

1 **The role of plant species and soil condition in the structural**
2 **development of the rhizosphere**

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20 Number of Tables: 0

21 Number of Figures: 7

22

23 Keywords: Rhizosphere, Root diameter, Soil structure, Structural development, X-
24 ray Computed Tomography (CT)

25

26 **Abstract**

27 Roots naturally exert axial and radial pressures during growth which alter the structural
28 arrangement of soil at the root-soil interface. However empirical models suggest soil
29 densification, which can have negative impacts on water and nutrient uptake, occurs at
30 the immediate root surface with decreasing distance from the root. Here we spatially
31 map structural gradients in the soil surrounding roots using non-invasive imaging, to
32 ascertain the role of root growth in early stage formation of soil structure. X-ray
33 Computed Tomography (CT) provided a means to not only visualise a root system in
34 situ and in 3-D but to assess the precise, root-induced alterations to soil structure close
35 to, and at selected distances away from the root-soil interface. We spatially quantified
36 the changes in soil structure generated by three common but contrasting plant species
37 (Pea, Tomato and Wheat) under different soil texture and compaction treatments.
38 Across the three plant types significant increases in porosity at the immediate root
39 surface were found in both clay loam and loamy sand soils and not soil densification,
40 the currently assumed norm. Densification of the soil was recorded, at some distance
41 away from the root, dependent on soil texture and plant type. There was a significant
42 soil texture x bulk density x plant species interaction for the root convex hull, a measure
43 of the extent to which root systems explore the soil, which suggested pea and wheat
44 grew better in the clay soil when at a high bulk density, compared to tomato which
45 preferred lower bulk density soils. These results, only revealed by high resolution non-
46 destructive imagery, show that while the root penetration mechanisms can lead to soil
47 densification (which could have a negative impact on growth), the immediate root-soil
48 interface is actually a zone of high porosity, which is very important for several key
49 rhizosphere processes occurring at this scale including water and nutrient uptake and
50 gaseous diffusion.

51 Keywords: Rhizosphere, X-ray Computed Tomography, Soil structure, Root
52 architecture, compaction

53 **Introduction**

54 The dynamic nature of the rhizosphere (the zone of soil surrounding a growing root
55 which is influenced by it) provides a niche environment which exhibits biophysical and
56 chemical gradients very different to those found away from the soil immediately
57 influenced by the root, referred to as the bulk soil. These gradients control root activity
58 through a combination of root-derived exudations and physical structural alterations,
59 influencing water and nutrient uptake, gaseous exchange, particle rearrangement and
60 wettability at the immediate root surface. Carminati et al. (2010) revealed the influence
61 of mucilage on the water holding capacity of the soil immediately around the root and
62 its implications for hydraulic continuity around the root system was demonstrated by
63 (Moradi et al. 2011). In compacted soils, the influence of plant derived exudates have
64 been highlighted to improve mechanical conditions for root penetration (Oleghe et al.
65 2017). Carminati and Vetterlein (2013) proposed the concept of rhizosphere plasticity to
66 help understand the bimodal hydraulic responses found at the root-soil interface under
67 different bulk soil moisture conditions. However soil structural dynamics, particularly
68 around an actively growing root, have been largely limited to theoretical models (Dexter
69 1988) or root analogue approaches (Aravena et al. 2011) due to the inherent difficulties
70 in observing a fragile, opaque system in situ.

71

72 As impeded roots elongate they undergo radial and axial elongations (Misra et al. 1986),
73 exerting compressive and shear forces on the surrounding soil in horizontal and vertical
74 directions (Bengough and MacKenzie 1994; Kolb et al. 2012). It is known that root
75 diameter varies in response to compaction and soil strength, with many studies
76 demonstrating an increased radial expansion of the root axes in dense soil (Atwell 1988;

77 Materechera et al. 1991; Tracy et al. 2012). These pressures, generated by the root, are
78 partly responsible for soil structural alterations in the rhizosphere, and they in turn
79 affect, the hydraulic continuity of the pore system (Aravena et al. 2011). However the
80 exact effect of root growth on soil structure, especially at the scale of the pore, is
81 uncertain, in large part due to the limited number of studies which have compared root
82 responses under contrasting physical soil conditions for different plant species (Iijima
83 and Kato 2007; Materechera et al. 1992). Aravena et al. (2011) reported decreased
84 porosity around growing roots using a root analogue technique which showed the radial
85 forces in wet soil reduce inter-aggregate pore space, impacting on the hydraulic contact
86 between aggregates. Contrary to this, Helliwell et al. (2017) recently reported an
87 increase in porosity at the immediate root surface at a resolution of 12 μm , surrounding
88 the growing roots of tomato in both coarse and fine soil textures, with a decrease in
89 porosity observed away from the root in the bulk soil.

90

91 The functioning of the rhizosphere, and in particular, its role in regulating the hydraulic
92 behaviour of plants has been an active area of research for many years. Carminati et al.
93 (2013) showed the importance of gap formation around roots in decreasing
94 transpirational demand in lupin. Likewise Berli et al. (2008) highlighted the potentially
95 beneficial role of rhizosphere densification in increasing hydraulic contact and
96 connectivity between neighbouring aggregates. Hence, understanding how plants
97 influence the precise arrangement of soil around a root in terms of densification, gap
98 formation and the resulting impact on water and nutrient flow towards roots is very
99 important from a plant developmental perspective. Ascertaining the role of root growth
100 on the structure of the rhizosphere is challenging due to the fragile nature of soils.
101 Previous attempts to address this have employed thin-section microscopy through resin
102 impregnation, to 'fix' and preserve the root and soil systems prior to analysis (Mooney

103 et al. 2007; Veen et al. 1992). However, these techniques are very laborious, still allow
104 for substantial root and soil disturbance and do not readily enable the study of the
105 system in 3-D. Non-invasive imaging such as X-ray Computed Tomography (CT), X-
106 ray Radiography, Neutron Radiography and Magnetic Resonance Imaging are now
107 accepted methods that are assisting us overcoming these limitations having been
108 successfully employed in studies of plant-soil interactions over the last decade (see
109 reviews by Helliwell et al. (2013); Mooney et al. (2012); Pires et al. (2010); Taina et al.
110 (2008)). Recent advances in X-ray detector efficiencies, X-ray source power and image
111 analysis methodologies have also highlighted X-ray CT as an exciting tool for mapping
112 microscale alterations to root architectures and soil structures (Helliwell et al. 2013;
113 Mooney et al. 2012), with previous limitations of coarse resolutions and poor image
114 quality greatly reduced.

115

116 The objective of this study was to take advantage of the recent advances in imaging
117 methodology to visualise the root-mediated soil structure in 3D (e.g. Helliwell et al.
118 2017) and gain a new insight into root-induced physical transformations in the
119 rhizosphere. The first aim was to assess how three different plant species with
120 contrasting root architecture modify the soil structure at the immediate soil surface in
121 comparison with the bulk soil. Secondly, we sought to investigate how the root response
122 to the soil was influenced by soil texture (or particle size) as this has often been ignored
123 in previous studies that have tended to focus on one soil type. Finally, we examined the
124 root response to soil structuring when grown in soils at different bulk densities to assess
125 the impact of compaction. Based on previous work we hypothesised that while root
126 growth mechanisms would generate zones of higher soil density, the root-soil interface,
127 a key zone for water and nutrient exchange, would be a zone of higher porosity
128 consistent across all species.

129 **Materials and Methods**

130 *Soil Core Preparation and Sampling*

131 Four replicate columns (80 mm height x 25 mm diameter) per soil texture and per bulk
132 density were uniformly packed to 1.2 Mg m⁻³ and 1.5 Mg m⁻³ with air dried sieved (<2
133 mm) Newport series loamy sand (sand 83.2%, silt 4.7%, and clay 12.1%; pH 6.35;
134 organic matter 2.93%; FAO Brown Soil) and Worcester series clay loam (sand 35.6%,
135 silt 31.5%, and clay 32.9%; pH 6.50; organic matter 5.19%; FAO Argillic Pelosol) soil
136 from the University of Nottingham farm at Bunny (Nottinghamshire, UK - 52.52°N,
137 1.07°W). The water retention curves for these soils can be found in Helliwell et al.
138 (2014). To ensure homogeneity in sample preparation and reduce any effects of soil
139 slumping following packing into the cores, the samples underwent one wetting and
140 drying cycle using tension table apparatus, before being maintained at a tension of -5
141 kPa on the tension table throughout seedling establishment and growth. Previous work
142 in Helliwell et al. (2017) showed that this was optimal for soil structure stabilisation
143 without inducing noticeable cracking through shrinkage. Surgical micropore tape (3M
144 United Kingdom PLC, Bracknell) was placed over the columns during soil preparation
145 to reduce soil surface evaporation and prevent sample contamination, whilst still
146 enabling gaseous exchange. Seeds of tomato *Solanum lycopersicum* cv. 'Ailsa Craig',
147 winter wheat *Triticum aestivum* cv. 'Cordiale' and common pea *Pisum sativum* cv.
148 'Kelvedon Wonder' were germinated in the dark on wetted filter paper for 48 hours
149 before being planted 5 mm below the soil surface in the replicate columns for each soil
150 texture and bulk density combination (n=48). Plants were grown under controlled
151 conditions (22 °C day / 16 °C night); 40% relative humidity; a 12 hour photoperiod with
152 a photosynthetic photon flux density at plant level of 330 μmol m⁻² s⁻¹ in a climate
153 chamber for a period of 8 days. During this 8 day period the plants are mainly using

154 nutrient seed reserves to support growth (Bouaziz and Hicks 1990) and there was
155 insufficient time for the development of nitrogen-fixing nodules on the pea roots.

156

157 *X-ray CT scanning procedure*

158 The samples were scanned using two X-ray microtomography systems, in order to
159 assess plant-induced structural development across two different spatial resolutions. All
160 samples were initially scanned using a Phoenix Nanotom 180NF X-ray micro-CT
161 scanner (GE Sensing and Inspection Technologies, Wunstorf, Germany). The source
162 had a 3 μm focal spot, with the centre of the sample 5.4 cm from the X-ray source and a
163 resultant imaged voxel size of 12 μm . The entire sample was imaged with a field of
164 view of 2308 x 2308 pixels using an X-ray energy of 110 kV, a current of 110 μA and
165 an exposure time of 750 ms. A 0.2 cm Cu filter was used and 1600 image projections
166 were taken, with each scan taking 70 minutes to complete. Each sample was scanned
167 once 8 days after planting, exposing each plant to a calculated dose of 6.33 Gy (Zappala
168 et al. 2013).

169

170 A subsection of two replicates per plant x soil texture x soil bulk density treatment were
171 further scanned using a Phoenix v|tome|x m 240kV X-ray micro-CT scanner (GE
172 Sensing and Inspection Technologies, Wunstorf, Germany). Due to an improved
173 detector efficiency (allowing enhanced X-ray projection image collection) and higher
174 X-ray flux in this system, scans at a voxel spatial resolution of 8.5 μm were possible,
175 with each scan taking 43 minutes to complete. An X-ray energy of 120 kV and current
176 of 60 μA was used, with 1981 projections taken at a timing of 333 ms per projection.
177 The centre of the sample was 3.48 cm from the X-ray source. Each sample was scanned
178 once, also after 8 days, exposing each plant to a calculated dose of 7.52 Gy.

179

180 *Image Processing, Segmentation and Analysis*

181 Image processing was performed in VG StudioMax[®] 2.2 software, using procedures
182 largely detailed in Helliwell et al. (2017). Briefly, segmentation of soil, root and pore
183 phases was undertaken after applying a median filter of radius 3 pixels to remove noise
184 but preserve structural borders. To segment pore and soil phases, the greyscale
185 histogram was calibrated (individually for each sample) against pore space and a
186 common aluminium reference object, segmenting solid material from pore and organic
187 (including root) material. At this high resolution and early growth stage, the roots were
188 readily segmented using an adaptive region growing algorithm, starting from the
189 greyscale value of the user-selected voxel and selecting all connected voxels within the
190 user defined range. The entire segmented root architecture from this point was analysed
191 as a whole. To assess changes to soil structure with distance from the root surface, the
192 surface mesh of the root region was three-dimensionally (3-D) dilated, creating discreet
193 regions moving away from the root in which pore and soil volumes could be calculated.
194 The first one-voxel dilation was subtracted from all subsequent dilations to prevent any
195 mischaracterisation at the immediate root surface due to partial volume effects or noise.
196 The ‘Volume Analyser’ tool was used to assess the volume of pore and soil material
197 within each dilated region, giving porosity profiles (where 1 voxel = 12 μm) for each
198 zone moving away from the root surface. This could be compared to a bulk soil value,
199 taken as the porosity of a large volume of soil observed at the furthest distance away
200 from the root, but without being influenced by the container wall (i.e. in most cases, c. 1
201 cm from the edge). The short growth period of the experiment meant that roots did not
202 interact with the boundary of the container, however to minimise any potential impact
203 of this we excluded material from the edges (c. 2 mm) from the analysis. By this method
204 we analysed the full root system of each plant. No roots overlapped for the imaging
205 undertaken at 8.5 μm , however for the wheat plants scanned at 12 μm , two samples had

206 instances of roots in close proximity or overlapping which were excluded from the
207 study, however as extra samples had been prepared and scanned, n=4 for each treatment
208 was maintained.

209

210 Root diameter was assessed by the novel application of an existing image analysis
211 protocol. A binary image stack of thresholded root material was exported from the VG
212 Studio Max v2.2 volume, and imported into Image J 1.47 (<http://rsbweb.nih.gov/ij/>).
213 Here, 3-D thickness measurements were made on root systems using the BoneJ plugin
214 (Doube et al. 2010). This plugin places sequentially smaller spheres inside the object of
215 interest and each sphere never overlaps the object border or each other. The mean
216 diameter of these spheres is deemed the ‘thickness’, giving a single value for each root
217 system. A subsequent colour heat map can be used to illustrate changes to relative
218 sphere size to give an indication of soil pore thickness change along the root axis.

219

220 Root convex hull can be used to provide a measure of potential soil exploration by
221 different plant root systems (Iyer-Pascuzzi et al. 2010), by assigning straight vertices
222 between the outer most points of the root system. Convex hull was determined by
223 importing the segmented root systems into *RooTrak* software (Mairhofer et al. 2013),
224 using the QuickHull algorithm (Barber et al. 1996) and estimating hull volume using
225 Monte Carlo Integration (Rubinstein 1981).

226

227 *Statistical analysis*

228 All data were analysed in GenStat Release 15.1 (VSN International) using a single-
229 variate linear mixed model (REML), containing all possible interactions as explanatory
230 variables and sample as a random effect. For soil porosity analysis, a REML analysis
231 containing plant species, soil texture, the distance from the root surface and soil bulk

232 density as the fixed model and sample as a random effect was used. Standard residual
233 plots were examined in GenStat to check data normality, with comparisons of means
234 based on least significant differences (L.S.D.) at the $P = 0.05$ and $P=0.01$ probability
235 levels.

236

237 **Results**

238 *The Influence of Root Growth on Rhizosphere Porosity*

239 There was a clear gradient in porosity surrounding the root systems in all treatments
240 after 8 days of growth (Figures 1 and 2), with an enhanced porous zone at the
241 immediate root surface in all samples and treatment specific localised
242 compaction/densification at increased distance from the root. ‘Densification’ was
243 considered as the point at which the porosity of an individual dilated region became
244 statistically the same or lower than that of the bulk soil. The interaction of bulk density
245 x plant species x soil texture x distance from the root surface was significant ($P<0.001$).

246

247 When averaged over all treatments there was a significant increase in soil porosity at the
248 immediate root surface compared to 48 μm away from the root (mean porosity of 47.3%
249 and 26.8 % respectively; Figure 1; Figure 2; $P<0.001$; SE’s available in supplementary
250 figures 1-4), with a significant interaction for plant species x distance from the root
251 ($P<0.05$), soil texture x distance from the root ($P<0.001$) and bulk density x distance
252 from the root ($P<0.001$). Scanning at a higher resolution revealed a clear gap formation
253 around tap and lateral roots in both soil textures (Figure 3), the diameter of which
254 approximately equalled the zones of increased porosity quantified in Figures 1 and 2.
255 Beyond this initial gap formation, changes to porosity at increased distance from the
256 root surface were explained by soil texture and bulk density.

257

258 At a bulk density of 1.2 Mg m^{-3} the loamy sand soil exhibited no further significant
259 change to porosity compared to the bulk soil at increasing distances away from the root
260 surface for any plant species (Figure 1a, c, e). At the same bulk density in the clay loam,
261 there was no significant change in porosity from the bulk soil value for the tomato
262 treatment (Figure 1d), but significant reductions in porosity of 7.5 and 9.5 % compared
263 to the bulk soil value to 23.6 and 23.1 % in the wheat and pea species respectively
264 (Figure 1b, f; $P < 0.001$). This localised densification compared to the bulk soil extended
265 to 0.36 and 0.42 mm from the root surface in the wheat and pea species respectively,
266 with the soil particularly compressed at the 0.1 mm location for both species compared
267 to the root-soil interface.

268

269 At 1.5 Mg m^{-3} , the tomato plants exhibited no further changes in porosity following the
270 initial increase at the immediate root surface in either soil texture (Figure 2c, d),
271 although the differences in the soil porosity profile between the two textures were the
272 most pronounced observed. However, there were significant decreases in porosity in
273 wheat and pea plants in both soil textures (Figure 2a, b, e, f; $P < 0.001$), the magnitude
274 of which were texture specific. In the loamy sand, the wheat and pea plants exhibited
275 decreases in porosity compared to the bulk soil of 5.6 and 4.0 % respectively, with
276 localised soil densification extending to 0.14 and 0.12 mm from the root surface. In the
277 clay loam the wheat and pea plants exhibited greater decreases in porosity of 8.1 and 7.6
278 % respectively compared to the bulk soil than in the loamy sand. Densification of the
279 soil surrounding the root extended further than in the loamy sand, to 0.42 and 0.22 mm
280 from the root surface in the clay loam for the wheat and pea treatments respectively.

281

282 The zone of influence of the root (i.e. the spatial degree of any change in porosity away
283 from the bulk soil) as an isolated dependent variable was significantly influenced by

284 plant species ($P < 0.001$) in the following the order wheat > pea > tomato (means of
285 694.7, 483.9, 21.2 mm³ respectively). Soil texture also significantly influenced the zone
286 of influence ($P < 0.05$), with clay loam having a much higher volume of 511.7 mm³
287 compared to 288.2 mm³ in the loamy sand. The bulk density x texture interaction was
288 significant ($P = 0.05$), with a larger zone of influence in the clay at 1.5 Mg m⁻³ than 1.2
289 Mg m⁻³ (mean values of 630.3 and 402.9 mm³ respectively) but in sand it was the
290 converse (mean values of 225.2 and 334.5 mm³ respectively). In comparison with the
291 lower density soil, the porosity at the root-soil interface and the bulk soil was reduced
292 by between 25-50% in the 1.5 Mg m⁻³ treatment.

293

294 *Impact of soil physical properties on root characteristics*

295 Representative images of root system architecture segmented from the X-ray CT images
296 for the three plant species are provided in Figure 4. Mean root thickness increased with
297 increasing bulk density (0.58 mm vs. 0.74 mm at bulk densities of 1.2 and 1.5 Mg m⁻³
298 respectively; $P = 0.001$), with a significant interaction of bulk density x plant species
299 (Figure 5; $P < 0.005$). Root thickness significantly differed between plant species with
300 the following the order: pea > tomato > wheat (mean thickness values of 1.16, 0.49 and
301 0.34 mm respectively; Figure 5; $P < 0.001$). Root thickness varied significantly with soil
302 type ($P < 0.001$), increasing in the finer textured clay loam (mean thickness of 0.74 vs.
303 0.58 mm in the clay loam and loamy sand textures respectively). The interaction of
304 species x texture was significant ($P = 0.01$). Averaged across all treatments there was no
305 significant effect of root thickness on porosity of the defined rhizosphere region, but a
306 significant interaction of plant species x root zone of influence ($P < 0.005$) and bulk
307 density x plant species x root zone of influence ($P = 0.001$). Note, this is based on
308 analysis of the soil around the roots hence where root architecture varied so did the
309 volume of soil assessed.

310

311 Mean values for convex hull volume were higher in the clay loam than loamy sand
312 (5607 vs. 4060 mm³; Figure 6; P<0.005), and were significantly affected by plant
313 species (convex hull volumes of 7077, 3940 and 3483 mm³ in the wheat, pea and tomato
314 respectively; P<0.001). There were significant interactions of bulk density x soil texture
315 (P<0.05) and bulk density x species x texture (P<0.05). There was a significant
316 relationship between convex hull volume and the volume of the root zone of influence
317 (P<0.001), with mean total volumes of both convex hull and the volume of root zone of
318 influence differing dramatically between plant species (P<0.001) and soil texture
319 (P<0.005) (Figure 6).

320

321 **Discussion**

322 Root growth has a significant impact on soil structure in the rhizosphere which we
323 observed here after very early growth. The extent of soil reorganisation is influenced not
324 only by the plant but also by the soil's physical properties. Previous work has indicated
325 that soil structure in the rhizosphere has key consequences for soil physical (Gregory
326 2006; Hinsinger et al. 2009) and hydraulic processes that directly influence root system
327 development (Carminati et al. 2013; Hallett et al. 2009). While, previous work, such as
328 Aravena et al. (2011), used root analogues to try disentangle the consequences of root
329 growth on structural development in the rhizosphere, an as assessment of real growing
330 roots in field soil on rhizosphere structure evolution has previously been considered not
331 possible. In this study we used X-ray CT to observe the structural development of the
332 rhizosphere across multiple plant species and soil treatments at scales down to 8.5 µm
333 on soil from the field that was structure-less. This approach offer new opportunities to
334 study in situ how plants influence the soil environment to their advantage/disadvantage
335 and how this is affected by different abiotic stresses.

336

337 *Impact of root growth on rhizosphere porous architecture*

338 There was a plant species independent increase in porosity immediately at the root
339 surface, which subsequently declined with distance from the root previously measured
340 by Helliwell et al. (2017). This contrasts with previous work using root analogues
341 (Aravena et al. 2011), which demonstrated a soil densification gradient at the immediate
342 root surface, increasing in porosity with distance from the root. Aravena et al. (2011)
343 acknowledge limitations to their balloon root analogue, in that it consists of an
344 unreactive non-dynamic interface, isolating lateral compressive forces due to radial
345 expansion. Therefore, in a real root system, more dynamic differences in the structural
346 gradients from the root to the bulk soil are expected. Beyond this zone of increased
347 porosity, an increase in densification of the soil was observed, governed by soil texture
348 (Figures 1 and 2). Figure 3b and c highlight the development of cracking behaviour in
349 the clay loam soil, with root-derived cracks radiating from the root surface in all plant
350 species. This is almost certainly due to shrinkage induced by soil drying (Hallett and
351 Newson 2005) and not a sample preparation artefact since great care was taken to
352 ensure the samples were packed as homogeneously as possible following the method of
353 Helliwell et al. (2017). The plastic nature of the clay loam can lead to the formation of
354 localised micro-cracks during root growth, corresponding to and accounting for the
355 increases in porosity quantified at the immediate root surface (Figure 1). The loamy
356 sand texture, which has a much smaller capacity to shrink than clay loam, exhibited a
357 smaller, but measurable shrinkage upon drying at the root surface, linked to a loss of
358 contact which was particularly pronounced in the thicker pea roots (Figure 3a).
359 However, as this soil did not crack, the magnitude of porosity increase, estimated from
360 the CT images, was smaller (Figure 2).

361

362 New lateral root growth was observed in crack shaped pores in the soil, with an
363 apparent preference for growth into pre-existing pore space as opposed to forging new
364 pathways. New root proliferation is known to exploit existing pore channels and fissures
365 where possible (Bengough et al. 2006), due to the relatively unimpeded pathways in
366 these regions compared to denser surrounding soil, although the extent of this can be
367 regulated by the overall soil bulk density (Colombi et al. 2017). Hence root growth
368 often becomes clustered in these channels that by-pass stronger regions of the soil
369 (White and Kirkegaard 2010), creating hotspots of intense water and nutrient uptake and
370 zones of relatively unaffected soil in poorly explored, impenetrable areas (Passioura
371 2002). It is likely that the increased yield observed in some zero tillage systems is due to
372 enhanced root penetration at depth due to an increased frequency of biopores and
373 enhanced pore connectivity (Pittelkow et al. 2015). Roots can also proliferate to locally
374 exploit patches of nutrients (Drew 1975). However, as the soil was homogenised before
375 packing into columns in this investigation we can discount root exploitation of pre-
376 existing nutrient patches. We observed that roots exhibited a clear strategy where lateral
377 roots explore newly formed fissures, potentially as an energy conservation mechanism.
378 This also accounts for a degree of gap formation immediately around the tap and lateral
379 roots (Figure 3b and c), as the roots often failed to fully fill the pores. The importance of
380 gap formation around growing roots was highlighted by Carminati et al. (2013), with
381 the shrinkage of roots responsible for air-filled gaps particularly pronounced around the
382 tap root. However, Carminati et al. (2013) and other previous investigations (Carminati
383 et al. 2009) demonstrated shrinkage of the root as opposed to the soil was the driver for
384 the gap development dynamics. It is possible that shrinkage of the soil was overlooked
385 in previous work due to the coarser resolution (*ca.* 100 μm) thus microscale structural
386 changes were not observed. Also the high sand content (92 %) used by Carminati et al.
387 (2013) would limit shrinkage of the soil itself, a likely factor influencing rhizosphere

388 structure development. The role of root hairs in structural formation is not considered
389 here due to an inability to observe them in these soils at the prescribed moisture content
390 (due to an overlap in X-ray attenuation rather than resolution), although Koebernick et
391 al. (2017) has shown this is possible in a coarse textured soil via synchrotron imaging
392 when considering air-filled pores only.

393

394 Beyond the initial increase in porosity at the immediate root surface, the contrasting
395 porosity changes at distances further from the root surface are also likely to be
396 influenced by the different cohesive properties of the soil. It follows that an apparent
397 lack of densification surrounding roots growing in coarser, less cohesive soil is due to
398 its relative ductility, with freely mobile particles able to be reorganised as the root
399 grows. Conversely the plastic nature of the clay soil creates a readily compressible
400 mass, clearly influenced by root size. The root effect on increasing densification away
401 from the root interface was greater in the highest bulk density treatment and was
402 consistent between the two soil types, though Figure 5 shows that this cannot be
403 explained by root diameter alone.

404

405 We hypothesised a relationship between the thickness of a root, soil bulk density, and
406 the degree and size of its impact on the surrounding physical soil environment, with
407 thicker roots under increased soil bulk density thought to contribute to an increased
408 deformation of rhizosphere soil. The non-significant effect of root thickness as a factor
409 determining the 'zone of influence' shows that root diameter, although reported to
410 increase the ability of roots to penetrate compacted soil (Bengough (1997), does not
411 account for the changes to structure we have observed, once the rhizosphere has
412 developed. The combination of thicker (Figure 4), blunter pea roots under the

413 appropriate soil texture exhibited increased soil deformation compared to tomato and
414 wheat (Figure 1f and Figure 2f). Whilst the degree of structural change was independent
415 of root thickness, the displacement of particles was less than one root diameter in all
416 treatments. This contrasts with Vollsnes et al. (2010) who showed compression of sand
417 in front of the root tip extending up to eight times the root diameter in maize using
418 particle image velocimetry. Aravena et al. (2011) reported lateral densification of *ca.* 8-
419 12% extending to one root diameter in wet aggregates at a resolution of 4.4 μm . In this
420 investigation we observed a similar degree of deformation of *ca.* 4-9% depending on the
421 soil texture and plant species, extending *ca.* 0.5x the root diameter (although root-
422 induced cracking often extended beyond this; Figure 4b and c). It is therefore clear that
423 investigations using artificial sand or a saturated medium may cause differences in the
424 size and magnitude of structural change observed not representative of field soil
425 conditions.

426

427 A common feature we observed was that immediately adjacent to the root, there was a
428 region of increased porosity. This was most likely due some combination of both soil
429 and root shrinkage alongside the thigmotropic response of root development. The way
430 in which particles, especially in structure-less samples, are arranged at the root-soil
431 interface has been proposed to account for the zone of higher porosity (Koebernick et al.
432 2018), and while we cannot discount that this as a contributing factor, it is clear from
433 Figures 3 b-d where a particulate structure is not observed, that this is unlikely to
434 explain our findings. At greater distances from the root there was a compacted region
435 (except for Fig. 1d), which was due to either (i) a legacy of soil deformation at the root
436 tip or (ii) microscale soil shrinkage due to water uptake by the root. Differences in root
437 exudate composition between the plant species are also thought to be important in
438 modifying the physical properties soil (Naveed et al. 2017).

439

440 *Implications for modelling of rhizosphere densification*

441 Dexter (1987) developed a model for the compression of soil surrounding a growing
442 root by assuming soil porosity is reduced adjacent to the root where compression is
443 greatest. This was based on work considering a metal probe as a root analogue entering
444 the soil and expanding to cause a porosity gradient which increased exponentially from
445 the object surface (Dexter and Tanner 1972). This was later supported by experimental
446 work using particle image velocimetry in pure sand at a spatial resolution of 0.5 mm
447 (Vollsnes et al. 2010), where the displacement of sand particles into pores in their
448 immediate vicinity was facilitated by root growth. Our work confirmed the predictions
449 by Dexter (1987) that following root-compression of soil to a minimum porosity and an
450 example of this behaviour is seen in Fig. 3d. However, we more commonly observed a
451 dual-zone impact of root growth on soil structure in the rhizosphere (Figs 1 and 2), with
452 the first corresponding to the increase in porosity at the immediate root surface to an
453 approximate distance of 50 μm , only observable by high resolution imaging and not
454 previously considered in similar modelling approaches. This high porosity zone where
455 root-soil contact is somewhat reduced could have profound implications for soil root
456 interaction: reduced hydraulic conductivity and water flow to the root due to a loss of
457 hydraulic connection, lower nutrient flux to the root especially nitrate and increased
458 aeration. The improved aeration could be of considerable benefit to the root while the
459 effects related to reduced water flux might be compensated by root mucilage production
460 (Carminati et al. 2009).

461 Plant roots donate carbon to encourage the development of beneficial
462 populations of microbes in the rhizosphere. For example, phosphate-solubilising
463 microorganisms can mobilise previously inaccessible pools of this important nutrient for
464 plants (Wang et al. 2016). Microorganisms growing on the root surface contribute to the

465 disruption of soil structure at the root surface that can aid aeration and the pathway for
466 nutrient and water delivery to the root surface (Helliwell et al. 2014). Our finding that
467 the extent of this root surface phenomenon, the zone of influence, differs between
468 species and depends on soil type and density (Figure 6) is worthy of further
469 investigation. For example, pea showed more sensitivity to the soil type when compared
470 with wheat and tomato at higher bulk density (Figures 6cd). In the thicker pea roots
471 (Figure 5) the production of specialised exudates particularly rich in hydroxyproline-
472 rich cell wall glycoprotein when compared with cereals (Knee et al. 2001) may be
473 depend on soil type. There may be the potential to improve this trait in future crop
474 breeding programmes by manipulating root exudate composition. In addition, the
475 considerable differences in root-induced structure around and away from the root
476 surface and the varied response to soil texture and bulk density highlights the needs for
477 plants breeders to undertake studies under more natural conditions when screening for
478 beneficial root traits.

479

480 **Conclusions**

481 Plants modify the soil environment in the rhizosphere very early on during plant root
482 growth. Soils with contrasting textures are deformed by roots in different ways,
483 depending on initial soil bulk density and plant species. X-ray microtomography of
484 loamy sand and clay loam soils showed an increase in the porosity of soil immediately
485 adjacent to the root in all three plant species examined, which was independent of root
486 diameter. Multi-scale scanning at higher resolutions revealed considerable micro-crack
487 formation around roots, attributable to soil shrinkage. However, subsequent deformation
488 and compaction created by root growth was spatially highly heterogeneous, and
489 dependent on a combination of root thickness, higher soil bulk density and finer
490 textured soils. Imaging approaches, such as those demonstrated here could provide a

491 basis for the future development of conceptual root-soil interaction models, especially
492 important as the soil structure in the rhizosphere has implications for the acquisition of
493 water and nutrients by plant roots as they engineer new hydraulic pathways through
494 soils. In addition, they could be used to support the efforts of plant breeders when
495 seeking to identify idealised root traits as the root-modulated soil porous architecture is
496 likely to play as an important a role in root development as the root system itself.

497

498 **Acknowledgements**

499 JRH is funded by a Lawes Agricultural Trust studentship and the University of
500 Nottingham. AJM is supported by grant funding BB/JJ004553/1 from the BBSRC and
501 the John Innes Foundation. WRW is supported at Rothamsted Research by the BBSRC
502 Designing Future Wheat project. SJM and CJS are also supported by the BBSRC
503 Designing Future Wheat project.

504

505 **References**

- 506 Aravena JE, Berli M, Ghezzehei TA, Tyler SW (2011) Effects of Root-Induced
507 Compaction on Rhizosphere Hydraulic Properties - X-ray Microtomography
508 Imaging and Numerical Simulations. *Environmental Science & Technology* 45:
509 425-431. doi: 10.1021/es102566j.
- 510 Atwell B (1988) Physiological responses of lupin roots to soil compaction. *Plant and*
511 *Soil* 111: 277-281. doi: 10.1007/bf02139953.
- 512 Barber CB, Dobkin DP, Huhdanpaa H (1996) The quickhull algorithm for convex hulls.
513 *ACM Transactions of Mathematical Software* 22: 469-483. doi:
514 10.1145/235815.235821.
- 515 Bengough AG (1997) Modelling Rooting Depth and Soil Strength in a Drying Soil
516 Profile. *Journal of Theoretical Biology* 186: 327-338.

517 Bengough AG, Bransby MF, Hans J, McKenna SJ, Roberts TJ, Valentine TA (2006)
518 Root responses to soil physical conditions; growth dynamics from field to cell.
519 *Journal of Experimental Botany* 57: 437-447. doi: 10.1093/jxb/erj003.

520 Bengough AG, MacKenzie CJ (1994) Simultaneous measurement of root force and
521 elongation for seedling pea roots. *Journal of Experimental Botany* 45: 95-102.
522 doi: 10.1093/jxb/45.1.95.

523 Berli M, Carminati A, Ghezzehei TA, Or D (2008) Evolution of unsaturated hydraulic
524 conductivity of aggregated soils due to compressive forces. *Water Resources*
525 *Research* 44: W00C09. doi: 10.1029/2007WR006501.

526 Carminati A, Moradi AB, Vetterlein D, Vontobel P, Lehmann E, Weller U, Vogel H-J,
527 Oswald SE (2010) Dynamics of soil water content in the rhizosphere. *Plant and*
528 *Soil* 332: 163-176. doi: 10.1007/s11104-010-0283-8.

529 Carminati A, Vetterlein D (2013) Plasticity of rhizosphere hydraulic properties as a key
530 for efficient utilization of scarce resources. *Annals of Botany* 112: 277-290. doi:
531 10.1093/aob/mcs262.

532 Carminati A, Vetterlein D, Koebernick N, Blaser S, Weller U, Vogel HJ (2013) Do
533 roots mind the gap? *Plant and Soil* 367: 651-661. doi: 10.1007/s11104-012-
534 1496-9.

535 Carminati A, Vetterlein D, Weller U, Vogel H-J, Oswald SE (2009) When Roots Lose
536 Contact. *Vadose Zone Journal* 8: 805-809. doi: 10.2136/vzj2008.0147.

537 Dexter AR (1987) Compression of soil around roots. *Plant and Soil* 97: 401-406. doi:
538 10.1007/BF02383230.

539 Dexter AR (1988) Advances in characterization of soil structure. *Soil and Tillage*
540 *Research* 11: 199-238. doi: 10.1016/0167-1987(88)90002-5.

541 Dexter AR, Tanner DW (1972) Soil deformations induced by a moving cutting blade,
542 an expanding tube and a penetrating sphere. *Journal of Agricultural Engineering*
543 *Research* 17: 371-375. doi: 10.1016/S0021-8634(72)80045-3.

544 Doube M, Kłosowski MM, Arganda-Carreras I, Cordelières FP, Dougherty RP, Jackson
545 JS, Schmid B, Hutchinson JR, Shefelbine SJ (2010) BoneJ: Free and extensible
546 bone image analysis in ImageJ. *Bone* 47: 1076-1079.

547 Gregory PJ (2006) Roots, rhizosphere and soil: the route to a better understanding of
548 soil science? *European Journal of Soil Science* 57: 2-12. doi: 10.1111/j.1365-
549 2389.2005.00778.x.

550 Hallett PD, Feeney DS, Bengough AG, Rillig MC, Scrimgeour CM, Young IM (2009)
551 Disentangling the impact of AM fungi versus roots on soil structure and water
552 transport. *Plant and Soil* 314: 183-196. doi: 10.1007/s11104-008-9717-y.

553 Helliwell JR, Miller AJ, Whalley WR, Mooney SJ, Sturrock CJ (2014) Quantifying the
554 impact of microbes on soil structural development and behaviour in wet soils.
555 *Soil Biology and Biochemistry* 74: 138-147. doi: 10.1016/j.soilbio.2014.03.009.

556 Helliwell JR, Sturrock CJ, Grayling KM, Tracy SR, Flavel RJ, Young IM, Whalley
557 WR, Mooney SJ (2013) Applications of X-ray computed tomography for
558 examining biophysical interactions and structural development in soil systems: a
559 review. *European Journal of Soil Science* 64: 279-297. doi: 10.1111/ejss.12028.

560 Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics,
561 biogeochemistry and ecological relevance. *Plant and Soil* 321: 117-152. doi:
562 10.1007/s11104-008-9885-9.

563 Iijima M, Kato J (2007) Combined Soil Physical Stress of Soil Drying, Anaerobiosis
564 and Mechanical Impedance to Seedling Root Growth of Four Crop Species.
565 *Plant Production Science* 10: 451-459. doi: 10.1626/pps.10.451.

566 Iyer-Pascuzzi AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, Weitz JS,
567 Benfey PN (2010) Imaging and Analysis Platform for Automatic Phenotyping
568 and Trait Ranking of Plant Root Systems. *Plant Physiology* 152: 1148-1157.
569 doi: 10.1104/pp.109.150748.

570 Kolb E, Hartmann C, Genet P (2012) Radial force development during root growth
571 measured by photoelasticity. *Plant and Soil* 360: 19-35. doi: 10.1007/s11104-
572 012-1316-2.

573 Mairhofer S, Zappala S, Tracy S, Sturrock C, Bennett MJ, Mooney SJ, Pridmore TP
574 (2013) Recovering complete plant root system architectures from soil via X-ray
575 mu-Computed Tomography. *Plant Methods* 9: 8.

576 Materechera SA, Dexter AR, Alston AM (1991) Penetration of very strong soils by
577 seedling roots of different plant species. *Plant and Soil* 135: 31-41.

578 Materechera SA, Dexter AR, Alston AM (1992) Formation of aggregates by plant-roots
579 in homogenized soils. *Plant and Soil* 142: 69-79.

580 Misra RK, Dexter AR, Alston AM (1986) Maximum axial and radial growth pressures
581 of plant roots. *Plant and Soil* 95: 315-326. doi: 10.1007/BF02374612.

582 Mooney S, Pridmore T, Helliwell J, Bennett M (2012) Developing X-ray Computed
583 Tomography to non-invasively image 3-D root systems architecture in soil.
584 *Plant and Soil* 352: 1-22. doi: 10.1007/s11104-011-1039-9.

585 Mooney SJ, Morris C, Craigon J, Berry P (2007) Quantification of soil structural
586 changes induced by cereal anchorage failure: Image analysis of thin sections.
587 *Journal of Plant Nutrition and Soil Science* 170: 363-372. doi:
588 10.1002/jpln.200622042.

589 Moradi AB, Carminati A, Vetterlein D, Vontobel P, Lehmann E, Weller U, Hopmans
590 JW, Vogel H-J, Oswald SE (2011) Three-dimensional visualization and

591 quantification of water content in the rhizosphere. *New Phytologist* 192: 653-
592 663. doi: 10.1111/j.1469-8137.2011.03826.x.

593 Oleghe E, Naveed M, Baggs EM, Hallett PD (2017) Plant exudates improve the
594 mechanical conditions for root penetration through compacted soils. *Plant and*
595 *Soil* 421: 19-30. doi: 10.1007/s11104-017-3424-5.

596 Passioura JB (2002) Soil conditions and plant growth. *Plant, Cell & Environment* 25:
597 311-318. doi: 10.1046/j.0016-8025.2001.00802.x.

598 Pires LF, Borges JAR, Bacchi OOS, Reichardt K (2010) Twenty-five years of computed
599 tomography in soil physics: A literature review of the Brazilian contribution.
600 *Soil & Tillage Research* 110: 197-210. doi: 10.1016/j.still.2010.07.013.

601 Rubinstein RY (1981) Simulation and the Monte Carlo Methods. John Wiley and Sons,
602 New York.

603 Taina IA, Heck RJ, Elliot TR (2008) Application of X-ray computed tomography to soil
604 science: A literature review. *Canadian Journal of Soil Science* 88: 1-20.

605 Tracy SR, Black CR, Roberts JA, Sturrock C, Mairhofer S, Craigan J, Mooney SJ
606 (2012) Quantifying the impact of soil compaction on root system architecture in
607 tomato (*Solanum lycopersicum*) by X-ray micro-computed tomography. *Annals*
608 *of Botany* 110: 511-519. doi: 10.1093/aob/mcs031.

609 Veen BW, Vannoordwijk M, Dewilligen P, Boone FR, Kooistra MJ (1992) Root-soil
610 contact of maize, as measured by a thin-section technique. 3. Effects on shoot
611 growth, nitrate and water-uptake efficiency. *Plant and Soil* 139: 131-138.

612 Vollsnes AV, Futsaether CM, Bengough AG (2010) Quantifying rhizosphere particle
613 movement around mutant maize roots using time-lapse imaging and particle
614 image velocimetry. *European Journal of Soil Science* 61: 926-939. doi:
615 10.1111/j.1365-2389.2010.01297.x.

616 White RG, Kirkegaard JA (2010) The distribution and abundance of wheat roots in a
617 dense, structured subsoil--implications for water uptake. *Plant Cell &*
618 *Environment* 33: 133-148. doi: 10.1111/j.1365-3040.2009.02059.x.

619 Zappala S, Helliwell JR, Tracy SR, Mairhofer S, Sturrock CJ, Pridmore T, Bennett M,
620 Mooney SJ (2013) Effects of X-Ray Dose On Rhizosphere Studies Using X-Ray
621 Computed Tomography. *PLoS ONE* 8: e67250. doi:
622 10.1371/journal.pone.0067250.

623

624 **List of Figures**

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627 moving away from the root surface. Error bars represent standard errors of 4 replicates.

628

629 Figure 2 – Porosity distributions at a bulk density of 1.5 Mg m^{-3} for wheat (a, b), tomato
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632

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641

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645

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651

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