental site is 3.12 ha, located at Harper 101 102 Adams University (HAU), Edgmond, Newport, England (52.779738 N, -2.426886 W). The HAU site is a loamy 103 sand soil consisting of the Olerton and Salwick series soils (Eutric Endoglevic Arenosol and Chromic Endostagnic 104 Luvisol respectively) (Millington et al., 2017). Further details of the soil properties are described in Table 1. To 105 highlight if any site variability existed across the site, soil properties were examined for fertility (pH and nutrient 106 levels), bulk density, soil strength and soil moisture. Particle size analysis (Gee and Or, 2002) was conducted to 107 determine soil texture classifications. The trial site was established in 2011 for previous studies with plots and 108 treatments carried out in the same location.

The experimental site was established in 2011 with each plot receiving the same tillage and traffic
treatment as this study. During the trial period, the site was treated with a standard crop rotation with winter wheat
(*Triticum aestivum L.*) harvested in 2012 followed winter wheat in 2013, winter barley (*Hordeum vulgare L.*)
2014, winter barley 2015, followed by a cover crop "TerraLife-N-Fixx" (DSV United Kingdom Ltd, 2015); Spring

- 113 oats 2016, spring wheat 2017 and winter beans 2018. In the year prior to this study, it was necessary to plant a
- break crop (2017/18) as part of a standard crop rotation to improve soil conditions and reduce diseases such as
- take all (Gaeumannomyces graminis var. tritici). A field bean (Vicia Fabia) break crop was planted, and yields
- 116 were assessed to ensure the trial site was uniform with no underlying issues. For this trial, winter wheat (*Triticum*.
- 117 *aestivum L. cv.* Graham) followed the bean crop and was drilled early October 2018 when the soil was dry, friable
- and soil temperatures >6 °C. The seeding rate was 250 seeds per m^2 and drilling took place on the 5th of October.
- **119** This is in line with local normal farming practice.
- 120

121	Table 1: Description of the topsoil (0-300 mm) properties for Harper Adams University trial site, Shropshire,
122	UK.

Property	Units	
Location	Latitude	52.779738 N
	Longitude	-2.426886 W
Soil type	Landis group*	Argillic brown earths, brown sands
	Landis series*	Salwick, Ollerton
	FAO	Luvisol & Arenosol
Sand (2000-65µm)	g g ⁻¹ dry soil	0.743
Silt (63-2µm)	g g ⁻¹ dry soil	0.115
Clay (<2µm)	g g ⁻¹ dry soil	0.143
Texture	SSEW class	Loamy sand
Organic matter (LOI)	g g ⁻¹ dry soil	0.044

123 *Landis Soil guide (University, 2021).

LOI, Loss of Ignition.

125

126 2.2 Experiment design

127 The experiment was a randomised 3 x 3 factorial arrangement of 9 treatments in four complete replicate blocks. 128 Each plot was 4 m wide x 84 m long with exception of block 4. Block 4 is 78.2 m long for operational reasons. 129 Tramlines were at a 90° angle to plots with 24 m spacing for fertilising and spraying operations throughout the 130 growing season. A split-plot design was used, half the plot (30 m) designated for sampling and the other half was 131 undisturbed for yield data collection. The half plot for sampling was sub-divided for the two sampling stages, ensuring sampling did not occur near the same location as the previous sample. Cultivation for spring beans in 132 133 2017 was performed at three depths, 250 mm for deep tillage, 100 mm for shallow tillage and direct into stubble 134 for zero tillage. In the winter wheat trial, soil cores were collected at tillering (Growth stage (GS) 25) and the 135 flowering stage (GS 61-69) (Zadoks et al., 1974) in July 2019.

Three commercial crop establishment systems were used consisting of three different tillage depths. The following tillage treatments are denoted as: Treatment 1 = Deep tine cultivator at 250 mm (DT) for deep tillage similar to (Ren *et al.*, 2019), treatment 2 = shallow disc cultivation at 100 mm (ST) and treatment 3 = zero tillage using a direct seed drill (ZT). In combination with the different tillage depths, three traffic regimes were used in 140 this study no traffic (NT), conventional tyre pressure (CP) and low tyre pressure (LP). Tillage depths were

141 combined with traffic management practices for the 9 treatments (DTNT, DTCP, DTLP, STNT, STCP, STLP, 142 ZTNT, ZTCP & ZTLP). Using GPS guidance and markers, trafficked areas of each plot were marked out to ensure

143 samples were taken from the correct location. A GPS (Trimble FMX display unit) was used to apply all tillage

144 and drilling applications. All wheelings from cultivation and drilling occurred in the same traffic lanes for the

145

duration of the trial. During drilling, the drill coulters directly behind the tractor wheeling were marked to aid 146 identification of trafficked crop rows. During harvest, it was necessary to avoid driving on non-trafficked areas

- 147 with the plot combine restricted the wheelway zones. This ensured a CTF system was replicated.
- 148

149 2.2.2 Tillage equipment and tyres

150 Primary cultivations in HAU involved a rigid tine and conical disc cultivator (Vaderstad Topdown) at 250 mm 151 depth to cut surface residues, loosen, mix, and consolidate the seedbed. The same implement was used for shallow tillage treatments with tines adjusted upwards to reduce tillage depth (100 mm). A 290 hp Massey Fergusson 8480 152 153 with a track width of 2.1 m was used. Increased flexion AxioBib tyres were fitted IF 650/85 R38 179D TL on the 154 rear axle and (IF 600/70 R30 159D TL) at the front. A pneumatic disc seed drill (Vaderstad Spirit) was used to 155 sow the crop with 167 mm row spacing. The same drill was used to sow the zero tillage plots with the tines and 156 discs lifted to minimise disturbance (Kaczorowska-Dolowy et al., 2019a).

157 For the tyre pressure treatment, the conventional tyre treatments were inflated to 1 bar for front and rear 158 tyres during cultivations. Low tyre pressure treatments and controlled traffic farming (CTF) plots operated on 0.7 159 bar front and 0.8 bar on the rear axle. A front weight block of 540 kg was applied to the tractor for tillage primary 160 cultivation. All operations were performed under the same wheel-ways to keep traffic free zones for CTF plots. 161 During harvest, a Claas Dominator combine operated on a 4-m header, matching plot sizes (Smith, 2016). Crop 162 husbandry was carried out in accordance to the AHDB guidelines and soil fertility test analysis (AHDB, 2018).

163 2.3.1 Soil physical properties

164 Soil bulk density samples were also collected within the trafficked and non-trafficked area of the plot, to represent 165 the bulk density of the tillage treatments. Samples were replicated three times. Each core sample was 50 mm in 166 width and 300mm in length. An Eijkelkamp® soil corer was used to take bulk densities samples. Each bulk density 167 sample was taken within 0.5 m of the location of the soil cores taken for X-ray CT. The objective was to represent 168 the physical constraints (or lack of) for root growth in each plot examined. The method used in this study involved 169 splitting the bulk density sample into three 100 mm sections (0-100 mm, 100-200 mm and 200 – 300 mm) similar 170 to (Smith, 2016). The corer was opened in the field and split using a knife and ruler.

171 The core sections were stored in resealable bags and labelled before transporting to the laboratory for 172 analysis. Intact fresh soil cores were weighed prior to drying to record sample fresh weights. Samples were placed 173 into an oven at 105°C for 24 h and reweighed to determine moisture % as per equation 1 and dry bulk density as 174 per equation 2 (Campbell and Henshall, 2000).

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Moisture % = fresh weight(g) – dry weight (g) / dry weight(g) *100 Equation 1 176 Dry bulk density (Mg m^{-3}) = dry soil weight (Mg)/ soil volume (m^{-3})

Equation 2.

178 2.3.2 Penetration resistance (PR)

Soil penetration resistance data were collected on each plot (in the wheel-ways and in the centre of the plot) down to 450 mm with a depth increment of 25 mm between each recorded penetrometer reading. A cone penetrometer (Data Field, Ukraine) was used, recording soil strength in kPa, the location and depth via built-in GPS device. Only the PR samples were recorded at 450 mm to complete a reading on the data logger. It is also widely known that roots penetrate past "tillage pans" (Bengough et al., 2011) Bengough et al., 2011). Five penetrations were made both under and between the wheel ways on each plot at GS 25 sampling to represent each treatment. PR was measured when soil conditions were at field capacity to ensure accuracy of each reading.

186

187 2.3.3 Soil porosity analysis

Before soil porosity analysis on ImageJ software (version 1.52) (Schneider et al., 2012) could commence, an 188 image stack was created in VG Studio Max[®] for each scan. The contrast was adjusted to improve the uniformity 189 190 and visibility of the soil pores. The register object tool corrected scan discrepancies for soil core angle. 191 Straightening the scan allowed a cylindrical shape to be cropped and the tube edges and air space outside of the 192 soil core removed. This enabled soil data to be captured throughout the soil core. A new volume was selected and extracted from the original. This created a separate cropped image volume to work from. The surface 193 determination tool in VG Studio Max[®] was used to threshold pore spaces within the solid matrix. The tool defines 194 195 the contour of objects, separating 3D data into regions, providing meaningful soil data (Borges de Oliveira et al., 196 2016). The image was then inverted to remove the extracted variables from the image and highlighting the pore 197 spaces in the soil core. The processed image was exported as an *.TIFF image stack for further analysis using 198 ImageJ software.

199 Soil pore characteristics were measured using X-ray CT to establish information about the 3D soil 200 environment for root growth without disrupting the structural integrity of the soil core. The original grey-scale X-201 ray CT images were analysed using ImageJ software. The scale was set for each dataset to define to spatial scale 202 of the active image. The unit of length was set in millimeters and the known distance was 0.045mm (45µm). Each 203 scanned core was cropped to remove the area outside of the soil column. The action of soil coring during sampling 204 had the effect of loosening the bottom 20 mm of the core, therefore 415 slices at the bottom of each scan were 205 discarded to remove the loosening effect due to the sampling process. The downward movement of the PVC pipe also caused a smearing effect on the soil at the outside edge of the core and this area was also removed by cropping. 206

The processed image was 1220 x 1220 pixels in size. Applying the contrast enhancement filter helped normalize all slices. The filter reduces the differences in pixel grey-level between slices known as beam hardening (Wildenschild et al., 2002). The ImageJ Huang automatic threshold algorithms were used for each scan to create binarized images and separate the air-filled pores from the background region. The binarized scans were despeckled twice to remove unwanted noise within each scanned image, improving analysis and accuracy of the investigated pores. The Look Up Table (LUT) was inverted to change the white pores to black, ensuring analysis
calculated the air-filled pores and not the soil matrix. The resulting binary images were analysed using the Analyze
Particles tool which provided information for average pore size, total area and percentage porosity for each
individual image.

216 2.4.1 Soil core sampling

217 Field soil core size was chosen to capture as much root material growing in the field as possible while minimizing 218 the trade-off that exists with the X-ray CT technology between image resolution and core size (Mooney et al., 219 2012; Zhou et al., 2021). The core dimensions were consistently 70 x 300 mm (diameter x depth) for each sample. 220 Soil cores were extracted from the field sites at GS 25 in February and again at GS 61 in June. Sampling was 221 carried out at GS 61 during wheat anthesis, when root growth is at its peak (Gregory et al., 1978). Due to high 222 moisture deficits in HAU (43 mm) during sampling at GS 61 in early July, the soil sample area was wetted with 223 2.5 L of water and allowed to infiltrate. This lubricated the soil, reduced soil fracturing, and allowed tube insertion 224 and soil core extraction to take place as smoothly as possible. Polyvinyl chloride (PVC) drainage pipes were cut 225 to size (70 x 300 mm) and these tubes were used to collect soil cores (as per Millington et al, 2017).

226 A single wheat plant sample was located at random in each plot. The selected plant was cut at the base 227 of the stem with a scissors and the above ground biomass discarded The PVC tube was placed (plant centred) 228 directly over the remaining plant stubble to maximise root system capture. Tubes were inserted into the soil using 229 a mallet in the crop rows in the centre of the plots between the wheel tracks (not trafficked by wheel) for 230 untrafficked samples for no traffic samples. A second core was taken in the wheel way for the tyre pressure 231 treatments. A small block of timber was used when hammering in the tube to protect tubes and soil cores from 232 damage. A total of 72 samples were extracted on each sampling occasion and examined in this study. The PVC 233 tubes were inserted into the soil to a depth of 300 mm. The soil core was extracted carefully using a spade and the 234 sample locations were backfilled with soil. Following sampling, cores were sealed (top and bottom) using tape, 235 labelled, and carefully placed into boxes protected with bubble wrap. Cores were tightly packed and insulated to 236 minimise movement and drying of samples during transit to the laboratory for analysis. Samples were transferred to refrigerated storage (<4°C) to prevent and reduce compositional changes to the soil through biological 237 238 degradation.

239 2.5.1 X-ray computed tomography (CT) – Root analysis

240 Soil cores were transferred to the University College Dublin (UCD) X-ray CT facility at the Rosemount 241 Experimental Research Station at Belfield Campus, UCD, Ireland. The soil cores were scanned using a Phoenix® 242 v|tome|x M 240 kV scanner (GE Measurement and Control solution, Wunstorf, Germany). The v|tome|x M was 243 set at a voltage of 90 kV and current of 400 µA to optimize contrast between background soil and root material. 244 A voxel resolution of 45 µm was achieved by using the 'Multi Scan option' to scan in 4 segments. A total of 1800 245 projection images per section were taken at 200 m/s per image using the 'Fast Scan option', which has the default values of an image averaging of 1 and 0 skip. No filters were used during scanning. The total scan time per core 246 247 was 24 minutes or 6 minutes per section. Once scanning was complete, the images were reconstructed using 248 Phoenix datos|x2 rec reconstruction software, the four scans were assembled into one 3D volume for the whole

core. Core samples were scanned within a week of the sampling date, the scanned core was 300 mm in length and
 70 mm diameter. The software corrected movements during the scanning process and removed noise from scanned
 images.

252 2.5.2 X-ray CT root segmentation

Image analysis for X-ray CT images was performed using the software VGStudioMax[®], version 3.2 (Volume 253 254 Graphics GmbH, Heidelberg, Germany) to segment roots and soil porosity. Roots were segmented by setting seed 255 points and using selected threshold values in the "Region grower" that enabled fast and accurate selection of grey-256 scale voxels (3D pixels) pertaining to root materials. The root system was extracted from the greyscale CT image 257 of soil using the VGStudioMax[®] semi-automated local adaptive thresholding "Region Growing" selection tool, 258 similar to (Tracy et al., 2013). Root volumes were calculated by segmenting the root region of interest (ROI). 259 Once the roots were segmented from the image, erosion and dilation tool was selected at 1 pixel using the *Region* 260 Growing tool. Root system architecture parameters such as root vertical depth, root volume and root surface area 261 were measured from the segmented root systems. Root vertical depth was calculated on the Z axis in 262 VGStudioMax® from the length of a complete root from the base seed point.

263

264 2.5.3 Destructive 2D root analysis

265 After the soil cores were scanned, the soil and root material were separated by root washing gently with a water 266 jet hose. Two sets of sieves with a mesh size of 2 mm and 1 mm collected root material. Roots were washed and 267 soil material removed before the roots were placed into a sealed and labelled bag filled with water. The washed root samples were placed into a freezer until scanning and analysis with WinRHIZO[™] scanning and software 268 (version 2016a Regent Instruments, Canada) commenced. The root samples were thawed before scanning with 269 270 the WinRHIZO[™] software. Large root stumps were removed from the sample prior to placing it inside the tray to 271 reduce root misrepresentation (Wang and Zhang, 2009). Roots were placed onto a clear transparent tray (30 cm x 272 20 cm) with water. A pair of plastic forceps were used to spread out root seminal and lateral roots. Images were 273 scanned at a resolution of 600 dpi (42 µm pixel size) with an Epson Perfection V800 scanning system. Root 274 images were measured for root length, root surface area, average root diameter and root volume for the total soil core. This output was used to verify the 3D root outputs from VGStudioMax[®] (Flavel et al., 2017; Tracy et al., 275 2012a). The WinRHIZO[™] software enabled rapid assessment of root parameters. It calculated the root volume by 276 277 determining the average root diameter and root length by pixel counting the 2D root image and then assuming the root shape was cylindrical. The WinRHIZO[™] used a skeletonization method for characterizing root systems 278 279 (Himmelbauer, Loiskandl and Kastanek, 2004). The software uses greyscale values in *.TIFF file format. The 280 output of the images was distinguished by global thresholding analyses for root diameter while root length was validated by skeleton images. After WinRHIZO[™] scanning, the roots were removed from the scanning tray using 281 282 forceps. The root samples were dried at 70°C for 24 hours and the root biomass samples were weighed.

283 2.6 Soil Moisture Deficit Model

- Soil Moisture Deficit (SMD) was calculated based on the SMD hybrid model for Irish grassland (Schulte et al.,
- 285 2005). The model is based on weather parameters and soil drainage classes. The inputs of the model include data
- on maximum and minimum temperatures, rainfall (mm), wind speed (m/s), sunshine hours which were taken from
- the nearest weather station located in Newport, Shropshire 6km from the site (Met office, 2019).
- 288

289 2.7 Statistics

- Data from the scanned (destructive and non-destructive) images and root biomass were not normally distributed.
 Non-normal data do not meet the assumptions underpinning ANOVA (Analysis of Variance); therefore, all data
 underwent log transformation (in Microsoft Excel) before being exported to Minitab 18[®] where analysis of
- variance (ANOVA) was performed to homogenize the variances of the compared means (Poorter and Garnier,
- **294** 1996). A two-way ANOVA was performed using the general linear model using the minitab software package.
- All means were analysed for normality before the test was run. When significant effects of rooting were detected,
- regression analysis was utilised to observe the relationship between the variables. For linear regression analysis,
- residuals of data were made to ensure that the assumptions of the analysis were met (normal distribution, constant
- variance, etc). Normality was tested using the Anderson-Darling test in Minitab 18[®].
- 299

300 **3. Results**

301 3.1 Growing conditions during crop season

In 2018, crops were established at low soil moisture levels, which may have reduced soil compaction caused by
 tillage operations across all site locations. From January to August (2019), 418.6 mm of rainfall was recorded at
 HAU, 68 mm in total for January and February. Soil moisture deficits reached 66.2 mm in HAU (Supplementary
 Figure 4.10) by early June 2019. High soil moisture deficits were recorded from early April to June, causing
 drought stress during rapid growth periods (Met office, 2019).

- 307 3.2.1 Soil properties Bulk density & Penetrometer resistance
- The calculated probability (P-value) using two way linear model analysis (ANOVA) results are shown in Table 308 309 2. In the top 0-100 mm, bulk density was significantly higher in DTCP (1.66 Mg m⁻³) and STCP (1.44 Mg m⁻³) treatments compared to ZTNT (0.994 Mg m⁻³) and DTNT (0.97 Mg m⁻³) (P<0.01). STNT (1.09 Mg m⁻³) was 310 significantly higher than ZTNT and DTNT and only significantly lower than DTCP. In the middle horizon (100-311 312 200 mm), a significant interaction between trafficking treatment was found. Bulk density was significantly lower in DTNT (1.07 Mg m⁻³) compared to DTCP (1.63 Mg m⁻³) and ZTCP (1.58 Mg m⁻³) treatments (P<0.05). In the 313 314 bottom 200-300 mm layer measured, no significant tillage x traffic interaction was found (P>0.05). Table 2 shows a significant traffic effect on soil bulk density in the 0-100mm layer and 100-200mm layer (P < 0.01). No traffic 315 316 in both layers revealed a lower bulk density compared to trafficked treatments.

317	Table 2: Variance of	of analysis for bulk	density x traffic.	tillage and tillage x traffic.

HAU	Deep	Shallow	Zero	Mean
Traffic/ Tillage 0 – 100 mm	•			
No traffic	0.971	1.099	0.994	1.058 _b
LP	1.351	1.625	1.230	1.401 _a
СР	1.661	1.444	1.282	1.462 _a
P < 0.01				
Traffic/Tillage 100 – 200 mm				
No traffic	1.079	1.406	1.353	1.279b
LP	1.389	1.509	1.552	1.483a
СР	1.637	1.437	1.583	1.553a
P < 0.01				
Traffic/Tillage 200 – 300 mm				
No traffic	1.429	1.466	1.404	1.433
LP	1.593	1.787	1.619	1.666
СР	1.537	1.548	1.548	1.544

319 *Significant difference between means is represented by different letters

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321 Penetration resistance (PR) was recorded in February 2019 when the soil was at field capacity. Measurements were grouped into three groups, 0-150 mm, 150-300 mm, and 300-450 mm depth layers and a 322 323 linear variance of analysis between tillage, traffic and tillage x traffic interaction conducted and shown in Table 324 3. Figure 1 depicts the combined three layers grouped into one 0-450 mm graph. The one-way ANOVA analysis 325 revealed highly significant differences for each layer. In the 0-150 mm layer, DTNT recorded the lowest kPa (kilopascals) readings and was significantly lower than ZTCP, STCP, STLP, ZTLP and ZTNT (P< 0.000). DTCP 326 and DTLP were significantly lower kPa than ZTLP, STLP, STCP and ZTCP. ZTCP recorded the highest kPa 327 328 reading and was significantly higher than ZTLP, ZTNT, STNT, DTLP, DTCP and DTNT. In the second layer (150-300 mm), similar trends were found and highly significant (P<0.000). STCP showed the highest kPa (3193.5 329 330 kPa) and was significantly higher than STNT, ZTNT, DTNT, DTLP and DTCP. In contrast, DTNT recorded the 331 lowest reading (1268.4 kPa) and was significantly lower than ZTNT, STNT, ZTLP, ZTCP, STCP and STLP. 332 STNT revealed significantly lower kPa than STLP, ZTCP and STCP. ZTNT penetrometer readings were 333 significantly lower than all trafficked ZT and ST treatments. In the lower depth (300-450 mm), DTNT was significantly lower than STLP, STCP, ZTCP, ZTLP and STNT (P<0.000). The results revealed a significant traffic 334 interaction for 0 - 150 mm (P < 0.001), 150 - 300 mm (P < 0.000) and 300 - 450 mm (P < 0.000). Again, no traffic 335 336 PR was significantly lower than trafficked treatments. When tillage was measured, a significant effect was observed for each layer studied (0 - 150 mm = P < 0.000, 150 - 300 mm = P < 0.000, 300 - 450 mm = P < 0.000). 337 In the top layer, deep tillage was significantly lower than shallow and zero. Further, shallow tillage was 338 339 significantly lower than zero tillage PR. In the second and third layer, deep tillage was lower than shallow and zero. A tillage v traffic interaction was observed in the first layer (0 - 150 mm) (P < 0.001) but the second and 340 341 third layer were not significant (P < 0.067, P < 0.313 respectively).

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- 343
- 344

Table 3: Analysis of variance table for penetration resistance x tillage, traffic and tillage x traffic interactions
 between 0 - 150 mm, 150 - 300 mm and 300 - 450 mm.

	0 – 150 mm 2019	150 – 300 mm 2019	300 – 450 mm 2019
Traffic			
No traffic	432.3ъ	1848b	3028.5b
СР	538.8a	2614a	3753.6 _a
LP	626.7 _a	2422a	3655.7 _a
P value	< 0.001	< 0.000	< 0.000
Tillage			
Deep	240c	1366.3ь	3135.2ь
Shallow	488.4 _b	2811.4a	3800a
Zero	869.4 _a	2706.7 _a	3502.7a
P value Tillage x traffic	< 0.000 0.001	< 0.000 0.067	< 0.000 NS

348 *Significant difference between means is represented by different letters

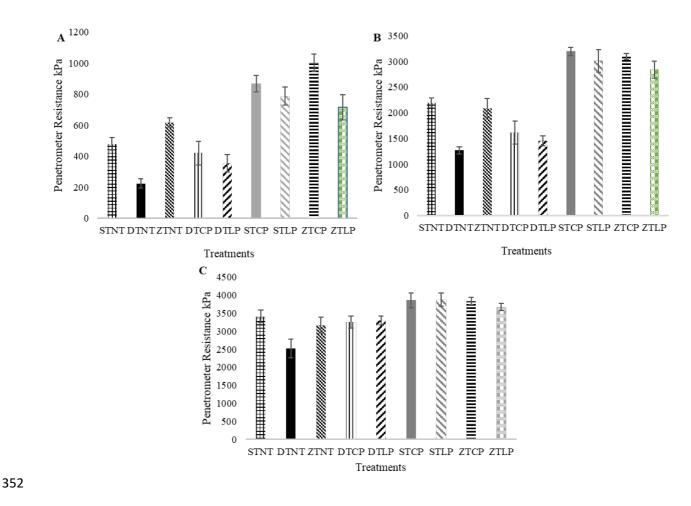


Figure 1: Penetration resistance for three layers (a) 0-150 mm (P<0.000), (b)150-300 mm (P<0.000) and (c) 300-450 mm (P<0.000) during wheat tillering (GS25). Soil moisture conditions were at field capacity during sampling.
 Bars represent the standard error of the mean.

357 3.2.2 Soil porosity

358 The results of the ANOVA analysis of the CT-measured porosity (0-220 mm) are presented in Table 4. Soil 359 porosity results were split into two soil layers of 0-100 mm and 100-200 mm respectively. In the top 0-100 mm 360 layer, DTNT showed significantly higher total pore space (P < 0.01) compared to all other treatments except 361 ZTNT. Tillage had a significant effect on soil porosity in the no traffic samples in the 0-100 mm layer (P < 0.05). 362 Deep tillage with no traffic had higher soil porosity (22.72%) than in shallow tillage (no traffic) (10.58%). There 363 was no significant difference between soil porosity under zero tillage and shallow tillage in the no traffic samples. 364 Trafficking had a significant effect on overall porosity. In deep tillage treatments, overall porosity 22.72% (no traffic) was reduced to 8.08% (under low tyre pressure) and 6.50% under conventional tyre pressure. Traffic had 365 366 little effect on shallow and zero tillage porosity in the top 0-100 mm when compared to the no traffic samples with small reductions in porosity. In the second examined layer, 100-200 mm zone, tillage and traffic were not 367 368 significantly different (P < 0.487). The percentage porosity shown in Table 4, indicate a sharp decline in the lower depth with only 9.02% in DTNT. DTCP treatments recorded the lowest porosity (3.96%). 369

ImageJ soil porosity % 0-100mm	n	No traffic	low tyre pressure	Conventional tyre pressure
Deep	4	22.72 a	8.08 b	6.50 b
Shallow	4	10.58 b	8.64 b	7.23 b
Zero	4	10.77 ab	8.41 b	8.49 b
P<0.01				
ImageJ Soil porosity % 100-				
200mm	n			
Deep	4	9.02	6.16	3.96
Shallow	4	4.06	6.44	5.32
Zero	4	2.895	6.44	5.32
P<0.487				

373 Table 4: Soil porosity for tillage x traffic for two soil layers.

374 *Significant differences between means are represented by different letters.

375

376 3.3.1 Destructive 2D root analysis

The interaction between tillage system and trafficking protocols using destructive root measuring methods 377 378 (WinRHIZOTM) are shown in Figure S2 for GS 25 and Figure S3 for GS 61 in the supplementary section. The variance of analysis results for WinRHIZO[™] are presented in Table 5. At GS25, no significant differences were 379 found between tillage, traffic, and traffic x tillage interactions. However, the WinRHIZO™ analysis revealed a 380 381 tendency towards increased root growth in no traffic treatments. At the later growth stage (GS61), Table 5 depicts 382 the results showing highly significant interactions between trafficking and tillage systems on root length density 383 (RLD) (P<0.001) and root length (P<0.001), root surface area (P<0.002) and a traffic effect on root volume (P< 384 0.05). Variance of analysis results showed a significant effect of tillage system (P < 0.01) for deep tillage compared 385 to shallow and zero tillage. When trafficking was considered, an even greater significance under was recorded (P < 0.000) for root length with no traffic significantly greater than conventional (CP) and low pressure (LP) 386 treatments. For root surface area (mm²), a significant tillage (P < 0.05) and traffic (P < 0.000) was found. Deep 387 388 tillage showed significance over shallow tillage but not zero. Root surface area was significantly lower in CP than 389 no traffic but not LP. Root volume (mm³) showed a significant traffic effect, but tillage was not significant. Indeed, no traffic was significantly greater than CP but not LP. For RLD mm³, a significant tillage (P < 0.01) and 390 trafficking effect (P < 0.000) was found. Deep tillage established greater RLD compared to zero and shallow 391 392 tillage while no traffic was significantly greater than CP and LP.

393 When traffic x tillage interactions were compared, no significant difference was found (Table 5). However,

individually, treatments were significantly greater than others. For example, DTNT showed significantly higher

- RLD, root surface area and root length compared to ZTCP, STCP and STLP. Root volume was significantly higher
- in DTNT over ZTCP and STCP. DTNT produced nearly double the root length compared to ZTCP. In contrast to

371

- 397 DTCP, root surface area reduced by 36% compared to untrafficked areas (no traffic samples). In shallow and zero
- tillage, root surface area was reduced by 32% and 63.6% respectively in conventional pressure samples compared
- 399 to untrafficked samples. There was no significant difference for root diameter and between all tillage and
- 400 trafficking regimes. The results demonstrate that there was no significant difference in RLD at the tillering stage,
- 401 nor could trends be found as roots were undeveloped. However, at anthesis, the RLD was significantly higher
- 402 under non-trafficked tillage treatments when compared to DTCP, STCP and ZTCP (Figure S3b).
- 403

Table 5: WinRhizo results of Tillage, traffic and Tillage x Traffic interactions with root traits at HAU during
 tillering (GS25) and anthesis (GS61). P values represent level of significance and 'NS' indicates non-significance.
 d.f. = degrees of freedom.

Root trait	Term	d.f.	GS25	GS61
Root length m	m			
C	Tillage	2	NS	< 0.01
	Traffic	2	NS	< 0.000
	Tillage x Traffic	35	NS	NS
Surface area mm ²	c .			
	Tillage	2	NS	< 0.05
	Traffic	2	NS	< 0.000
	Tillage x Traffic	35	NS	NS
Root diameter mm				
	Tillage	2	NS	NS
	Traffic	2	NS	NS
	Tillage x Traffic	35	NS	NS
Root volume mm ³	C			
	Tillage	2	NS	NS
	Traffic	2	NS	< 0.05
	Tillage x Traffic	35	NS	NS
RLD mm ³			210	0.01
	Tillage	2	NS	< 0.01
	Traffic	2	NS	< 0.000
	Tillage x Traffic	35	NS	NS

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410 3.3.2 X-ray CT root analysis results

Significant differences were found between trafficking treatments at GS61 for RLD and vertical root depth using 411 non-destructive VGStudioMax 3.2 (Table 4). The X-ray CT scans revealed significantly longer vertical rooting 412 413 (measured via the Z axis in VGStudioMax®) in ZTNT (112.7 mm) compared to DTCP (60.44 mm), DTLP (66.96 mm), STLP (65.39 mm) treatments (P<0.001). ZTNT showed significantly greater RLD (0.000098 mm/m³) over 414 DTCP (0.000052 mm/m³), DTLP (0.000058 mm/m³), STLP (0.000058 mm/m³) and ZTCP (0.000060 mm/m³) 415 treatments (P<0.001). Root volume and surface area showed no significant difference using X-ray CT. However, 416 similar trends were found to the conventional WinRHIZO[™] method. Trafficking had more of an influence on 417 rooting than tillage method which did not have any significant effect on root parameters. As RLD is an important 418

- 419 root trait commonly measured to estimate water uptake (White et al., 2015), linear regression was used to verify
- 420 the relationship between root depth and RLD. A significant relationship (P < 0.001) was found with a coefficient
- 421 of determination $R^2 = 0.54$ (Supplementary Fig. S4).
- 422
- 423

424 **Table 6:** Root system architecture using non-destructive method.

			n Architecture growth stage	
Tillage x traffic	Root volume mm3	Root surface area mm2	Length (Z) axis (mm3)	Root length density (mm/m3)
DTNT	3900.00	23448	96.1 ab	0.000083 ab
STNT	2648.00	17350	88.4 abc	0.000077 ab
ZTNT	3048.00	17907	112.7 a	0.000098 a
DTCP	2276.00	12114	60.44 c	0.000052 b
DTLP	3525.00	20269	66.96 bc	0.000058 b
STCP	2900.00	18052	67 abc	0.000058 ab
STLP	2358.00	14211	65.39 bc	0.000057 b
ZTCP	2533.00	15040	69.43 abc	0.000060 b
ZTLP	4480.00	25104	97.89 ab	0.000085 ab
P value	NS	NS	0.001	0.001

425 *Significant differences between means are represented by different letters.

Figure 2 shows root biomass results for GS25 and GS61. No significant differences between treatments at GS25 (P<0.848) were found. However, root biomass was significantly different for tillage x traffic with high confidence level (P<0.001) at GS61. DTNT (0.829 g) showed significantly (P<0.001) greater root biomass, than STCP (0.437 g) and ZTCP (0.4530 g) treatments. DTNT did not significantly differ from ZTLP (0.7992 g), ZTNT (0.7939 g), DTLP (0.6837 g), STNT (0.4991 g) and STLP (0.4923 g). The results show that, DTNT, ZTLP and ZTNT resulted in nearly 50% greater root biomass over STCP and ZTCP treatments. Tillage treatments (center line where there was no traffic effect) did not differ significantly with respect to root biomass.

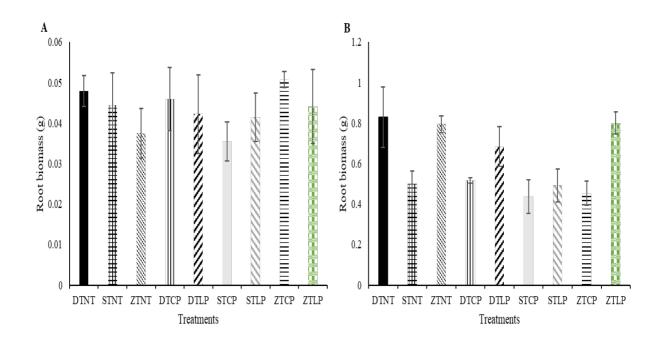




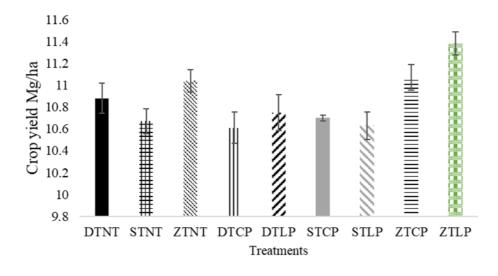
Figure 2: Root biomass at tillering (GS25) and flowering (GS61) for traffic and tillage treatments. Treatments
represented by initials (Tillage: D = Deep, S = Shallow, Z = Zero), (Traffic: NT = No traffic, LP = Low pressure
tyre, CP = Conventional pressure tyre).

438 3.4 Crop yield

Crop yield was highly significant between trafficking treatments and tillage (P <0.01) shown in Figure 3. ZTLP 439 440 had the highest yield (11,385 kg ha-1) and was significantly greater than DTLP (10,757 kg ha-1), STCP (10,700 441 kg ha-1), STNT (10,678 kg ha-1), STLP (10,638 kg ha-1) and DTCP (10,613 kg ha-1). All three zero tillage 442 treatments trended higher than deep tillage and shallow tillage treatments. ZTLP showed a 500 kg ha-1 yield advantage over DTNT (NS) and between 628 - 772 kg ha-1 over trafficked treatments and STNT with high 443 444 significance. In general, this study did not show a trend in yield between conventional and low tyre pressure 445 treatments. For deep tillage, conventional tyre pressure reduced crop yield compared to low tyre pressure by 144 446 kg ha-1) (1.34%). When compared to the no traffic sample, conventional tyre pressure consistently reduced yield by 272 kg ha-1) (2.5%) in deep tillage. Although not significant, trafficking trended towards improving yield by 447 448 30 kg ha-1) (0.03%) using conventional tyre pressure and 340 kg ha-1) (3.07%) using low tyre pressure. No trends 449 were found in shallow tillage treatments. Linear regression of root depth using X-ray CT showed a significant relationship to crop yield (P < 0.001) and positive correlation (r = 0.54). However, the coefficient of determination 450 451 was low R2 = 0.3094 (Figure S5). Moreover, regression analysis also showed a significant relationship between 452 root biomass and crop yield (P < 0.01). However, the correlation between the two variables was weaker (r = 0.43)

453 (coefficient of variance R2 = 0.1859. This indicates that root depth is a stronger predictor of crop yield.

454



457 **Figure 3:** Crop yield in Mg/ha for traffic x tillage treatments.

- 458
- 459

460 4. Discussion

461 4.1.1 Soil physical responses to tillage

462 Previous studies have shown that zero tillage systems increase in bulk density, penetration resistance and reduce 463 in porosity in the early years of adoption from conventional tillage systems (Christian and Ball, 1994; Six et al., 464 2004; Mangalassery et al., 2014b; Smith, 2016). Indeed, Soane et al., (2012) reported that significant regeneration of soil structure requires a three-year period from tillage depending on previous historic land management 465 466 practice. Moreover, values decrease in the long term with multiple benefits including improved saturated conductivity, soil organic matter and air permeability in lower soil horizons. Arvidsson, 1998 showed that soils 467 with <30 g kg⁻¹ of organic matter were likely to suffer 11% higher crop yield loss due to compaction using uniaxial 468 469 compression tests. It is plausible that the actions of soil fauna such as earthworms and old root channels could 470 have reduced bulk density over time (Figure 4) as identified by (Angers and Caron, 1998). Roots promote soil 471 structural formation through increasing soil aggregation. Root mucilage production, root hair formation, and 472 localised wetting and drying cycles encourage a reduction in soil bulk density (Bengough, 2012).

473 4.1.2 Soil porosity in response to tillage

474 Sandy soils due to their adhesive and coarse grain nature, have reduced porosity, including lower levels of

475 micropores compared to loamy soils (Arvidsson, 1998). The aggregation potential in this sandy loam soil is low.

476 In the presence of plants, porosity and pore connectivity as shown to reduce further compared to clay cohesive

- soils which tend to increase in porosity through flocculation and aggregation (Bacq-Labreuil et al., 2018). Here,
- 478 we found soil porosity to be low in general across all treatments. When comparing cultivation systems, we found
- that shallow tillage in the 0-100 mm layer had significantly lower porosity (10.58%) compared to deep tillage

480 (22.72%). Although zero tillage recorded low porosity values also (10.72%), it was not significantly different to
481 the other two systems.

A key characteristic of zero tilled soils is a change in soil pore architecture with vertically orientated fissures 482 connected down through the soil profile created by biopores (Figure 4). Similar findings have resulted in reduced 483 CO₂ fluxes and increased saturated hydraulic conductivity by surface-connected porosity (Cooper et al., 2021). 484 485 The same study found similar soil porosity levels between conventional and zero tillage with zero tillage total porosity ranging from <5%, 10% and 12% on average over 1-5, 6-10 and 11-15 years respectively. The significant 486 487 increase in deep tillage soil porosity substantially increases soil respiration, resulting in up to 13.8 times higher CO₂ emissions through increased oxidation and carbon breakdown (Reicosky et al., 1999). The lower porosities 488 489 in zero and shallow tilled soils reduces space for gas exchange, reducing soil respiration and supporting carbon 490 sequestration, thus increasing recalcitrant levels of carbon in soil. Mangalassery et al., (2014) found similar 491 porosity results using X-ray CT methods to measure the effect of tillage method on greenhouse gas emissions, 492 finding significantly higher porosity in tilled soil (13.6%) compared to zero tilled soil (9.6%) in the top 0-100 mm 493 layer. However, in deeper soil horizons, no difference could be found between tillage system. The findings in this 494 experiment agree with that study, showing both tillage methods did not differ significantly in the 100-200 mm

layer with lower soil porosities recorded.

496 4.1.3 Root system architecture responses to tillage

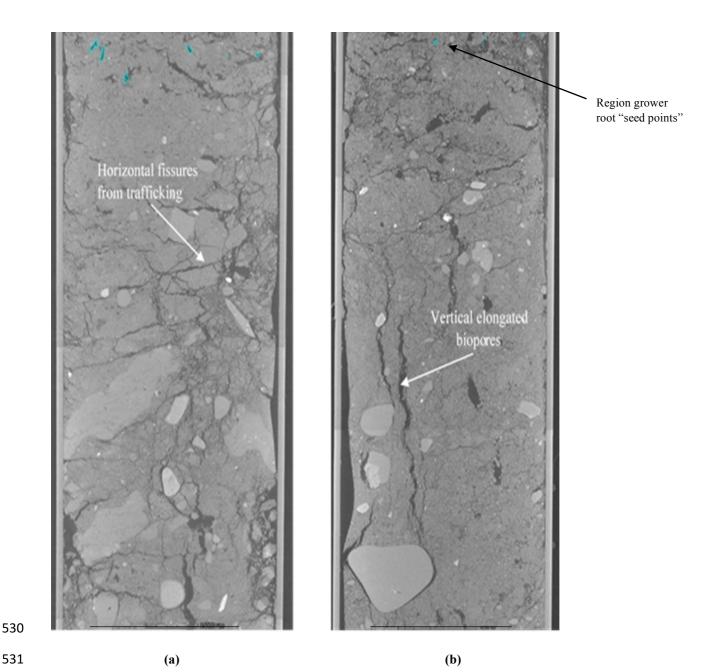
The 'hidden half' (i.e. roots) of plants are difficult to interpret in field studies (Lynch and Brown, 2001). A large 497 root system is characterized by large biomass, root length and root length density (Ehdaie et al., 2010; Hamblin 498 499 and Tennant, 1987). Root biomass was an important indicator of root size, showing treatment effect at anthesis 500 compared to the tillering stage. In general, root biomass had a positive relationship with grain yield. Zero tillage 501 treatments both untrafficked and trafficked at low pressure had greater root biomass over all shallow tillage 502 treatments and deep till trafficked at conventional pressure. Although deep tillage treatments had the highest root 503 biomass by GS61, it did not achieve the highest yield. No significant difference in root biomass was found between 504 tillage treatments, confirming that roots are more sensitive to trafficking than tillage method. RLD in shallow 505 tillage treatments and zero tilled treatments trafficked at conventional pressure. RLD is an important parameter 506 for characterizing root growth (Doussan et al., 2006) and has been used in previous studies as a key root parameter 507 for modelling water uptake (Tinker and Nye, 2000; Javaux et al., 2013). Munos-Romero et al., (2010) and 508 Chakraborty et al., (2008) results indicate that RLD is a positive predictor of crop yield. Although RLD had a 509 positive correlation with crop yield in this study, root depth (using X-ray) displayed a much stronger relationship 510 with crop yield (Figure S5).

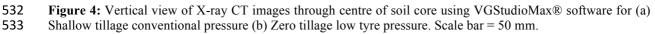
511

513 4.2.1 Soil physical responses to traffic

514 In line with this papers hypothesis, trafficking effects were more influential on crop and root performance than tillage system. The presence of wheeled areas in both zero and deep cultivation treatments increased soil bulk 515 516 density significantly in deep tillage treatments (Table 2). Our data shows similar findings with zero and deep 517 tillage significantly reduced bulk density values in untrafficked zones. However, in trafficked treatments, high 518 tyre pressure combined with deep tillage treatments resulted in higher bulk density values due to the loss of 519 inherent strength by tilled soil, resulting in compression of soil particles (Raper, 2005; Soane, Godwin and Spoor, 520 1986). Chan et al., (2006) observed that trafficking after deep tillage increased bulk density values from 1.27 Mg m⁻³ to 1.54 Mg m⁻³, emphasizing the effect of trafficking on the reduced bearing capacity of the deep tilled soil. 521 522 The optimum soil density has been reported to differ between soil types in previous studies. Indeed, Czyż, (2004) 523 established a soil type interaction between crop yield, bulk density and root mass concluding with sandy loam

- 524 soils (similar to this study) having an optimum bulk density value of 1.54-1.66 Mg m⁻³. Yet, in this study, root
- 525 biomass was significantly reduced with treatments displaying similar soil density values to that reported optimum.
- 526 Although conventional pressure tyres significantly affected zero tillage in the 100 200 mm layer, trafficking
- 527 affected the 0 200 mm later under deep tillage. In shallow tillage treatments, the top 0- 100 mm layer was
- 528 considerably impacted by high tyre pressure.





535 4.2.2 Soil porosity in response to traffic

Compared to non-trafficked treatments, trafficked soil in general caused a sharp decline in soil porosity in the top
0-100 mm layer. Tyre inflation pressure is one of the key contributors to soil stress in the 100 to 1000 mm layer

538 (Botta et al., 2008). The effect of re-compaction from trafficking after cultivation was often worse in deep tillage

- treatments, with a lower percentage porosity than in zero and shallow tillage (Table 4 for DTLP and DTCP
- 540 treatments). In deeply cultivated soils, water infiltration rates can be reduced by up to 82% after a single
- 541 wheeling's (Chyba, 2012), which has agronomic implications such as reduced water and nutrient use efficiency
- 542 by up to 22% thus, potentially resulting in crop yield penalties of up to 38% (Ishaq et al., 2001). Yield effects by

trafficking were modest in our study due to low soil moisture conditions during sowing in autumn 2018 (Met office, 2019). Dry soil has increased soil strength, reducing the effects of soil compaction as the soil load support

545 capacity would have increased thus, increasing permissible ground pressure (Hamza and Anderson, 2005).

546

547 4.2.3 Penetrometer responses to traffic

Penetrometer resistance (PR) is a useful parameter for evaluation of soil physical resistance to root growth (Otto 548 et al., 2011). In general, trafficking had a considerable influence on soil PR in this study as depicted in Figure 6. 549 550 The greatest contrast in soil penetration resistance was between trafficked and un-trafficked soil with zero tillage 551 showing the highest resistance under conventional tyre pressure. Recent studies have shown that roots can exploit 552 pores and bypass layers of strong soil (Atkinson et al., 2020). Axial pressure from repeated trafficking in ZTCP 553 resulted in the highest PR values. However, root depth was less affected in contrast to STCP and DTCP. This might explain why roots could exploit existing pore networks in undisturbed soils compared to tillage treatments. 554 555 In the middle layer examined, shallow till conventional pressure treatments suffered from a tillage pan effect shown in Figure 5. In fact, all trafficked zero and shallow tillage systems resulted in PR values beyond 2,000 kPa, 556 557 a threshold level which several studies show there is a reduction in root growth (da Silva et al., 1994; Lapen et 558 al., 2004; Tormena, da Silva and Libardi, 1999). A compact zone at shallow depths is detrimental to plant growth 559 and crop yield in rainfed temperate climates when short term droughts occur (Campbell, Reicosky and Doty, 560 1974).

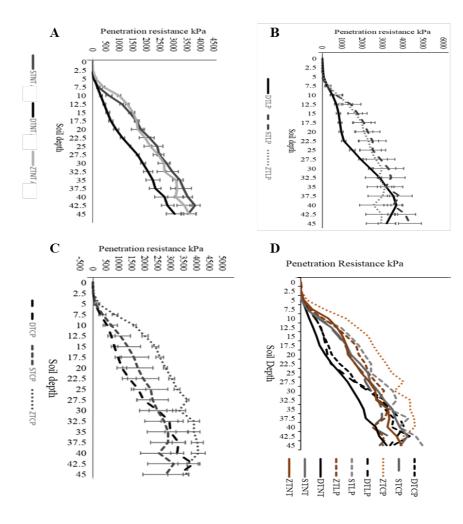




Figure 5: Penetration resistance (kPa) for tillage and traffic treatments at soil depths of 0 - 450 mm. X axis depicts
soil depth. Y axis depicts Soil penetration resistance (kPa). Treatments represented by initials (Tillage: D = Deep,
S = Shallow, Z = Zero), (Traffic: NT = No traffic, LP = Low pressure tyre, CP = Conventional pressure tyre). A
no traffic, B low tyre pressure, C conventional tyre pressure and D traffic x tillage treatments combined.

568 4.2.4 Root system architecture responses to traffic

569 Traffic significantly affected root volume, root surface area, root length and when comparing the highest root 570 biomass (under deep tillage with no traffic) and bulk density results in the 100-200 mm layer, we found a reduction 571 in root biomass when trafficked under conventional pressure by 28% in deep tillage under conventional pressure $(BD = 1.66 \text{ g cm}^{-3})$, 37% in shallow till conventional pressure (1.437 g cm $^{-3}$) and 39% in zero tillage conventional 572 pressure (1.583 g cm⁻³) treatments. The compaction effects of trafficking on soil structure exacerbated the impact 573 574 on rooting in general. Typically, studies report shallower rooting, increases in root diameter and decreased axial 575 and lateral rooting (Grzesiak et al., 2014). Shallow tillage had the lowest root biomass in both trafficked and 576 untrafficked treatments. Shallow tillage treatments suffered from visible horizontal fissures or "tillage pan" in 577 Figure 5, causing significantly reduced rooting compared to deep tillage treatments. Moreover, a combination of 578 <10% porosity and PR reaching >2,000 kPa in the 100-200 mm layer, it is likely that roots may also have suffered 579 from anaerobic conditions due to poor infiltration rates through the tillage pan during heavy rainfall events. 580 Conversely, root impedance may have occurred during drought periods through May and June (Batey, 2009).

Alameda, Anten and Villar, (2012) proposed that axial growth suffers more than radial root growth. These effects
of increased PR and soil bulk density were observed underin the current study. However, the increase in root
diameter reported by several authors was not detected here (Chen et al., 2014; Lipiec et al., 2012; Tracy et al.,
2012a; Alameda et al., 2012).

585 4.2.5 Tillage and trafficking effects on rooting and crop yield

586 In the present study, it was found that long term zero tillage plots under low tyre pressure increased yield by up to 0.772 Mt ha⁻¹ compared to the deep tillage conventional tyre pressure treatments. All zero tillage treatments 587 yielded over 11 Mt ha⁻¹ compared to deep and shallow tillage treatments (10.71 Mt ha⁻¹mean). Evidence using 588 589 data collected from the X-ray CT scans showed deeper vertical rooting in zero tillage plots compared to shallow 590 and deep tillage treatments (Figure 6). Coupled with deeper rooting, zero tillage no traffic treatments had 591 significantly lower bulk density than deep tillage conventional pressure plots. Munoz-Romero et al., (2010) 592 reported a yield increase of 0.5 Mt ha⁻¹ in zero tillage compared to conventional tillage which was associated with greater water use and increased water use efficiency, similar to (Chakraborty et al., 2008). Improvements in 593 594 moisture retention, soil pore structures and reduced soil compaction under zero-tillage, may also have contributed 595 to a yield increase over conventionally tilled treatments.

596 It is possible that the lower levels of porosity found in zero tillage aided with water retention during drought 597 periods on the highly sandy soil in this trial. Coupled with the development of vertically oriented soil structural 598 characteristics attributed to earthworm activity and old root channels (Figure 4), the zero tillage treatments may 599 also have had increased access to water by roots at lower soil horizons. Indeed, biopores benefit root growth by 600 altering the surrounding chemical, physical and biological properties of soil (Stroud et al., 2017; Banfield et al., 601 2017). Thus providing macropore pathways with lower mechanical resistance in which deeper rooting 602 preferentially grow towards (Zhou et al., 2021). In contrast, deep cultivation created a porous structure which has 603 shown to increase respiration of aerobic microorganisms, improving the flow of air and water thus increasing CO2 emissions (Mangalassery et al., 2014a). Crop yield was influenced less in zero tillage treatments by 604 605 trafficking than the other tillage treatments. The lower sensitivity to compaction in zero tillage is attributed to an elastic behavior or increase in bearing capacity, with soil acquiring similar structural properties to grassland soil 606 607 (Ehlers and Claupein, 1994).

608

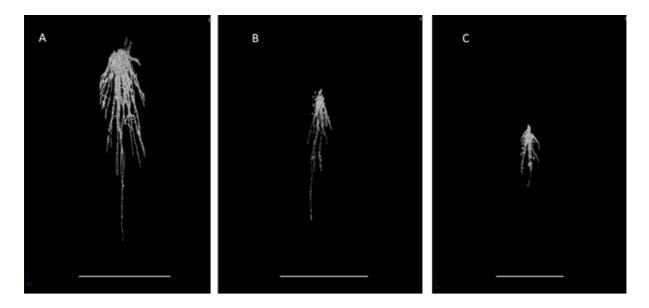
609 4.3 2D & 3D imaging for studying root-soil relationships

610 Due to the complexity of measuring root systems, two methods were conducted to provide comprehensive 611 analysis. Important topology (root networks) and geometrical (physical positions) characteristics of wheat rooting 612 using X-ray CT were found in this study. A strong significant relationship between RLD (WinRHIZO™) and root 613 depth (X-ray CT) was found (Figure S4) validating the suitability of image analysis methods in field studies. 614 Further, root depth showed the strongest correlation with crop yield compared to root biomass and RLD (Figure 615 S5). Moreover, the large environmental variance (low r number) in root relationships may have been caused by 616 spatial effects reported in previous studies (Guo et al., 2020; Zhou et al., 2021). Visualizing important behaviors 617 of wheat rooting in field scale trials, highlights the significance of root depth to sustain high yields in drought conditions. Figure 6 depicts significantly longer root length in zero tillage treatments compared to trafficked deep
and shallow tillage, with trafficked treatments roots were generally confined to the top 0-50 mm of soil. In general,
root length rarely surpassed 100 mm in depth. This was partly due to insufficient resolution available with the X-

621 ray CT scanner to capture finer root materials (Pfeifer et al., 2015).

In general, both root analysis methods showed agreement in the results. Zero tillage treatments had significantly 622 deeper rooting over shallow tillage and deep tillage trafficked treatments. Using the WinRhizo[™] method, 623 untrafficked deep tillage treatments showed superior root length. Similar disagreements in findings between 624 625 methods could be explained by the difference in methodology between the two imaging approaches as X-ray CT 626 is 3D and scans roots in soil whilst, WinRhizo[™] is 2D and scans washed roots (Tracy et al., 2012a). Root volume and surface area were also examined using X-ray CT. In contrast to the WinRhizo[™] analysis, no significant 627 628 differences could be detected between treatments. The root volumes obtained by the WinRhizo™ were much 629 greater than the volumes attained from the X-ray CT scan. The difference can be attributed by much clearer 630 contrasts between air and root material with the destructive method compared to limitations with resolution and 631 density differences between soil, root and organic materials (Mooney et al., 2012) in the X-ray CT scan images.

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Figure 6: Root system architecture of winter wheat during anthesis for (a) Deep tillage no traffic, (b) Zero tillage
low tyre pressure and (c) deep tillage conventional tyre pressure. (a) and (b) showed significantly longer root
length on the primary axis compared to (c) deep tillage trafficked treatments. Scale bar = 70 mm.

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638 5. Conclusion

639 The results from this research highlight the importance of traffic management for improving crop productivity.
640 Physical and visual implications of soil compaction on the soil profile were demonstrated in this study, signifying
641 the implications of tyre pressure on root growth. Traffic significantly reduced root development in all tillage
642 treatments with tyre pressure having no significant effect on mitigating compaction effects on soil and roots.

643	Moreover, deep, and shallow tillage systems were more influenced by compaction than zero tillage with roots
644	confined to the top 0-60 mm thus, reducing primary vertical rooting and inhibiting roots access to deeper soil
645	moisture reserves. The highly significant impact on crop yield was highlighted by the strong relationship between
646	root depth and crop yield. The visible effects of trafficking on the soil profile depicted through X-ray CT, provides
647	evidence of the damage modern farm machinery can cause for root resource capture, leading to potential increased
648	drought stress and yield loss in crop production. This long-term trial site has shown that zero tillage does not affect
649	root growth, in fact, reduced bulk density, improved grain yield and rooting depth significantly through deeply
650	connected vertical soil pore fissures created by earthworms and old root channels, allowing roots to access deeper
651	soil moisture reserves. These findings suggest that scientists and farmers should focus on designing improved
652	zero tillage cropping systems, managing field trafficking protocols with controlled traffic farming. Moreover,
653	further investigation on tracks and dual radial tyres are required to quantify practical compaction mitigation
654	measures. Furthermore, this research shows that the combination of X-ray CT scanning along with traditional
655	destructive methods provide a robust method for assessing in field rooting for future crop breeding initiatives and
656	soil management practice. This research concludes that trafficking has more profound effects for root growth and
657	crop yield than tillage method.
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660	Supplement. The supplement related to this article is available in a separate word file as per submission.
661	
662	Author contributions. KMc and ST conceived the experiment. DH & MH carried out sampling and soil analysis.
663	DH processed and analysed all samples. DH analysed and interpreted the data and wrote the manuscript. All
664	authors contributed to the data, providing interpretation and comments to the manuscript.
665	
666	Competing interests. The authors declare that they have no conflict of interest
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668	Disclaimer.
669	
670	Acknowledgements. This research took samples from a long term tillage and traffic experiment site in Harper
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