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2018. Phacelia tanacetifolia affects soil structure differently depending on  
soil texture. *Plant and Soil*.

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# Plant and Soil

## Phacelia (*Phacelia tanacetifolia* Benth.) affects soil structure differently depending on soil texture --Manuscript Draft--

<b>Manuscript Number:</b>	PLSO-D-18-01941R2	
<b>Full Title:</b>	Phacelia ( <i>Phacelia tanacetifolia</i> Benth.) affects soil structure differently depending on soil texture	
<b>Article Type:</b>	Manuscript	
<b>Keywords:</b>	cover crop; Phacelia; soil pore connectivity; soil porosity; x-ray computed tomography; 3D image analysis	
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	Karl Ritz	
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<b>Funding Information:</b>	Biotechnology and Biological Sciences Research Council (BBS/E/C/00010310)	Prof John Crawford
	Biotechnology and Biological Sciences Research Council (BBS/E/C/00010130)	Prof John Crawford
<b>Abstract:</b>	<p><b>Aims:</b> We studied the effects of <i>Phacelia tanacetifolia</i>, increasingly used as a cover-crop species in arable agricultural systems, upon soil structural properties in the context of two contrasting soil textures. We hypothesised there would be differential effects of the plants upon soil structure contingent on the texture.</p> <p><b>Methods:</b> A sandy-loam and a clay soil were destructured by passing through 2 mm sieves, and planted with <i>Phacelia</i> in a replicated pot experiment, with associated unplanted controls. X-ray Computed Tomography was used to visualise and quantify the soil pore networks in 3D.</p> <p><b>Results:</b> For the sandy-loam soil, there was no impact of plants upon aggregate size distribution porosity, pore connectivity, and pore surface density decreased in the presence of plants, whereas for the clay, there was a significant increase of aggregates &lt;1,000 µm, the porosity was constant, the pore-connectivity decreased, and surface density increased in the presence of plants.</p> <p><b>Conclusions:</b> Plants can impact the structural genesis of soil depending on its inherent textural characteristics, leading to a differential development of pore architecture in different contexts. These results have implications both from an ecological perspective and in terms of the prescription of plants to remediate or condition soil structure in managed systems.</p>	
<b>Response to Reviewers:</b>	Comments from the section editor	

Your paper was sent for review, because there were inconsistencies in the manuscript, which I outline below. Unfortunately, none of the original reviewers were available. However, I ask that you take account of these additional comments during your revision. Some of the points of the reviewer reflect seem confusion over porosity. In my view, the fact that your porosity is for pores >40um still does not come across clearly.

There are inconsistencies in your paper about the effect on aggregation.

On Line 25 you say “The presence of plants did not affect the aggregate size distribution for both textures during the time frame of the experiment (6 weeks).”  
> Apologies, a slip here and this sentence was incorrect, as for the clay soil, plants did impact the ASD. And this then leads to the subsequent confusion. We have amended texts appropriately. (Line 25-29)

“For the sandy-loam soil, there was no impact of plants upon aggregate size distribution porosity, pore connectivity, and pore surface density decreased in the presence of plants, whereas for the clay, there was a significant increase of aggregates <1,000 µm, the porosity was constant, the pore-connectivity decreased, and surface density increased in the presence of plants.”

But in the results section, you do report effects of plant growth on aggregate size distribution.

Line 229 “At Week 0, the aggregate size distribution of the sandy loam showed an increasing proportion of aggregates in size class 53-500 µm, followed by a reverse of this trend for aggregates >2,000 µm (Fig. 5a).” For other soils you also report effects on aggregate size distribution.

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“At Week 6, this pattern was still manifest, and there was no significant effect of plants (P>0.05; Fig. 5b)”

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THIS NEEDS TO BE SORTED OUT. YOU CAN NOT CLAIM ONE RESULT AND THEN DISCUSS A COMPLETELY DIFFERENT RESULT! – Your comment on line 350 agrees with your results, but the discussion does not.

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L56 delete “Moreover”

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Line 74 what does “need to grow” mean. Plants cannot decide to grow.

> We have modified the wording (Line 73)  
"root systems must grow deeper in order to access water"

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> A vugh by definition is a 'small cavity. In soil micromorphology terms it is classified as an 'irregular shaped pores' Bullock and Murphy (1983) Soil Micromorphology. This has been added to the text to make clearer (Line 193-194).

"In micromorphology terms a vugh is classified as an "irregular shaped pores" (Bullock and Murphy 1983)."

Line 244 delete "As would be expected" and "profoundly"

> Done

Line 360 from "Sandy soils" onwards is not a conclusion of your study.

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I have decided on minor revisions, but I do expect all of this points, including those of the reviewer to be dealt with and a full response submitted.

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Specific comments

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> The center of the column was extracted, i.e. 3 cm at the top and 2 cm at the bottom were excluded from the analysis.

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This explanation has been added to the text (Line 153-160).

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> Here, we choose to do a very long scan time to have a better image quality which enabled us to have a clear difference between the grayscale of root and pore space.

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> The process was sieving, as described in M&M. We favour the 'destructuring' term since it emphasizes that the structure of the soil was experimentally altered and detection of restructuring was then indicative of a genesis of new structure. We have added 'sieving' as well to clarify (Line 192).

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distribution at week 6 experienced on cycle of wet and dry because the soils were maintained at -30 Kpa at tension table in plant growing period and air-dried before aggregate size distribution measurement at week 6.

> Yes, but this was the same for week 0, the column at week 0 were packed, saturated and drained on the tension table and settle prior sowing as well. There were destructively harvested at the same time as the other column were sown. And here, the wet and dry cycle was referring the wet and dry cycle during the experiment (while the plants were growing through the soil).

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> Yes, but as the editor pointed out in the first round of revision "It will not be possible for roots to elongate in soils with a porosity as low as 10% (see fig. 2); this would be a density in the region of 2.4 g/cm<sup>3</sup>", so it means that if there was a decrease of the porosity of the pores > 40 µm then it should be an increase of the pore < 40 µm because the bulk density was not modified during this experiment.

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> We state this since it encapsulates the key finding of the study.

Fig.1 Some examples of 3D pore networks can be presented as well.

>We appreciate the referee's point that such data as we have collected can be also presented as a 3D visualization. In this case however, because of the very high numbers of small pores, these images are not easily interpreted and as such do not add to the narrative. We believe the single 2D slices we show exhibit the treatment differences most clearly for the reader. In previous studies some authors have shown 3D pore networks but removed all of the small pores to make treatment differences clearer, however this would become an artefact in this case as our observations are focused at this fine scale.

**Aurelie Bacq-Labreuil**  
University of Nottingham  
Sutton Bonington Campus  
Loughborough  
Leicestershire LE12 5RD  
United Kingdom

Thursday, 10<sup>th</sup> of April 2019

Dear Richard Whalley,

Re: PLSO-D-18-01941

Thank you for your further correspondence in relation to our manuscript. We are pleased to submit a revised MS and narrative to the review comments for your further attention. We would like to thank the editor and reviewer who scrutinised the manuscript.

Thank you for your attention.

Yours faithfully,

A handwritten signature in black ink, appearing to read 'A. Bacq-Labreuil', with a horizontal line underneath.

Aurelie Bacq-Labreuil (on behalf of all authors).

## Comments from the section editor

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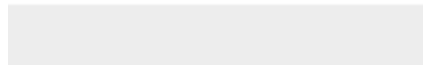
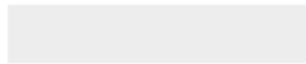
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3

4 NAME(S) OF AUTHOR(S): A. Bacq-Labreuil<sup>a</sup>, J. Crawford<sup>b</sup>, S. J. Mooney<sup>a</sup>, A.L.

5 Neal<sup>b</sup>, K. Ritz<sup>a</sup>

6 Affiliation:

7 <sup>a</sup>Division of Agriculture & Environmental Sciences, School of Biosciences, University  
8 of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK

9 <sup>b</sup>Department of Sustainable Agriculture Science, Rothamsted Research, West Common,  
10 Harpenden, AL5 2JQ, UK

11

12 \*Corresponding Author: [aurelie.bacqlabreuil@gmail.com](mailto:aurelie.bacqlabreuil@gmail.com)

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15

16 **Abstract**

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25 **Results:** For the sandy-loam soil, there was no impact of plants upon aggregate size  
26 distribution porosity, pore connectivity, and pore surface density decreased in the  
27 presence of plants, whereas for the clay, there was a significant increase of aggregates  
28 <1,000  $\mu\text{m}$ , the porosity was constant, the pore-connectivity decreased, and surface  
29 density increased in the presence of plants.

30 **Conclusions:** Plants can impact the structural genesis of soil depending on its inherent  
31 textural characteristics, leading to a differential development of pore architecture in  
32 different contexts. These results have implications both from an ecological perspective  
33 and in terms of the prescription of plants to remediate or condition soil structure in  
34 managed systems.

35 **Introduction**

36 In terrestrial systems, soil is the fundamental base which supports vegetation growth  
37 (van Breemen 1993), but plants also affect the nature of their belowground habitat both  
38 directly and indirectly. In agricultural systems, the use of cover crops is increasing  
39 (Storr et al. 2019) in order to increase the sequestration of carbon (Reicosky and  
40 Forcella 1998; Scott et al. 2017), soil macro-porosity (Abdollahi et al. 2014; Bodner et  
41 al. 2014; Burr-Hersey et al. 2017; Cercioğlu et al. 2018) and decrease soil erosion  
42 (Reicosky and Forcella 1998; Storr et al. 2019). Furthermore, cover crops have an  
43 impact on the biota of the soil, increasing microbial diversity and richness (Patkowska  
44 and Konopiński 2013; Fernandez et al. 2016) and the abundance of saprophytic and  
45 mycorrhizal fungi (Six et al. 2006; Duchene et al. 2017; Finney et al. 2017). In a  
46 restored grassland, roots and fungi increased the proportion of carbon sequestered in  
47 aggregate (Scott et al. 2017), however, there was no measurement of the pore network,  
48 and the characterisation of the soil structure was via aggregate size. Bodner et al. (2014)  
49 showed that cover crops with different root architectures induced different porosity and  
50 pore size distributions determined via water infiltration (i.e. a destructive method). The  
51 physical structure of the soil was not visualised. X-ray Computed Tomography is a non-  
52 destructive method which image the soil structure as well as the roots (Zhou et al. 2016;  
53 Cercioğlu et al. 2018; Rabot et al. 2018; Schlüter et al. 2018). A recent study revealed  
54 contrasting responses between species in their root morphology to changes in bulk  
55 density (Burr-Hersey et al. 2017), but presented little information on associated soil  
56 structure. Cover crops and biofuel crops can improve soil pore characteristics via  
57 increasing the macro-porosity and decreasing soil bulk density (Cercioğlu et al. 2018).  
58



59 Soil structure is classically defined as the arrangement of soil particles and organic  
60 materials (Tisdall and Oades 1982), typically creating a dynamic and heterogeneous  
61 pore network within the soil matrix (Dexter 1988). The nature of this pore network is to  
62 a large extent underpinned by soil texture, but it can also be affected by other factors  
63 such as the actions of living organisms, wet:dry and freeze:thaw cycles, etc. (Ritz &  
64 Young, 2011). A recent study revealed tomato root architecture was markedly different  
65 for plants after 8 days of growth dependant on soil texture: plants developed a thick tap  
66 root in sandy loam soil but grew thinner roots with more laterals in clay soil (Helliwell  
67 et al. 2017). Furthermore, the porosity of the rhizosphere of the sandy loam soil was  
68 decreased whereas for the clay loam soil it was increased. Thus, the root growth  
69 strategies of plants are influenced by the surrounded environment. In non-cohesive and  
70 coarser soil, root systems generally develop to greater depth and are thicker than roots  
71 growing in a cohesive, finer textured soil (Hacke et al. 2000; Jackson et al. 2000; Li et  
72 al. 2005). Non-cohesive and coarser soil dries at greater rates in the upper layer,  
73 therefore the root systems must grow deeper in order to access water (Jackson et al.  
74 2000). The influence of plants on soil structural dynamics is also dependant on soil  
75 texture: in a silty-clay soil the presence of plant can increase the porosity and pore  
76 connectivity compared to a sandy soil where the presence of plants can decrease the  
77 porosity and pore-connectivity (Bacq-Labreuil et al. 2018). However, the effects of soil  
78 texture upon the impact of plants upon soil structural dynamics is not well understood.  
79 Hydraulic properties in finer textured soils are considerably different due to the  
80 enhanced water holding in finer pores (Saxton et al. 1986). Plant roots modify the  
81 aggregation of soil particles, generally acting to generate and stabilise aggregates  
82 (Tisdall and Oades 1982). This occurs by processes of enmeshment of soil particles and  
83 excretion of mucilage and other extra-cellular polymeric substances which adhere

84 constituents together (Bronick and Lal 2005; Erktan et al. 2018; Gould et al. 2016).  
85 Indirect mechanisms are mediated by interactions with soil biota also serve to drive  
86 aggregation processes such as excretion of extracellular substances (Haynes and Beare  
87 1997; Rillig et al. 2002; Ritz and Young 2011). Root mucilage stabilises aggregates by  
88 increasing cohesion and decreasing wetting rates of aggregates (Czarnes et al. 2000).  
89 The inherent diversity of plant species means that the soil is frequently exposed to an  
90 increase in the diversity of root architecture within the matrix (e.g. tap, fibrous, fine  
91 roots), an increase in the quality and quantity of carbon inputs, and considerable  
92 differentiation in the microbial communities associated with the root systems (Haynes  
93 and Beare 1997; Chan and Heenan 1999; Rillig et al. 2002; Gould et al. 2016).

94

95 The aim of this study was to establish the effect of soil texture and plant growth on early  
96 stage soil structural genesis. We grew *Phacelia tanacetifolia*, a herbaceous plant  
97 commonly used as a cover crop in arable rotations and apocryphally thought to be  
98 particularly effective in conditioning soil structure, in a sandy loam and clay soil, along  
99 with unplanted control treatments. We hypothesised that (i) the plant roots have a  
100 contrasting effect on soil structure (via the modification of aggregate distribution and  
101 pore network) depending on the soil texture; and (ii) the presence of a plant increases  
102 the porosity, pore-connectivity, and diversity of pore sizes.

103

104

## 105 **Materials and methods**

106 Preparation of soil cores

107 Soil from the Newport series, a sandy loam (clay: 9.5%, silt: 26.1%, sand: 65.3%;

108 organic matter 2.9%, pH 6.3; FAO Brown Soil) and soil from the Worcester series, a

109 clay (clay: 43.3%, silt: 28.4%, sand: 28.3%; pH 6.5, organic matter 5.2%, pH 6.5; FAO  
110 Argillic Pelosol) were collected from the top 50 cm of arable fields situated in Bunny,  
111 Nottinghamshire, UK (52.52 °N, 1.07 °W). After collection, the soils were spread and  
112 left to air-dry over two days before being thoroughly mixed and broken down by  
113 passing through a 2-mm mesh sieve. Columns comprised of polypropylene tubes (170  
114 mm height x 68 mm diameter) with a 0.1 mm mesh affixed to the base were packed  
115 with soil to a bulk density of 1.2 g cm<sup>-3</sup>. Columns were placed on a tension table for  
116 saturation for 24 h and then equilibration for 3 days at -3 kPa prior to seed sowing  
117 which is equivalent to a moisture of 30 % (± 2 %) for the clay and 20 % (± 1 %) for the  
118 sandy loam. Pre-germinated seeds of *Phacelia tanacetifolia* Benth. cv. “Angelia” were  
119 planted in the soil surface and adjusted to provide one emergent plant per column. Four  
120 planted and four unplanted replicates of each soil type were established and arranged in  
121 a randomised block design in a growth chamber providing 16:8 h light:dark cycle at  
122 21°C:50% humidity, 15°C:75% humidity respectively and the moisture content was  
123 kept constant by maintaining the plants on a tension table at -3kPa. Plants were grown  
124 for 6 weeks since at this age they were fully pot-bound.

125

## 126 X-ray Computed Tomography (CT)

127 All columns were X-ray CT scanned prior to sowing seeds, and at 2, 4 and 6 weeks  
128 thereafter, using a Phoenix v | tome | x M scanner (GE Measurement and Control  
129 solution, Wunstorf, Germany) set at a voxel resolution of 40 µm, the voltage of 180 kV  
130 with a current of 180 µA. A total of 2,160 projection images were collected for each  
131 scan at an exposure time of 250 ms period using an averaging of 3 images and skip of 1,  
132 resulting in a total scan time of 90 min. The scanning time was chosen to optimise the

133 image processing with greater quality of image. Scans occurred over 4 days with  
134 treatments randomly allocated over this period but consistent between the three  
135 occasions.

136 All scanned images were reconstructed using Phoenix datos | x2 rec reconstruction  
137 software. The scanned images were optimised to correct any sample movement during  
138 the scan and reduce noise using the beam hardening correction algorithm, set at 8. Here,  
139 beam hardening was set at 8, due to previous tests which gave the best image quality.

140 As a multi-scan routine was performed on the core samples, VG StudioMax<sup>®</sup> 2.2 was  
141 used to merge the top, middle and bottom scans to obtain a single 3D volume for each  
142 complete core. Image sequences of 40 x 40 x 120 mm were extracted for image  
143 analysis.

144

145 Image analysis

146 Pre-processing of the image sequences was performed using Image J (Schneider et al.  
147 2012). This step was used to crop the image sequence, apply a median filter (averaging  
148 2 pixels), enhance brightness and contrast, and selected two threshold values manually.

149 The threshold and the 3D calculation was implemented in QuantIm (Vogel et al. 2010),  
150 following a standard method detail in Bacq-Labreuil et al. (2018), described briefly  
151 here. The segmentation of the pore networks was realised in 3D, and only included the  
152 pores and left out the root materials. The threshold was facilitated by the long scanning  
153 procedure which enhanced the image quality. The threshold used here is a 3D threshold  
154 using an neighbour-algorithm, i.e. the software requires 2 threshold values ( $T_1 < T_2$ ) and  
155 compares every voxel greyscale value ( $T_i$ ) to this two values. If  $T_i < T_1$ ,  $T_i$  is attributed  
156 to the pore phase, if  $T_i > T_2$ ,  $T_i$  is attributed to the solid phase and if  $T_1 < T_i < T_2$ ,  $T_i$  is  
157 attributed to the fuzzy regions. When all the voxels are attributed to each of the three

158 phases, then the software compares the voxel from the fuzzy regions to their  
159 neighbours: if one of  $T_i$  neighbour belongs to the pore space, then  $T_i$  is attributed to the  
160 pore phase otherwise  $T_i$  stays in the fuzzy region. This step is repeated until no changes  
161 can be made, all the voxel in the fuzzy region is attributed then to the solid phase. The  
162 quantification of the 3D pore network was performed by QuantIm (Vogel et al. 2010).  
163 In summary, the following Minkowski function which characterised 3D pore network,  
164 were collected using QuantIm: porosity of the selected volume was the percentage of  
165 the pores greater than 40  $\mu\text{m}$ , here referred as the porosity; pore size distribution,  
166 expressed here as a cumulative value, was the proportion of each size class in the  
167 volume; pore connectivity expressed by the Euler number, with a negative Euler  
168 number is associated with greater pore connectivity; pore surface density which is the  
169 pore-solid interface, a greater surface density suggests a larger roughness of the pore  
170 edges (Vogel et al. 2010).

171

## 172 Sampling and measurements

173 After 6 weeks, the columns were destructively harvested, and the soil air-dried.  
174 Aggregate size distribution was determined by passing 250 g of air-dried soil through a  
175 sieve series of 2000, 1000, 710, 500, 425, 300, 212 and 53  $\mu\text{m}$ , via horizontal shaking  
176 for 3 minutes at 300 rotations  $\text{min}^{-1}$ . The mass of aggregates retained on each sieve was  
177 determined and normalized to the total mass (Kézdi 1974).

178

## 179 Statistical analysis

180 All statistical analyses were conducted using Genstat version 17.1 (VSN International  
181 Ltd., 2014). For aggregate size distribution, at Week 0, a one-way analysis of variance  
182 (ANOVA) was performed to assess the difference in soil mass between size classes at

183 Week 6, and for porosity a two-factor repeated-measures RM-ANOVA was used to  
184 assess the effects of plant status and either size class or time. A three-way RM-ANOVA  
185 was performed on all primary variables using a split-plot design with soil type, plant  
186 status and size classes of pores as factors.

187

## 188 **Results**

189 Both soils showed contrasting pore architectures (Fig. 1a, c). For the sandy soil, the  
190 pores were primarily compound-packing pores that were typically a similar small and  
191 well distributed through the soil profile (Fig. 1a). However, for the clay soil, pores were  
192 larger as a result of the destructuring (sieving) process, typically vugh-shaped and more  
193 heterogeneously distributed than the sand soil (Fig. 1c). In micromorphology terms a  
194 vugh is classified as an “irregular shaped pores” (Bullock and Murphy 1983). The  
195 growth of *Phacelia* after 6 weeks induced cracks in the soil surrounding the primary  
196 root, but were more apparent in the clay soil (Fig. 1b, d, e). Cracks were apparent,  
197 principally associated with primary roots within the soil profile (Fig. 1b, d) or with  
198 lateral roots growing through aggregates in the clay soil (Fig. 1e).

199

### 200 Pore characteristics

201 In the sandy loam soil, porosity decreased between Week 0 and Week 2 but not  
202 thereafter for the unplanted soil, whilst in planted soils there was a consistent decrease  
203 in porosity across Weeks 0-6 (time x treatment interaction  $P < 0.05$ ; Fig. 2a). In the clay  
204 soil, porosity was less in planted treatments at Week 0, similar at Week 2 and greater in  
205 planted soils at Week 6 than unplanted treatments (time x treatment interaction  
206  $P < 0.001$ ; Fig. 2b).

207 Minkowski functions only showed significant changes with respect to pore diameters of  
208 <0.3 mm for both sandy loam and clay soils (Figs. 3 & 4). For sandy loam there was a  
209 significant pore size diameter x treatment x time interaction term with respect to all pore  
210 size distribution, pore connectivity and pore surface density ( $P \leq 0.01$ ). Whilst this effect  
211 was statistically significant with respect to pore size distribution, in numerical terms the  
212 effects were minor, and barely discernible when plotted (Fig. 3 a-c). Approximately  
213 90% of the pore sizes in all cases were  $\leq 0.16$  mm (Fig. 3 a-c). The connectivity function  
214 of unplanted soils decreased significantly between Weeks 0 and 2, with only a modest  
215 increase by Week 6. However, on these occasions, plant effects on connectivity differed  
216 depending on pore size. At Week 2, pores <0.1 mm were more connected in planted  
217 soils but not above this size. By Week 6 this relationship changed such that pores <0.1  
218 mm were less connected, and those in the range 0.1-0.25 mm were more connected in  
219 planted soils. Pore surface density decreased for both unplanted and planted soils  
220 between Week 0 and Week 2 but with a greater magnitude for unplanted soils, and with  
221 this decline continuing in planted soils to Week 6 (Fig. 3 j-l).

222 For the clay soil, there was no significant three-way interaction term with respect to  
223 pore size distribution ( $P > 0.05$ ; Fig. 4 a-c), but there was for pore connectivity and pore  
224 surface density ( $P < 0.001$ ; Fig. 4 d-l). Overall, approximately 80% of the pore sizes for  
225 both treatments were  $\leq 0.25$  mm (Fig. 3 a-c). At Week 0, the pore connectivity of the  
226 unplanted soils was substantially greater than the planted soils for pores in the 0.05-0.1  
227 mm size range (Fig. 4d). Over the subsequent 6 weeks, pore connectivity in planted and  
228 unplanted soils converged to parity (approximately  $0.23 \text{ mm}^{-1}$ ; Fig. 4 d-f), leading to a  
229 significant interaction. Pore surface density of unplanted soils was greater than planted  
230 soils by up to 0.3 mm at Week 0. By Week 2, pore surface density functions had

231 decreased and converged for both treatments, and by Week 6 was significantly smaller  
232 for pores <0.2 mm in unplanted soils (Fig. 4 j-1).

233

234 Aggregate size distribution

235 At Week 0, the aggregate size distribution of the sandy loam showed an increasing  
236 proportion of aggregates in size class 53-500  $\mu\text{m}$ , followed by a reverse of this trend for  
237 aggregates >2,000  $\mu\text{m}$  (Fig. 5a). This trend was interrupted at 425-500  $\mu\text{m}$ , where this  
238 size class constituted a significantly smaller proportion than neighbouring classes (Fig.  
239 5a). There was an extremely low proportion of aggregates > 2,000  $\mu\text{m}$  (approximately  
240 0.4%, Fig. 5a). At Week 6, this pattern was still manifest, and there was no significant  
241 effect of plants ( $P>0.05$ ; Fig. 5b). For the clay soil, there was a general trend of an  
242 increase in proportion of aggregates with increasing size class, but a substantial increase  
243 for pores >1,000  $\mu\text{m}$ , with the greatest proportion >2,000  $\mu\text{m}$  (Fig. 5c). This pattern  
244 persisted at Week 6, where there was a significant effect of plants with respect to  
245 aggregates >1,000  $\mu\text{m}$ ; planted soils had a significantly greater proportion of aggregates  
246 1-2 mm than unplanted soils, but this pattern was reversed for aggregates >2,000  $\mu\text{m}$   
247 ( $P<0.05$ ; Fig. 5d).

248

## 249 **Discussion**

250 Whilst the organic matter content was lower in the sandy soil, this is essentially  
251 inevitable for similarly-managed and co-located clay versus sandy arable soils, and the  
252 primary difference between the soils used in this study was textural. The nature of the  
253 aggregate size distribution was different between the textures: approximately 80 % of  
254 all aggregates were >1,000  $\mu\text{m}$  for the clay, whereas in sandy loam soil the aggregate  
255 sizes were more evenly distributed throughout the sizes <2,000  $\mu\text{m}$  with 0.5 % of



256 aggregate sizes  $>2,000 \mu\text{m}$  (Fig. 5). For the clay soil, the larger proportion of aggregates  
257  $>1,000 \mu\text{m}$  can be attributed to the greater proportion of clay particles due to their  
258 capacity to bound together (Tisdall and Oades 1982; Dexter 1988; Blake et al. 2003).  
259 The presence of plants did not impact the aggregate size distribution in the sandy loam  
260 soil. This may be due to a lack of any substantial wet:dry cycles imparted, which is  
261 known to stabilise aggregate (Bronick and Lal 2005) as the samples were held at a fixed  
262 water potential in this experiment. During wetting, water can disperse or swell clay  
263 particles which leads to increased contact between clay and other particles, and  
264 therefore binding during the drying phase (Singer et al. 1992). Furthermore, sandy loam  
265 soil contained a low proportion of clay (9.5%), which is representative of a non-  
266 cohesive soil. Thus in non-cohesive soil, the binding due to the presence of clay is  
267 reduced leading to a reduction of the root action on the aggregation (Degens et al. 1994;  
268 Six et al. 2004). We wished to avoid such effects in this study in order to investigate the  
269 inherent effects of the plant on structural genesis. Hence in both soils, the water regime  
270 was constant during the experiment, thus the change in wet and dry cycles were not  
271 responsible for the greater proportion of aggregates  $>2,000 \mu\text{m}$  observed in the  
272 unplanted treatment for the clay soil. Thus, the aggregation in the unplanted treatment  
273 might be due to other biotic factors, such as microbial activity. The planted soils  
274 showed a decrease in the percentage of aggregate sizes  $>2,000 \mu\text{m}$  and an increase in  
275 the percentage of aggregate sizes  $1,000\text{-}2,000 \mu\text{m}$  (Fig. 5). The greater proportion of  
276 aggregates sizes between  $1,000\text{-}2,000 \mu\text{m}$  in the planted soil might have resulted from  
277 fragmentation of bigger aggregates by root penetration or development via root action,  
278 and localised wet-dry cycles induced by the presence of plants (Materechera et al. 1994;  
279 Chan and Heenan 1996; Jin et al. 2013). However, the moisture content of the column  
280 was kept constant during the experiment via the use of a tension table, and the

281 transpiration rates of plants was not measured. Such localised effects might have  
282 induced a rearrangement of the clay particles around the roots and modified the  
283 aggregate size distribution (Reid and Goss 1982; Six et al. 2004; Gregory et al. 2009).  
284 Therefore, in the more cohesive soil, roots appear to generate fragmented aggregates,  
285 which may facilitate water infiltration or drainage within the aggregates (Fig. 1e;  
286 Materechera et al. 1994). This in turn would have arguably positive effects upon water  
287 availability to the plants through the generation of a wider pore sizes from sizes  
288 between 0.05 and 0.16 mm, which are associated to the transmission pores (Metzger  
289 and Yaron 1987; Watts and Dexter 1997).

290 For both soil textures, a decrease in porosity was observed in unplanted soil at Week 2  
291 (from 14.9 to 8.9% for the sandy loam soil and from 10.4 to 8.2% for the clay soil)  
292 which maintained constant until Week 6 (Fig. 2) which is most likely a consequence of  
293 settling of the soil due to gravity. Moreover, the presence of cracks observed in both  
294 columns was attributed to the root action as the water content was controlled (Fig. 1).  
295 This observation corroborates with a recent study that showed cracks associated with  
296 root formation (Helliwell et al. 2019). However, soil texture profoundly influenced the  
297 soil structural development of planted soil: in sandy loam soil, porosity decreased  
298 constantly over the 6 weeks (from 15.4 to 7%) whereas, in clay soil, the porosity stayed  
299 constant over the 6 weeks (approximately 7.8%). For the sandy soil, the decrease of the  
300 porosity could have been induced by the rearrangement of soil particles which increased  
301 pores  $<40\ \mu\text{m}$  and these pores were not included in the measured porosity. Furthermore,  
302 the results from the sandy loam soil was consistent with a previous study which  
303 observed, a decrease of porosity in rhizosphere soil induced by root growth of tomato  
304 plants for the same soil texture (Helliwell et al. 2017). However, the results for clay  
305 soils are divergent from Helliwell et al. (2017) who detected an increase of rhizosphere

306 porosity in this case. The impact of plants on the bulk soil, here measured for pores >40  
307  $\mu\text{m}$  resolution, could be slower compared to the rhizosphere porosity, measured at >12  
308  $\mu\text{m}$  resolution (Helliwell et al. 2017). This observation was also observed at the field  
309 level: the presence of plants decreased the porosity of a sandy soil compared to the  
310 increase of the porosity for a clay soil (Bacq-Labreuil et al. 2018). Therefore, the  
311 indications are that a plant can modify soil structure differently depending on the soil  
312 texture. The results for the sandy loam soil was consistent with another study which  
313 showed plants growing at a bulk density of  $1.2 \text{ g cm}^{-3}$  decreased the soil porosity  
314 (Martin et al. 2012). However, these results are divergent from Feeney et al. (2006) for  
315 the soil of the same textural class, at a bulk density of  $1.3 \text{ g cm}^{-3}$ , where the presence of  
316 plants and soil microbiota increased the porosity. Our results suggest that the initial  
317 configuration of the pore network, defined by soil texture and bulk density, affects  
318 subsequent root growth responses and the associated impacts of roots on soil structural  
319 genesis.

320 The results obtained via X-ray CT imaging contrasted with those of the aggregate size  
321 distributions. In the sandy loam soil, there was no significant impact of the plants upon  
322 soil aggregation whereas plants significantly affected the pore network. In comparison,  
323 for the clay soil, there was a significant increase of aggregates <1,000  $\mu\text{m}$ , while the  
324 plants induced a constant porosity. These observations show that the aggregate size  
325 distribution metrics concealed information regarding the *in situ* soil structure.

326 Neither soil texture showed a significant plant effect on pore size distribution or pore  
327 connectivity after 6 weeks growth. A longer experiment might have revealed a greater  
328 influence of plants on soil structural genesis. In the sandy loam soil, the presence of  
329 plants decreased the pore surface density, i.e. decreasing pore-solid interfaces (Fig. 3 g-  
330 i). This meant the presence of plants reduced the irregular shaped-pores or elongated

331 pores within the pore network (Vogel et al. 2010; Bacq-Labreuil et al. 2018). In clay  
332 soil, the pore solid interface increased in the planted soils (Fig. 4 g-i), which suggests  
333 that elongated or irregular shaped-pores increased within the pore network. The  
334 formation of more irregular-shaped pores would likely influence the microbial  
335 community due to the creation of new habitats and a wider range of niches (Holden  
336 2011). A more diverse pore structure and heterogeneity in pore morphology can also  
337 affect soil hydrology, via modifying water flow at a local scale and the nature of water  
338 film continua. Therefore, the same plant genotype had two distinctive effects upon the  
339 modification of pore morphology depending on the inherent soil texture. Therefore, the  
340 prescription of crops for specific characteristics such as root morphology,  
341 rhizodeposition, might be better informed by consideration of the soil texture in which  
342 they are grown. Especially that the same plant species is affected differently depending  
343 on soil textures. This characteristic might be important for breeders and farmers in order  
344 to prescribe plant species that are optimal for the needs of the farmers and depending on  
345 the soil texture.

346 Therefore, farmers, depending on their requirements (such as water management,  
347 compaction, etc) could prescribe different plant species depending on their  
348 characteristics, but taking in account the soil texture. Sandy soils are usually free  
349 draining, thus there may be an adaptive advantage where roots reduce the porosity in  
350 soils in which they are growing, which will likely increase the retention of water.

351 Therefore, cover crops could potentially be used to prime soil structure before sowing  
352 the main crop, specifically in sandy soil to enhance the retention of water, and in clay  
353 soils to increase water transmission. Further studies are required to understand whether  
354 different plant species affect such soil structural dynamics in different ways (Ehrmann  
355 and Ritz 2013; Erktan et al. 2018). We postulate this is likely given the diversity of root

356 morphologies, rhizodeposition patterns and higher-order interactions between plants and  
357 soil biota. These observations also have implications from an ecological perspective, for  
358 example in the way vegetation may modulate soil structural dynamics during  
359 successional processes, which appears to have been barely considered.

360

### 361 **Conclusions**

362 This study revealed a contrasting effect of soil textural characteristics on soil structural  
363 genesis. The results confirmed our hypothesis that a plant can modify soil aggregate  
364 size distribution and pore networks differently depending on the inherent soil texture,  
365 manifest by different aggregate size distributions, and the contrasting effect of plants in  
366 both textural classes. However, the second hypothesis was not fully supported for both  
367 soils. For the sandy loam soil, the presence of roots decreased porosity, pore surface  
368 density, but had no significant impact on pore size distribution and pore connectivity  
369 after 6 weeks of growth. For the clay soil, the presence of roots maintained the porosity  
370 constant over the 6 weeks, but had no effect on the pore connectivity, contradicting the  
371 second hypothesis, but increased the pore surface density, which supported it. These  
372 results showed that impact of plants on soil pore architecture depends on textural  
373 characteristics.

374

### 375 **Acknowledgements**

376 We thank Pr. Hu Zhou for his assistance with the software QuantIm, and Paul Brown  
377 for supplying seed of *Phacelia*. This work was performed at the University of  
378 Nottingham Hounsfield facility. The University of Nottingham Hounsfield Facility  
379 receives funding from BBSRC (Swindon, UK), and The Wolfson Foundation (London,  
380 UK). This work is supported by the BBSRC-funded Soil to Nutrition strategic

381 programme (BBS/E/C/000I0310) and jointly by the Natural Environment Research  
382 Council and BBSRC as part of the Achieving Sustainable Agricultural Systems research  
383 programme (BBS/E/C/000I0130). We also thank the anonymous reviewers for their  
384 insight and suggestions for improvement.

385

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523

524 **Figure captions**

525 **Fig. 1** 2D X-ray attenuation images of soils (40  $\mu\text{m}$  resolution; darker shades relate to  
526 lower attenuation; a sharpening algorithm has been passed over these images to increase  
527 contrast of features) from (a, c) unplanted at Week 0 and (b, d, e) soil planted with  
528 phacelia after 6. (a, b) sandy clay soils; (c, d) clay soils. (e) example of effect of lateral  
529 root (LR) growing from a primary root (R) through aggregate in the clay soil and  
530 resulting in crack (C), growing through the soil matrix (S). P represents isolated pores.

531 **Fig. 2** Total soil porosity in unplanted and planted soils (spatial resolution 40  $\mu\text{m}$ ). (a)  
532 sandy loam soil; (b) clay soil. Bars denote means (n=4) expressed as the percentage of  
533 pores relative to the total volume, whiskers denote pooled standard errors.

534 **Fig. 3** Minkowski functions of sandy loam soils for the unplanted and planted soils at  
535 Week 0 (a, d, g), Week 2 (b, e, h) and Week 6 (c, f, i): (a - c) cumulative pore  
536 distribution of cores; (d - f) connectivity; (g - i) surface density. Points denote means  
537 (n=4), whiskers denote pooled standard errors.

538 **Fig. 4** Minkowski functions of clay soils for the unplanted and planted soils at Week 0  
539 (a, d, g), Week 2 (b, e, h) and Week 6 (c, f, i): (a - c) cumulative pore distribution of  
540 cores; (d - f) connectivity; (g - i) surface density. Points denote means (n=4), whiskers  
541 denote pooled standard errors.

542 **Fig. 5** Soil aggregate size distribution showing the starting condition at Week 0 (a, c) and  
543 the effect of plants at Week 6 (b, d) for the sandy loam soil (a - b) and the clay soil (c -  
544 d). Bars denote means (n=4) expressed as the percentage of aggregates relative to the total  
545 volume, whiskers denote pooled standard errors.

