

## Significant structural development of a long-term fallow soil in response to agricultural management practices requires at least 10 years after conversion

Journal:	European Journal of Soil Science
Manuscript ID	EJSS-084-20.R2
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
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Keywords:	Porosity, X-ray Computed Tomography, 3D pore characteristics, soil structure, agricultural management practices
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- 2 management practices requires at least 10 years after conversion
- 3 Running title: Structural development of long-term fallow soils
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### 19 Abstract (250 words, currently 232)

20 Agricultural practices can have significant effects on the physical and biological properties of 21 soil. The aim of this study was to understand how the physical structure of a compromised 22 soil, arising from long-term bare-fallow management, was modified by adopting different 23 field management practices. We hypothesised that changing agricultural practice from bare-24 fallow to arable or grassland would influence the modification of pore structure via an 25 increase in porosity, pore connectivity, and a more homogenous distribution of pore sizes; 26 and that this change exerts a rapid development of soil structure following conversion. Soil 27 aggregates (< 2 mm) collected in successive years from field plots subjected to three 28 contrasting managements were studied; viz. bare-fallow, bare-fallow converted to arable, and 29 bare-fallow converted to grassland. Soil structure was assessed by X-ray Computed 30 Tomography on the aggregates at 1.5  $\mu$ m resolution, capturing detail relevant to soil biophysical processes. The grassland system increased porosity, diversity of pore sizes, pore-31 32 connectivity and pore-surface density significantly over the decade following conversion. 33 However, measured at this resolution, the development of most of these metrics of soil 34 structure required approximately 10 years post-conversion to show a significant effect. The 35 arable system did not influence soil structural development significantly. Only the pore size distribution was modified in grassland in a shorter time frame (2 years post-conversion). 36 37 Hence development of the soil structural characteristics appears to require at least a decadal 38 timescale following conversion to grassland.

39

### 40 Key words:

Soil structure, 3D pore characteristics, agricultural management practices, X-ray Computed
Tomography, porosity

44		
45	Highlights:	
46	- The physical structure of a compromised soil was modified by adopting plant-based	
47	field management practices.	
48	- Conversion to grassland increased pore size diversity after 2 years.	
49	- Porosity, pore connectivity and pore surface density showed a significant	
50	modification between 7 to 10 years after conversion.	
51	- Bare fallow soil management for this extreme period (> 50 years) is detrimental to	
52	physical soil properties and the regeneration of the soil structure requires more than	
53	10 years after being reconverted to arable and grassland.	
54		
55	Introduction	
56	Agricultural practices can have beneficial or detrimental effects on soil functions when	
57	applied for decades, depending on the nature of such practices (Ashworth et al., 2017;	
58	Bronick and Lal, 2005; Denef et al., 2009; Pagliai et al., 2004). Agricultural management	
59	generally aims to increase - or at least stabilise - crop yield, but intensive farming can lead to	
60	soil degradation, erosion, compaction and pollution (Bronick and Lal, 2005). Conventional	
61	tillage can lead to a decline in soil aggregation and soil structure (Watts et al., 2001), as well	
62	as depletion of nutrients and organic carbon within soil (Coleman et al., 1997). Addition of	
63	organic matter or crop rotations can prevent soil disruption from tillage by improving soil	
64	porosity and aggregation (Abdollahi et al., 2014; Pagliai et al., 2004). In some cases,	
65	modification of crop management can have beneficial impacts on soil functions. For example,	
66	after 50 years of continuous cultivation, a desert aeolian sandy soil was managed into a	
67	sustainable agricultural soil by increasing silt and clay content (a determinant for aggregate	

68 formation), soil organic matter and nutrient retention (Su et al., 2010). Moreover, soil

aggregate stability is a key factor for soil fertility and physical resilience from external forces,
e.g. wind and water (Abivent et al. 2009).

71 Soil structure plays a fundamental role in the distribution of carbon, soil microorganisms, 72 water and nutrient accessibility (Rabot et al. 2018; Smith et al. 2017). Analysis of soil 73 structure indicates that pore size distribution, assessed by X-ray Computed Tomography 74 (CT), plays an important role in aggregate stability (Menon et al. 2020). Increased diversity 75 of pore sizes (i.e. a more homogenous distribution of pores) is associated with a more 76 complex pore network. This leads to an increase in the number of storage and transmission 77 pores resulting in greater water and nutrient flux (Kravchenko et al. 2014; 2019). The modification of pore size distributions appears to also play a key role in the decomposition of 78 79 organic matter (Quigley et al. 2018; Smith et al. 2017; Toosi et al. 2017a; 2017b). For 80 example, the presence of small (13-32  $\mu$ m) and large (136-260  $\mu$ m) pores decreases organic 81 matter decomposition within macro-aggregates (Toosi et al. 2017b). Pore connectivity is also 82 one of the most important factors, alongside porosity and pore size distribution, to understand 83 soil functions (Rabot et al. 2018). Modification of pore connectivity can have a significant 84 effect upon the distribution and the transport of gas and water (Lucas et al. 2020; Müller et al. 85 2019; Pires et al. 2019). Pires et al. (2017) demonstrated that pore connectivity was enhanced in zero tillage systems over conventional tillage systems, especially in the upper 10 cm. 86 87 In the field, long-term management practices can have substantial impacts on soil structural 88 dynamics (Bacq-Labreuil et al. 2018; Müller et al. 2019; Pires et al. 2019). For example, 50-89 years of management of a typical silty clay loam soil as bare fallow resulted in significant 90 reductions of carbon and nitrogen, and in the abundance of biological communities (Hirsch et 91 al. 2009) with soil structure also severely compromised (Bacq-Labreuil et al. 2018). 92 Conversion from bare fallow to arable or grassland increased soil organic carbon, soil

93 nitrogen and the population of meso-fauna and fungi within 3 to 5 years following conversion 94 (Hirsch et al. 2017), although, soil structure modification was not assessed in this experiment. 95 The aim of this study was to establish how the micro-structure of a compromised silt-clay 96 loam soil is modified by altered field management over time using soil aggregates (< 2 mm 97 diameter). Three treatments were studied from the converted field of the long-term 98 experiment: continuous bare-fallow, bare-fallow converted to arable, and bare-fallow 99 converted to grassland. We hypothesised that: (1) plants are an active factor in increasing soil 100 porosity, diversity of pore sizes and pore connectivity, and (2) structural development is more 101 rapid in grassland than arable converted systems due to the greater and more persistent presence of vegetation. The precise time for soil structural development is unclear *a priori*, 102 103 and we aimed to determine this by measuring structural properties on several successive 104 years after conversion. 105 106 **Materials and Methods** 

107 Soil aggregate sampling

108 Samples were obtained from conversion plots of the long-term Highfield Ley-Arable

109 experiment at Rothamsted Research, Harpenden, UK (LATLONG 51.8103 N, -0.3748 E).

110 The soil is a silty-clay loam (clay: 27%, silt: 58,4, sand: 14.6%; Jensen et al. 2020b)

111 developed on clay-with-flints over Eocene London Clay (Batcombe series), classified as

112 Chromic Luvisol by FAO criteria (Avery and Catt, 1995; FAO, 2006; Watt and Dexter,

113 1997). In October 2008, plots of soil managed as bare-fallow by regular tillage to remove any

- 114 plants since 1952, were converted to arable and grassland managements. The conversion
- 115 plots had similar soil characteristics. The conversion of the plots from bare-fallow to arable
- and grassland is explained in details in Hirsh et al. (2017). Arable soil was placed under
- 117 continuous wheat rotation (winter wheat, Triticum aestivum L., c.v. 'Hereward' seed coated

118 with Redigo® Deter® combination insecticide/fungicide treatment, Bayer CropScience) 119 receiving ammonium nitrate fertilization to provide approximately 220 kg-N ha<sup>-1</sup> y<sup>-1</sup>. For the 120 arable and grassland plots additional fertilizers 250 kg-K ha<sup>-1</sup> and 65 kg-P ha<sup>-1</sup> was added 121 every three years. Grassland plots were maintained as a managed sward of mixed fescue 122 (Festuca pratensis L.), Timothy grass (Phleum pratense L.) and white clover (Trifolium 123 repens L.) (30 kg ha<sup>-1</sup>). To remove weeds, bare-fallowed plots were maintained with regular 124 tillage or rotavation at least four times per year. Arable and bare-fallowed plots were tilled to 125 a standard depth of 23 cm. Plots have been sampled annually using cores (10 cm height and 3 126 cm diameter) in October, except in 2018 where the plots were sampled in June. Following 127 sampling, soil was air-dried and sieved at 2 mm before being archived at room temperature. 128 Aggregates (< 2 mm diameter) from continuous bare fallow (bare-fallow), bare fallow 129 converted into arable (arable) and bare fallow converted to grassland (grassland) were 130 randomly selected from samples collected in 2008, 2010, 2012, 2015 and 2018, representing 131 0-, 2-, 4-, 7- and 10- years post conversion. The replication of treatments was a total of 9 132 scanned aggregates (< 2 mm) *per* year and *per* treatment where randomly selected from 3 133 independent plots *per* treatment and 3 aggregates (< 2 mm) *per* plot (i.e. 3 replicates *per* plot) 134 to be X-ray CT scanned.

135

136 X-ray Computed Tomography

Aggregates (< 2 mm diameter) were scanned using a Phoenix Nanotom<sup>®</sup> (GE Measurement and Control solution, Wunstorf, Germany) set at a voltage of 90 kV, a current of 65  $\mu$ A and at a resolution of 1.50  $\mu$ m (thus pores below this size were not considered) at the Hounsfield Facility at The University of Nottingham. A total of 1,440 projection images were taken at a 500 ms period using an averaging of 3 images and skip of 2. The total scan time per sample was 60 minutes. Scanned images were reconstructed using Phoenix datos x2 rec reconstruction software. They were optimised to correct for any movement of the sample
during the scan and subjected to noise reduction using the beam hardening correction
algorithm, set at 8.

146

147 *Image analysis* 

148 Image analysis was performed using two software packages, ImageJ (Schneider et al., 2012) and QuantIm (Vogel et al., 2010) following the method from Bacq-Labreuil et al. (2018). 149 150 Briefly, all the images were thresholded using the bin bi-level threshold developed by Vogel 151 and Kretschmar (1996). QuantIm was used to output the 3D characteristics of the pore 152 network calculated from the Minkowski functions where the total porosity (referred to as 153 porosity from here) is the percentage of all the pores >1.5  $\mu$ m; pore size distribution is the 154 proportion of each size class in the volume normalised to the total pore volume, expressed 155 here as a cumulative value; pore connectivity was calculated from the Euler number and 156 normalised to the total volume (the more negative the Euler number, the greater the pore-157 connectivity); the pore surface density represents the roughness of the surface of pores: a 158 lower surface density means a lower roughness, i.e. less surface to be colonised by living 159 organisms (Vogel et al., 2010). The Gini-coefficient (G), a statistical measure of distribution, 160 was also determined. It is commonly applied in economics research to estimate the statistical 161 dispersion of income or wealth, and commonly used as a measurement of inequality (Bellù 162 and Liberati, 2006). Here, G was applied to measure the distribution of pore size classes as an 163 indicator of the equality of the pore size distribution.  $G \approx 0$  represents an equitable 164 distribution of pores amongst all pore size classes meaning that the soil pores have a 165 homogenous distribution of the pore sizes.  $G \approx 1$  represents a heterogeneous distribution of 166 pores which means that a majority of pores have the same sizes.

#### 168 *Statistical analysis*

169 A standard analysis of variance (ANOVA) was performed using Genstat v 17.1 (VSN 170 International Ltd., 2014) on the porosity. A two-factor ANOVA was conducted on each 171 Minkowski function divided by year using a split plot design with the treatment and the 172 diameter of pores as factors. For total porosity, G, the connected porosity and pore surface 173 area, an analysis of co-variance (ANCOVA), was also performed between the arable and 174 grassland with years' post-conversion as a co-variate using SigmaPlot for Windows ver. 14.0 175 (Systat Software Inc., San Jose, CA). In the case of pore surface area, pore diameter was 176 employed as a second covariate. Parameters were tested following either square root or  $log_{10}$ 177 transformation where necessary to conform to model assumptions of normality (tested using 178 the Shapiro-Wilk test) and homogeneous variances (tested using Levene's test). In each case, 179 ANCOVA was used to test for homogeneity of slopes associated with the change of total and 180 connected porosities and G with years' post-conversion of bare fallow to either arable or 181 grassland management. Soil managed as bare fallow throughout the experiment was used to 182 account for temporal changes in soil parameters under continuous management. Post hoc pair-wise comparisons were performed employing the Copenhaver & Holland multiple 183 comparisons procedure (Holland and Copenhaver, 1987). 184

185

#### 186 **Results**

#### 187 Visual appraisal of soil structures

188 Representative 2D images showed that after 1 and 3 years, all three treatments had similar 189 pore architectures in terms of size and shape (Fig. 1a-f). After 5 years, arable and grassland 190 started to display different pore configurations clearly manifest by a greater proportion of 191 larger pores (>40  $\mu$ m; Fig. 1g-i). The evolution of the pore characteristics over time was 192 apparent, after 7- and 10-years *post* conversion for the arable and grassland treatments especially for vugh (i.e. irregular) and crack shaped pores (Fig. 1j-o). In contrast the size anddistribution of pores was relatively consistent over time.

195

196 Total Porosity

Before the conversion (in 2008) and after 2 and 4 years, there were no significant treatment 197 198 effects on porosity (P > 0.05; Fig. 2a-c) compared to 7- and 10-years post conversion (respectively P=0.029 and P=0.002; Fig. 2d, e). After 7 years, the porosity in the grassland 199 200 soil was greater than in bare-fallow or arable soils which were similar (Fig. 2d). However, 201 after 10 years, porosity increased in the presence of plants according to the ranking; bare-202 fallow < arable < grassland (Fig. 2e). No significant change in  $\log_{10}$  total porosity was 203 observed in the continuous bare-fallow soil over the 10 years (slope = 0.026, t = 0.104, p =204 0.917) (Supplementary Fig. 1). However, total porosity in soils converted to arable (slope = 0.713, t = 3.4, p = 0.0014) and grassland (slope = 0.41, t = 3.7, p < 0.001) managements 205 206 increased over the same period (Fig. 1f). ANCOVA comparing the arable and grassland soils 207 identified a significant time response in  $\log_{10}$  porosity ( $F_{1.86} = 31.0, p < 0.001$ ) but no 208 significant difference in the rates of change (slope) in total porosity ( $F_{1,86} = 0.8, p = 0.36$ ). 209 The resulting equal slopes model identified a significant difference in the adjusted mean  $\log_{10}$ 210 total porosity of each treatment ( $F_{1.87} = 23.5$ , p < 0.001) with grassland accumulating significantly greater  $\log_{10}$  porosity (0.985 ± 0.025, equivalent to 9.66 ± 1.05%, adjusted mean 211 212  $\pm$  standard error of the mean) than arable soil (0.848  $\pm$  0.021, equivalent to 7.05  $\pm$  1.05%) 213

214 *Pore size distribution* 

215 Before the conversion (in 2008), there was no significant treatment effect on the cumulative

216 pore size distribution (P > 0.05; Fig. 3a). Between 2- to 10-years post conversion, there was a

217 significant diameter by treatment interaction with respect to the cumulative pore size

218 distribution (2 and 7 years: P<0.001; 4 and 10 years: P<0.05; Fig. 3b-e). After 2 years post 219 conversion, there was a greater proportion of smaller pores under bare-fallow and arable 220 treatments than grassland: for bare-fallow and arable, approximately 50% of pores were smaller than 3.56 µm and 70% of pores smaller than 5.97 µm compared to grassland where 221 50% of pores were smaller than 5.97  $\mu$ m and 70% smaller than 14.9  $\mu$ m. Moreover, the 222 223 proportion of pores larger than 42 µm was greater under grassland (13% of pores) than bare-224 fallow and arable (respectively 1% and 2% of pores; Fig. 3b). After 4 years, this trend was 225 not apparent: the difference between grassland compared to bare-fallow and arable was less 226 significant than after 2 years. The proportion of pores smaller the 9.26 µm was greater under bare-fallow and arable compared to grassland but the proportion of pores larger than 42 µm 227 228 was not significant between all treatments (Fig. 3c). After 7 years, the trend observed after 2 229 years was more apparent: the proportion of pore sizes smaller than 14.9 µm was greater 230 ranking from bare-fallow > arable > grassland and the proportion of pore sizes over 42  $\mu$ m 231 was greater under arable and grassland (respectively 7% and 10% of pores) than bare-fallow 232 (2% of pores; Fig. 3d). After 10 years post conversion, this trend was also observed, but only 233 for pores smaller than 9.26 µm, where the proportion of pores followed the ranking; bare-234 fallow > arable > grassland (Fig. 3e). Beyond this pore size, the proportion of pore sizes was 235 not significantly different between bare-fallow and arable. The proportion of pore sizes 236 greater than 42 µm was highest under grassland (15% of pores) than bare-fallow and arable 237 (respectively 4% and 2% of pores; Fig. 3e). 238 The general trend in all three treatments was a shift to a more even distributions of pore sizes,

239 manifest as a decrease in G over time (Fig. 3f). ANCOVA indicated equal rates of change of

240 *G* between treatments ( $F_{2,129} = 0.18$ , p = 0.834). Using an equal slopes model, there was a

significant effect of land management upon  $G(F_{2,131} = 9.1, p < 0.001)$  with grassland having

a significantly lower adjusted mean  $G(0.420 \pm 0.033)$  than either bare-fallow (adjusted mean

 $G = 0.566 \pm 0.028; t = 3.6, p < 0.001) \text{ or arable (adjusted mean } G = 0.571 \pm 0.024; t = 3.7, p$ < 0.001). There was no significant difference in adjusted mean G between arable and barefallow (t = 0.13, p = 0.900).

246

#### 247 *Pore connectivity*

248 Before the conversion (in 2008) and after 7-years post conversion, there was no significant pore diameter by treatment interaction with regards to pore connectivity (P = 0.05; Fig. 4a, d). 249 However, there was a significant pore diameter by treatment interaction after 2-, 4- and 10-250 251 years (with 2- and 10-years:  $P \le 0.001$ ; 4-years:  $P \le 0.05$ ; Fig. 4b, c, e). After 2- and 4- years, the difference was significant only for the pore sizes smaller than 3.56 µm. After 2-years, 252 253 pore connectivity was greater ranking from bare-fallow > grassland > arable (Fig. 4b) and 254 after 4-years, pore connectivity was greater under arable and grassland than bare-fallow (Fig. 4c). After 10-years post-conversion, the same trend as after 4-years was shown with a greater 255 256 difference in the values (Fig. 4e). There was no significant trend in square root transformed 257 connected porosity (Supplementary Fig. 2) in bare-fallow (slope = -0.00023, t = 0.051, p =258 0.959). However, both arable (slope = 0.017, t = 4.0, p = 0.0002) and grassland (slope = 0.022, t = 5.3, p < 0.0001) showed increases in connected porosity with time. ANCOVA 259 260 comparing arable and grassland indicated a significant influence of time post conversion upon square root transformed connected porosity ( $F_{1,85} = 43.9$ , p < 0.001) but no significant 261 262 heterogeneity of slopes ( $F_{1.85} = 0.98$ , p = 0.326). Using an equal slopes model, a significant 263 effect of management was detected ( $F_{1.85} = 4.4$ , p = 0.039): grassland was associated with greater connected porosity ( $0.048 \pm 0.0002\%$ , adjusted mean  $\pm$  standard error) than arable 264 265  $(0.011 \pm 0.0002\%).$ 

266

267 *Pore surface density* 

268 Before the conversion (in 2008) and at 4- and 7-years post conversion, there was no 269 significant pore diameter by treatment interaction with regards to pore surface density 270 (P>0.05; Fig. 5a, c, d). There was a significant diameter by treatment interaction after 2- and 271 10-years (respectively P < 0.05 and P < 0.001; Fig. 5b, e). After 2-years, the difference in pore surface density was greater ranking from bare-fallow > arable > grassland for the pore sizes 272 273 equal to 1.86 µm, and the difference between arable and grassland was not significant for the 274 pore sizes equal to 3.56 µm. Beyond this pore size, there was no significant difference 275 between treatments (Fig. 5b). Ten years post conversion, pore surface density was greater 276 ranking from grassland > arable > bare-fallow, for all pore sizes smaller than 14.9  $\mu$ m, there 277 was no significant difference beyond this pore size (Fig. 5e). Years post-conversion and pore 278 diameter were both used as covariates in ANCOVA analysis of pore surface area. 279 Accounting for these two covariates, an equal slopes model identified a significant effect of 280 land management upon pore surface area ( $F_{2,2020} = 9.1$ , p < 0.001). Post hoc pair-wise 281 comparison of adjusted means indicated that grassland supported a greater pore surface area  $(0.00784 \pm 0.000493 \ \mu\text{m}^2 \ \mu\text{m}^{-3})$  than either arable  $(0.00617 \pm 0.000452 \ \mu\text{m}^2 \ \mu\text{m}^{-3})$ ; difference 282 = 0.00167, t = 3.4, p = 0.001) or bare-fallow (0.00593 ± 0.000443 µm<sup>2</sup> µm<sup>-3</sup>; difference = 283 0.00191, t = 3.9, p < 0.001). There was no significant difference in pore surface area between 284 285 arable and bare-fallow (difference = 0.000240, t = 0.495, p = 0.621). 286

#### 287 Discussion

The plots studied here were derived from long-term bare-fallow management converted to arable and grassland. A lack of a significant treatment effect on porosity until 7-years post conversion suggests that modification of micro-porosity takes several years (Fig. 2). Another study on the same soil 2 and 4 years post conversion found some recovery of meso-faunal populations after 2-years of conversion and an increase in soil organic matter and microbial 293 abundance after 4- and 2-years respectively (Hirsch et al., 2017). However, our study showed 294 that development of micro-scale porosity apparently takes longer. This might be related to 295 carbon cycling processes which are modified by the microbial communities and plants (via 296 decomposition of organic matter and rhizodeposition). This is likely to affect soil structure at the micro-scale, but not instantaneously. Increased pore formation under grassland compared 297 298 to arable is consistent with a previous study, which showed greater resistance to, and 299 development from physical stresses of soil structure from grassland (Gregory et al., 2009). 300 They posited that the greater proportion of organic matter enhanced the elastic recovery of 301 soil structure (Gregory et al., 2009). 302 Pore size distributions showed a more rapid response to altered management than porosity for

303 the grassland treatment: after only 2-years of conversion (in 2010), a greater diversity of pore 304 sizes was observed, and this trend was also recorded in the data after 7- and 10-years (Fig. 3). 305 The Gini-coefficient indicated that soil converted to grassland established a more even 306 distribution of pore sizes than the other treatments, meaning that grassland treatment had a 307 greater diversity of pores after 2-, 7- and 10-years post conversion (Supplementary Fig. 2) 308 leading to enhanced functionality. This increase in pore size diversity might be due to the 309 increase of presence of plants, active organisms and organic matter (Hirsch et al., 2017) as 310 well as the absence of tillage. A study focused on the soil organic carbon on the same 311 experiment found that the conversion of the bare-fallow soil to grassland led to an increase of 312 soil organic carbon (+46 %) 7-years post conversion (Jensen et al. 2020a). There is no data 313 regarding the conversion from bare-fallow to arable. Thus, the increase of organic matter in 314 the converted soil may play a role in the more homogenous distribution of the pore sizes. 315 Indeed, in a silty clay soil, the greater organic matter content increases the proportion of 316 pores between 0.5 to 500 µm (Metzger and Yaron, 1987; Watts and Dexter, 1997) leading to a more equitable distribution of pore sizes, i.e. a greater diversity of pore sizes. The greater 317

318 diversity of pore sizes under soil converted to grassland was consistent with a previous study 319 describing the long-term effect of grassland management on the same field experiment 320 (Bacq-Labreuil et al., 2018). After 4-years the pore size distribution did not follow this trend (Fig. 3c), which could be due to weather conditions prior sampling in that year. Indeed, 2008 321 and 2012 (at the start and 4-years post conversion respectively) were the wettest years during 322 323 the experimental period (Supplementary Fig. 3). In the presence of water, clay particles can 324 swell, and the compression of entrapped air in capillary pores can disrupt the pore 325 architecture, and affect the pore size distribution (Denef et al., 2001; Grant and Dexter, 326 1990). Pore networks are re-structured upon re-wetting due to the nature of soil particles. Changes in pore size between 2-, 4- and 7-years post-conversion raises the question of the 327 328 dynamics of this mechanism. The pore size distribution may have had a heterogeneous 329 response over time due to the impact of the wet year 4-years post-conversion, which shows 330 the rapid development of the pore size distribution after a sustained wet period compared to 331 the impact of agricultural practices (Supplementary Fig. 3). For the arable treatment, this 332 trend was not observed even 10-years post conversion, which might be due to the associated 333 tillage practices. 334 For pore connectivity, the conversion to grassland had a small effect after 2- and 4-years 335 compared to after 10-years post conversion (Fig. 4 b, c, e). However, pore connectivity data 336 after 10-years post conversion, for both arable and grassland converted soils (Fig. 4e), 337 suggested the pore network was less connected compared to Bacq-Labreuil et al., (2018). 338 This indicated that a longer time may be required to develop the connectivity of a pore network than the overall porosity. Increased connectivity of pores promotes water, gasses and 339 340 nutrient flows within the pore structure (Dexter, 1988; Tisdall and Oades, 1982). Therefore,

341 subtle increases in pore connectivity might increase water, gas and nutrient flux within the

342 soil. As well as the pore connectivity, the pore surface density was significantly increased in

343 the grassland and arable 10-years post-conversion (Fig. 5e). Our results are congruent with 344 Bacq-Labreuil et al. (2018), which showed grassland managed consistently for over 200 years 345 has an increased pore surface density compared to arable and bare-fallow soils *i.e.* the pore-346 solid interface which led to a greater surface of the pore where micro-organisms and plant roots can colonise and water films can develop. A greater pore surface density in the 347 348 converted plot means that the grassland and the arable have a more complex structure of 349 pores than the bare-fallow soil (Müller et al. 2019). The greater surface density for the 350 grassland compared to the arable might be induced by the greater SOC content and the 351 absence of tillage for this treatment (Hirsch et al. 2017; Jensen et al. 2020a). This can lead to 352 the formation of new habitats and niches which can be beneficial for microbial community 353 diversity (Holden, 2011). The greater pore surface area might increase water and nutrient 354 uptake by the microbial community and plants. This study suggests that conversion of degraded bare-fallow soil to grassland requires at least 355 356 10-years after conversion before being effective in terms of significant development of soil 357 structure at aggregate scale, as assessed by the overall and connected porosity. Moreover, the 358 conversion from bare-fallow to arable had no significant effect on soil structural properties 359 after a decade. In general, the recovery of meso-fauna and organic matter (Griffiths et al., 360 2000; Hirsch et al., 2017) were more rapid than the recovery of soil structure (Gregory et al., 361 2009). The pore size distribution was the only characteristic which was more sensitive to 362 changes induced by wetting and drying cycles and living organisms.

363

Our first hypothesis was supported since porosity, pore size diversity, pore connectivity, and pore surface area density were all enhanced in grassland soil. Moreover, the conversion to arable management did not affect soil structural development significantly. The conversion to grassland increased the range of pore sizes after 2-years, consistent with our second 368 hypothesis. However, all other Minkowski functions (porosity, pore connectivity and pore 369 surface area density) responded to change more slowly. The mechanisms behind the 370 development of pore sizes appeared to be dynamic and possibly dependent upon weather 371 conditions before sampling. Apart from the pore size distribution, the magnitude of the 372 grassland effects on all other Minkowski functions was lower than the difference observed 373 after a minimum of 50 years of management (Bacq-Labreuil et al., 2018). In this study, the 374 effect of grassland upon porosity and pore connectivity were two-fold greater than bare-375 fallow management. Here, the difference was significant but not as major. Bare-fallow soil 376 management for this long period (> 50 years) is detrimental to both physical and biological soil properties and the development of the soil structure after this requires more than 10 years 377 after the conversion the grassland 378

379

### 380 Conclusions

The soil structural development of a degraded silt-clay loam soil, as quantified by micro-scale 381 382 topological metrics, requires at least 10-years of a grassland management before showing any 383 significant effects.. These observations raise the question on the application to certain 384 managements in agricultural practices. For example, instead of applying a bare-fallow 385 treatment in a crop rotation, it would be beneficial for the soil characteristics to apply a 386 vegetation cover *i.e.* cover crops which increase organic matter inputs and influence soil 387 structure, leading to a 'conditioning' of soil physical and biological characteristics for the 388 next crop. This would prevent further degradation of soil and help its development if the soil 389 characteristics were compromised. Moreover, the development of soil structure is apparently 390 a long process in the context of current agricultural practices and perceived imperatives. 391 Thus, a modification of cropping managements should be anticipated to require some time 392 before the observation of beneficial impacts on soil structural dynamics.

393	
394	Acknowledgements
395	Work at Rothamsted is supported by the BBSRC-funded Soil to Nutrition strategic
396	programme (BBS/E/C/000I0310) and jointly by the Natural Environment Research Council
397	and BBSRC as part of the Achieving Sustainable Agricultural Systems research programme
398	(NE/N018125/1 LTS-M). Access to the Highfield Ley-Arable experiments is supported by
399	the UK's Long-Term Experiment National Capability, funded by a Biotechnology and
400	Biological Sciences Research Council Grant (BBS/E/C/000J0300). We thank two anonymous
401	reviewers for their insightful scrutiny of manuscripts and suggestions for improvement of this
402	paper. The data support the findings of this study are available from the corresponding author
403	upon reasonable request.
404	
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Figure 1: Representative 2D X-ray attenuation images of soils subjected to different forms of
management over 10-years following conversion to each treatment. Base resolution is 40 μm;
(P) pores are the darker shades and (S) soil matrix are the lighter shades which relate to the
attenuation of the X-ray (a sharpening algorithm has been passed over these images to
increase contrast of features); (a, d, g, j, m) bare-fallow; (b, e, h, k, n) arable; and (c, f, i, l, o)
grassland.

**Figure 2:** Porosity (based on resolution of 1.5  $\mu$ m) in bare fallow, arable and grassland soils in the years following: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Bars are means (n = 9) expressed as the percentage of pores relative to the total volume, whiskers denote pooled standard errors. (f) Porosity evolution 0- to 10-years *post* conversion, data points represent means (n = 9), whiskers denote pooled standard errors for clarity and trend lines are linear regressions.

543

Figure 3: Cumulative pore size normalized to the total volume in relation to bare-fallow, arable and grassland in the years following conversion: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Data points indicate means (n=9), whiskers denote pooled standard errors. (f) Gini coefficient development 0- to 10-years post conversion, data points represent means (n = 9), whiskers denote pooled standard errors for clarity and trend lines are linear regressions.

550

Figure 4: Pore connectivity normalized to total volume in continuous bare fallow, arable and grassland soils following conversion: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-

553 years. Data points indicate means (n=9), whiskers denote pooled standard errors.

- 555 **Figure 5:** Pore surface density in continuous bare fallow, arable and grassland soils following
- 556 conversion: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Data points indicate
- 557 means (n = 9), whiskers denote pooled standard errors.

558

# Appendix

## Significant structural development of a long-term fallow soil in response to agricultural management practices requires at least 10 years after conversion

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**Supplementary Figure 1:**  $Log_{10}$  total porosity, points represent the adjusted means generated from an analysis of covariance, employing time *post* conversion (years) as the covariate. Errors represent the 95% confidence intervals associated with the adjusted means

**Supplementary Figure 2:** Square root transformed connected porosity: points represent the replicates and the lines are a linear regression for each treatment.

**Supplementary Figure 3:** Cumulative rainfall (mm) on the Highfield from (a) September to October for 2008 to 2015; and (b) May to June for 2018, as the sampling time were different.

Review



**Supplementary Figure 1:**  $Log_{10}$  total porosity, points represent the adjusted means generated from an analysis of covariance, employing time *post* conversion (years) as the covariate. Errors represent the 95% confidence intervals associated with the adjusted means



**Supplementary Figure 2:** Square root transformed connected porosity: points represent the replicates and the lines are a linear regression for each treatment.



**Supplementary Figure 3:** Cumulative rainfall (mm) on the Highfield from (a) September to October for 2008 to 2015; and (b) May to June for 2018, as the sampling time were different.



200 µm

Representative 2D X-ray attenuation images of soils subjected to different forms of management over 10years following conversion to each treatment. Base resolution is 40  $\mu$ m; (P) pores are the darker shades and (S) soil matrix are the lighter shades which relate to the attenuation of the X-ray (a sharpening algorithm has been passed over these images to increase contrast of features); (a, d, g, j, m) bare-fallow; (b, e, h, k, n) arable; and (c, f, i, l, o) grassland.

190x234mm (96 x 96 DPI)



Porosity (based on resolution of 1.5 μm) in bare fallow, arable and grassland soils in the years following: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Bars are means (n = 9) expressed as the percentage of pores relative to the total volume, whiskers denote pooled standard errors. (f) Porosity evolution 0- to 10-years post conversion, data points represent means (n = 9), whiskers denote pooled standard errors for clarity and trend lines are linear regressions.

179x244mm (96 x 96 DPI)



Cumulative pore size normalized to the total volume in relation to bare-fallow, arable and grassland in the years following conversion: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Data points indicate means (n=9), whiskers denote pooled standard errors. (f) Gini coefficient development 0- to 10-years post conversion, data points represent means (n = 9), whiskers denote pooled standard errors for clarity and trend lines are linear regressions.

177x244mm (96 x 96 DPI)



Pore connectivity normalized to total volume in continuous bare fallow, arable and grassland soils following conversion: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Data points indicate means (n=9), whiskers denote pooled standard errors.

181x243mm (96 x 96 DPI)



Pore surface density in continuous bare fallow, arable and grassland soils following conversion: (a) 0-year; (b) 2-years; (c) 4-years; (d) 7-years; (e) 10-years. Data points indicate means (n = 9), whiskers denote pooled standard errors.

176x244mm (96 x 96 DPI)

1	<u>Significant</u> S <u>s</u> tructural <u>recovery development</u> of a long-term fallow soil in response to
2	annual or perennialagricultural management practices cropping requires at least 10
3	years after conversion
4	
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#### 20 Abstract (250 words, currently 23023312) 21 Agricultural practices can have significant effects on soil-the physical and biological soil 22 properties of soil. Crop rotation and modification of cropping systems can lead to marked 23 effects on these properties and enhance plant growth, sequestration of carbon and reorganise 24 soil structure. The aim of this study was to understand how the physical structure of a 25 compromised soil, arising from a-long-term bare-bare-fallow periodmanagement, was 26 modified by adopting different field management practices. Soil aggregates collected on 27 successive years from field plots subjected to three contrasting management regimes were 28 studied, viz. bare fallow, bare fallow converted to arable, and bare fallow converted to 29 grassland. We hypothesised that a changeing of plant inputs agricultural practices from bare-30 fallow to arable andor grassland would influence the modification of pore structure via an 31 increase of in porosity, pore connectivity, and a more homogenous distribution of pore sizes; diversity of pore sizes and pore connectivity; and that thise effect of plantschange exerts 32 33 a rapid recovery-development of soil structure after following conversion. Soil aggregates (< 34 2 mm) collected oin successive years from field plots subjected to three contrasting 35 managements were studied; viz. bare-fallow, bare-fallow converted to arable, and bare-36 fallow converted to grassland. -Soil structure was assessed by X-ray Computed Tomography 37 of 2 mmon the aggregates at 1.5 µm resolution, to captureing detail relevant to key soil 38 biophysical processes. The greatest presence of plants, here represented by tThe grassland 39 system, increased significantly porosity, diversity of pore sizes, pore-connectivity and pore-40 surface density significantly over the decade following conversion. -However, measured at 41 this resolution, the recovery development of most of these metrics of soil structure required 42 approximately 10 years post-conversion to show a significant effect of plant presence after the 43 conversion to grassland. The arable system did not affectinfluence the soil structureal 44 development significantly. Only the pore size distribution was modified in grassland in by

4	5	plants in a shorter time frame (2 years post-conversion). Hence Full-dDevelopmentrecovery
4	6	of the soil structural characteristics, therefore, appears to require at least a decadal time-
4	7	scaletimescale after being converted following conversion to grassland.
4	8	
4	9	Key words:
5	0	Soil structure, soil recovery, 3D pore characteristics, cropping systemsagricultural
5	1	management practices, X-ray Computed Tomography, porosity
5	2	
5	3	
5	4	Highlights:
5	5	- <u>How t</u> he physical structure of a compromised soil was modified by adopting
5	6	different plant-based field management practices.
5	7	- The presence of plantseConversion to grassland increased the pore size diversity of
5	8	pore sizes-after only-2 years post-conversion.
5	9	- Porosity, pore connectivity and pore surface area density showed recovery a
6	0	significant modification between 7 to 11-10 years post-after conversion.
6	1	- Bare fallow soil management for this extreme period (> 50 years) is detrimental to
6	2	both physical and biological soil properties and the recovery regeneration of the soil
6	3	structure requires more than 10 years after being reconverted to arable and grassland.
6	4	
6	5	Introduction
6	6	When applied for decades to soil, aAgricultural practices can have both beneficial or
6	7	detrimental effects on soil function properties, when applied for decades, depending on the
6	8	nature of such practices (Ashworth et al., 2017; Bronick and Lal, 2005; Denef et al., 2009;
6	9	Pagliai et al., 2004). Agricultural management generally aims to increase or at least
1		
70	stabilise crop yield, but intensive farming can lead to soil degradation, erosion,	
----	---	
71	compaction and pollution (Bronick and Lal, 2005). Conventional tillage can lead to <u>a</u> decline	
72	of in soil aggregation and soil structure (Watts et al., 2001), as well as a depletion of	
73	nutrients and organic carbon within soil (Coleman et al., 1997). The aAddition of organic	
74	matter or crop rotations can prevent soil disruption from tillage by improving soil porosity	
75	and aggregation (Abdollahi et al., 2014; Pagliai et al., 2004). In some cases, mModification	
76	of cropping management can have beneficial impacts on soil properties functions. For	
77	example, after 50 years of continuous cultivation, a desert aeolian sandy soil was managed	
78	into a sustainable agricultural soil by increasing silt and clay content - which was (a	
79	determinant for aggregate formation), soil organic matter and the content of silt and clay	
80	(caused by irrigation) leading to an increase of aggregation nutrient retention (Su et al., 2010).	
81	Moreover, soil aggregate stability is a key factor for soil fertility and physical resilience from	
82	external forces, e.g. wind and water (Abivent et al. 2009).	
83	Soil structure plays a fundamental role in the distribution of carbon, soil microorganisms,	
84	water and nutrient accessibility (Rabot et al. 2018; Smith et al. 2017). The aAnalysis of soil	
85	structure led to suggest indicates that pore size distribution, assessed by X-ray Computed	
86	Tomography (X-ray (CT), plays an important role in aggregate stability (Menon et al. 2020).	
87	The greaterIncreased diversity of pore sizes diversity (i.e. a more homogenous distribution of	
88	pores) ereates is associated with a more complex pore network. This might leads to an	
89	increase of thein the number of storage and transmission pores resulting in an increase	
90	ofgreater water and nutrient flowsflux (Kravchenko et al. 2014; 2019). The modification of	
91	the-pore size distributions appears to also play also a key role in the decomposition of organic	
92	matter (Quigley et al. 2018; Smith et al. 2017; Toosi et al. 2017a; 2017b). For example, the	
93	presence of small (13-32 µm) and large (136-260 µm) pores decreases the organic matter	
94	decomposition within macro-aggregates (Toosi et al. 2017b). The pPore connectivity is also	

95	one of the most important factors, alongside-with porosity and pore size distribution, to
96	understand soil functions (Rabot et al. 2018). The mModification of pore connectivity can
97	have a significant effect upon the distribution and the transport of gas and water (Lucas et al.
98	2020; Müller et al. 2019; Pires et al. 2019). Pires et al. (2017) demonstrated that pore
99	connectivity was enhanced in zero tillage systems over conventional tillage systems,
100	especially in the upper 10 cm.
101	In the field, long-term
102	Furthermore, microbial biomass - a fundamental component of soil fertility - is highly
103	sensitive to tillage (Ashworth et al., 2017). Drijber et al. (2000) showed that on bare-fallow
104	plots microbial biomass was reduced by approximately 30% compared to cropped plots after
105	25 years. The composition of the bacterial communities is more closely related to soil
106	characteristics (such as pH and soil texture) than cropping management, and the fungal
107	community is more associated with nutrients within the soil than cropping management
108	(Lauber et al. 2008). By contrast, a recent study showed that crop management did not have
109	any impact on the microbial community structure, but had an effect on the distribution of
110	genes coding for different functions (Neal et al., 2017). This study focused on phosphatase-
111	coding genes, and showed that bare-fallowed soil contained more genes coding for
112	extracellular and outer-membrane associated enzymes compared to grassland and arable
113	soils, leading to a community with greater foraging potential to access nutrients from a
114	greater distance under the bare-fallow (Neal et al., 2017). Moreover, different
115	mMmanagement practices can have a-substantial impacts on soil structuraldynamics (Bacq-
116	Labreuil et al. 2018; Müller et al. 2019; Pires et al. 2019). For example, After-50-years under
117	a of management of a typical silty clay loam soil as bare fallow treatment resulted in
118	significant reductions of carbon and nitrogenn was markedly reduced, and in the the
119	abundance of biological communities_was reduced (Hirsch et al. 2009) and sSwith soil
I	

120	structure was-also severely compromised (Bacq-Labreuil et al. 2018). Conversion from bare
121	fallow to arable or grassland increased soil organic carbon, soil nitrogen and the population
122	of meso-fauna and fungi within 3 to 5 years after following conversion (Hirsch et al. 2017),
123	Howeveralthough, soil structure modification was not assessed in this experiment.
124	The aim of this study was to establish how the micro-structure of a compromised silt-clay
125	loam soil is modified by altered field management over time using soil aggregates (<2 mm
126	diameter). Three treatments were studied from the converted field of the long-term
127	experiment: continuous <u>bare-bare-</u> fallow, barefallow converted to arable, and <u>bare-bare-</u>
128	fallow converted to grassland. We hypothesised that: (1) plants are an active factor in
129	the modification of soil pore structure via an increaseing insoil porosity, diversity of pore
130	sizes and pore connectivity, and (2) structural recovery development would beis more rapid
131	in grassland than arable converted systems due to the greater and presence of more persistent
132	presence of vegetationplant populations. The precise time for recovery soil structural
133	development is unclear <i>a priori</i> , and we aimed to determine this by measuring structural
134	<b>Development</b> is unclear <i>a priori</i> , and we annee to determine this by measuring structural properties on several successive years after conversion. <b>Materials and Methods</b> Soil aAggregatees samplinges           Samples were obtained from conversion plots of the long-term Highfield Ley-Arable
135	
136	Materials and Methods
137	Soil aAggregatees samplinges
138	Samples were obtained from conversion plots of the long-term Highfield Ley-Arable
139	experiment at Rothamsted Research, Harpenden, UK (LATLONG 51.8103 N, -0.3748 E).
140	The soil is a silty-clay loam (clay: 27%, silt: 58,4, sand: 14.6%; Jensen et al. 2020b)
141	developed on clay-with-flints over Eocene London Clay (Batcombe series), classified as
142	Chromic Luvisol by FAO criteria (Avery and Catt, 1995; FAO, 2006; Watt and Dexter,
143	1997). In October 20072008, plots of soil managed as bare-fallow by regular tillage to
144	remove any plants since 1952, were converted to arable and grassland managements. The

Commented [Ab1]: À ajouter

145	conversion plots hadve similar soil characteristics. The conversion of the plots from bare-
146	fallow to arable and grassland iwas explained in details in Hirsh et al. (2017). Arable Briefly,
147	aArable soil was placed under continuous wheat rotation (winter wheat, Triticum aestivum L.,
148	c.v. 'Hereward' seed coated with Redigo® Deter® combination insecticide/fungicide
149	treatment, Bayer CropScience) receiving ammonium nitrate fertilization to provide
150	approximately 220 kg-N ha <sup>-1</sup> annumy <sup>-1</sup> , and. For the arable and grassland plots additional
151	fertilizers 250 kg-K ha-1 and 65 kg-P ha-1 wais added every three years, and grassland
152	Grassland plots weare maintained as a managed sward of mixed grasses and forbsfescue
153	(Festuca pratensis L.), &Timothy-grass (Phleum pratense L.) and white clover (Trifolium
154	repens L.) (30 kg ha <sup>-1</sup> ). To remove weeds, bare-fallowed plots weare maintained with regular
155	ploughed, tillage andor rotavatedion at least four times per year. Arable and bare-fallowed
156	plots weare tilled withto a standard depth of 23 cm. Plots have been sampled annually using
157	cores (10 cm height and 3 cm diameter) in October, except in 2018 where the plots were
158	sampled in Junce 2018, Following sampling, using cores (10 cm height and 3 cm diameter),
159	the and soil was air-dried and sieved ( <u>at 2 mm</u> ) before being archived at room temperature.
160	Aggregates (< 2 mm diameter) from continuous bare fallow (bare-fallow), bare fallow
161	converted into arable (arable) and bare fallow converted to grassland (grassland) were
162	randomly selected from the years; samples collected in 2008, 2010, 2012, 2015 and 2018,
163	representing <u>40-</u> , <u>32-</u> , <u>54-</u> , <u>8-7-</u> and <u>44-10-</u> years <i>post</i> conversion. The replication of
164	treatments was: a total of 9 scanned aggregates (< 2 mm) per years and per treatments where
165	randomly selected from 3 independent plots per treatments and 3 aggregates (< 2 mm) per
166	plots (i.e. 3 replicates per plot) were randomly selected to be X-ray CT scanned, therefore a
167	total of 9 scanned aggregates per year, per treatment were assessed by this approach.
168	

169 X-ray Computed Tomography

170	Aggregates (< 2 mm diameter) were scanned using a Phoenix Nanotom <sup>®</sup> (GE Measurement
171	and Control solution, Wunstorf, Germany) set at a voltage of 90 kV, a current of 65 $\mu A$ and
172	at a resolution of 1.50 $\mu$ m (thus pores below this size were not considered in this study) at the
173	Hounsfield Facility at the The University Of of Nottingham. A total of 1,440 projection
174	images were taken at a_500 ms period using an averaging of 3 images and skip of 2. The total
175	scan time per sample was 60 minutes. Scanned images were reconstructed using Phoenix
176	datos x2 rec reconstruction software. They were optimised to correct for any movement of
177	the sample during the scan and subjected to noise reduction using the beam hardening
178	correction algorithm, set at 8.
179	
180	Image analysis
181	Image analysis was performed using two software packages, ImageJ (Schneider et al., 2012)
182	and QuantIm (Vogel et al., 2010) following the method from Bacq-Labreuil et al. (2018).
183	Briefly, all the images were thresholded using the bin bi-level threshold developed by Vogel
184	and Kretschmar (1996). QuantIm was used to output the 3D characteristics of the pore
185	network calculated from the Minkowski functions where the total porosity (ealled
186	here <u>referred to as</u> porosity <u>from here</u> ) is the percentage of all the pores >1.5 $\mu$ m; pore size
187	distribution is the proportion of each size class in the volume normalised to the total pore
188	volume, expressed here as a cumulative value; pore connectivity was calculated from the
189	Euler number and normalised to the total volume (the more negative the Euler number, the
190	greater the pore-connectivity); the pore surface density represents the roughness of the
191	surface of pores: a lower surface density means a lower roughness, i.e. less surface to be
192	colonised by living organisms (Vogel et al., 2010). The Gini-coefficient $(G)_{a-is}$ a statistical
193	measure of distribution, was also determined. It is commonly applied in economics research
194	to estimate the statistical dispersion of income or wealth, and commonly used as a

195	measurement of inequality (Bellù and Liberati, 2006). Here, $G$ was applied to measure the	
196	distribution of pore size classes as an indicator of the equality of the pore size distribution $\hat{G}$	
197	$\approx$ 0 represents an equitable distribution of the pores amongst all pore size classes meaning	
198	that the soil pores have a homogenous distribution of the pore sizes and, $G \approx 1$ represents a	
199	heterogeneous distribution of pores which means that a majority of pores haves the same	
200	sizes.	
201	Statistical analysis	
202	Statistical analysis	
203	A standard analysis of variance (ANOVA) was performed using Genstat v 17.1 (VSN	
204	International Ltd., 2014) on the porosity. A two-factor ANOVA was conducted on each	
205	Minkowski function divided by year using a split plot design with the treatment and the	
206	diameter of pores as factors. For total porosity, G, the connected porosity and pore surface	
207	area, an analysis of co-variance (ANCOVA), was also performed between the arable and	
208	grassland with years' post-conversion as a co-variate using SigmaPlot for Windows ver. 14.0	
209	(Systat Software Inc., San Jose, CA). In the case of pore surface area, pore diameter was	
210	employed as a second covariate. Parameters were tested following either square root or $\log_{10}$	
211	transformation where necessary to conform to model assumptions of normality (tested using	
212	the Shapiro-Wilk test) and homogeneous variances (tested using Levene's test). In each case,	
213	ANCOVA was used to test for homogeneity of slopes associated with the change of total and	
214	connected porosities and $G$ with years' post-conversion of bare fallow to either arable or	
215	grassland management. Soil managed as bare fallow throughout the experiment was used to	
216	account for temporal changes in soil parameters under continuous management. Post hoc	
217	pair-wise comparisons were performed employing the Copenhaver & Holland multiple	
218	comparisons procedure (Holland and Copenhaver, 1987).	

219

## 220 Results

221 Visual appraisal of soil structures

Representative 2D images showed that after 1 and 3 years, all three treatments had similar pore architectures in terms of size and shape (Fig. 1a-f). After 5 years, arable and grassland started to display different pore configurations clearly manifest by a greater proportion of larger pores (>40 µm; Fig. 1g-i). The evolution of the pore characteristics over time was apparent, after 8 and 11 years 87- and 101-years *post*-conversion for the arable and grassland treatments especially for vugh (i.e. irregular) and crack shaped pores (Fig. 1j-o). In contrast the size and distribution of pores is was relatively stable consistent over time.

229

## 230 Total Porosity

231 Before the conversion (in 2008) and aAfter 1, 3-2 and 5-4 years, there were no significant 232 treatment effects on porosity (P > 0.05; Fig. 2a-c) compared to 8 and 11 years 87- and 140-233 years post- conversion (respectively P=0.029 and P=0.002; Fig. 2d, e). After 8-7 years, the 234 porosity of in the grassland soil was greater than the porosity of in bare-fallow and or arable 235 soils which were similar (Fig. 2d). However, after 11-10 years, porosity increased in the 236 presence of plants according to the ranking; bare-fallow < arable < grassland (Fig. 2e). No 237 significant change in log<sub>10</sub> total porosity was observed in the continuous bare-fallow soil over 238 the  $\frac{11}{10}$  years (slope = 0.026, t = 0.104, p = 0.917) (Supplementary Fig. 1). However, total 239 porosity in both-soils converted to arable (slope = 0.713, t = 3.4, p = 0.0014) and grassland 240 (slope = 0.4107, t = 3.7, p < 0.001) managements increased over the same period (Fig. 1f). 241 ANCOVA comparing the arable and grassland soils identified a significant time response in 242  $\log_{10}$  porosity ( $F_{1,86} = 31.0, p < 0.001$ ) but no significant difference in the rates of change 243 (slope) in total porosity ( $F_{1,86} = 0.8$ , p = 0.364). The resulting equal slopes model identified a 244 significant difference in the adjusted mean  $log_{10}$  total porosity of each treatment ( $F_{1,87} = 23.5$ ,

245

246	equivalent to 9.66 $\pm$ 1.05%, adjusted mean $\pm$ standard error of the mean) than arable soil
247	$(0.848 \pm 0.021, \text{ equivalent to } 7.05 \pm 1.05\%)$
248	
249	Pore size distribution
250	After 1 year <u>1-year postBefore the</u> -conversion (in 2008), there was no significant treatment
251	effect on the cumulative pore size distribution ( $P > 0.05$ ; Fig. 3a). Between <u>32-</u> to <u>4410-</u> years
252	postconversion, there was a significant diameter by treatment interaction with respect to the
253	cumulative pore size distribution (3-2 and 8-7 years: $P < 0.001$ ; $45$ and $11-10$ years: $P < 0.05$ ;
254	Fig. 3b-e). After <u>3-2</u> years post-conversion, there was a greater proportion of smaller pores
255	under bare-fallow and arable treatments than grassland: for bare-fallow and arable,
256	approximately 50% of pores were smaller than 3.56 $\mu m$ and 70% of pores smaller than 5.97
257	$\mu m$ compared to grassland where 50% of pores were smaller than 5.97 $\mu m$ and 70% smaller
258	than 14.9 $\mu$ m. Moreover, the proportion of pores larger than 42 $\mu$ m was greater under
259	grassland (13% of pores) than bare-fallow and arable (respectively 1% and 2% of pores; Fig.
260	3b). After 5-4 years, this trend was not apparent: the difference between grassland compared
261	to bare-fallow and arable was less significant than after $\frac{3-2}{2}$ years. The proportion of pores
262	smaller the 9.26 $\mu$ m was greater under bare-fallow and arable compared to grassland but the
263	proportion of pores larger than 42 $\mu$ m was not significant between all treatments (Fig. 3c).
264	After 8-7_years, the trend observed after 3-2_years was more apparent: the proportion of pore
265	sizes smaller than 14.9 $\mu$ m was greater ranking from bare-fallow > arable > grassland and the
266	proportion of pore sizes over 42 $\mu m$ was greater under arable and grassland (respectively 7%

p < 0.001) with grassland accumulating significantly greater log<sub>10</sub> porosity (0.985 ± 0.025,

and 10% of pores) than bare-fallow (2% of pores; Fig. 3d). After <u>H-10</u> years postconversion, this trend was also observed, but only for pores smaller than 9.26 μm, where the

proportion of pores was followed the ranking; from bare-fallow > arable > grassland (Fig.

270 3e). Beyond this pore size, the proportion of pore sizes was not significantly different 271 between bare-fallow and arable. The proportion of pore sizes greater than 42 µm was highest 272 under grassland (15% of pores) than bare-fallow and arable (respectively 4% and 2% of 273 pores; Fig. 3e). 274 The general trend in all three treatments was a shift to a more equitable even distributions of 275 pore sizes, manifest as a decrease in G over time (Fig. 3f). ANCOVA indicated equal rates of 276 change of G between treatments ( $F_{2,129} = 0.18$ , p = 0.834). Using an equal slopes model, 277 there was a significant effect of land management upon G ( $F_{2,131} = 9.1, p < 0.001$ ) with 278 grassland having a significantly lower adjusted mean  $G(0.420 \pm 0.033)$  than either bare-279 fallow (adjusted mean  $G = 0.566 \pm 0.028$ ; t = 3.6, p < 0.001) or arable (adjusted mean G = $0.571 \pm 0.024$ ; t = 3.7, p < 0.001). There was no significant difference in adjusted mean G 280 281 between a able and bare-fallow (t = 0.13, p = 0.900). 282 283 Pore connectivity 284 Before the conversion (in 2008) and Aafter 1 and 8 years1- and 87-years post- conversion, 285 there was no significant pore diameter by treatment interaction with regards to pore 286 connectivity (P = 0.05; Fig. 4a, d). However, there was a significant pore diameter by 287 treatment interaction after 32-, 54- and 11-10-years (with 3-2- and 11-10-years: P < 0.001; 5288 4-years: P < 0.05; Fig. 4b, c, e). After 3-2- and 5-4- years, the difference was significant only 289 for the pore sizes smaller than 3.56 µm. After 3-2-years, pore connectivity was greater 290 ranking from bare-fallow > grassland > arable (Fig. 4b) and after 5-4-years, pore connectivity 291 was greater under arable and grassland than bare-fallow (Fig. 4c). After 11-10-years post-292 conversion, the same trend as after 5-4-years was shown with a greater difference in the 293 values (Fig. 4e). There was no significant trend in square root transformed connected porosity

294 (Supplementary Fig. 2) in bare-fallow (slope = -0.00023, t = 0.051, p = 0.959). However,

295	both arable (slope = 0.017, $t$ = 4.0, $p$ = 0.0002) and grassland (slope = 0.022, $t$ = 5.3, $p$ <
296	0.0001) showed increases in connected porosity with timeANCOVA comparing arable and
297	grassland indicated a significant influence of time post-conversion upon square root
298	transformed connected porosity ( $F_{1,85} = 43.9$ , $p < 0.001$ ) but no significant heterogeneity of
299	slopes ( $F_{1,85} = 0.98$ , $p = 0.326$ ). Using an equal slopes model, a significant effect of
300	management was detected ( $F_{1,85} = 4.4$ , $p = 0.039$ ): grassland was associated with greater
301	connected porosity (0.048 $\pm$ 0.0002%, adjusted mean $\pm$ standard error) than arable (0.011 $\pm$
302	0.0002%).
303	0.0002%). Pore surface density
304	Pore surface density
305	Before the conversion (in 2008) and After Aat 1-, 5 and 8 years 54- and 87-years post-
306	conversion, there was no significant pore diameter by treatment interaction with respect
307	<u>regards</u> to pore surface density ( $P > 0.05$ ; Fig. 5a, c, d). There was a significant diameter by
308	treatment interaction after 3-2- and $11-10$ -years (respectively $P < 0.05$ and $P < 0.001$ ; Fig. 5b,
309	e). After 3-2-years, the difference in pore surface density was greater ranking from bare-
310	fallow > arable > grassland for the pore sizes equal to 1.86 $\mu$ m, and the difference between
311	arable and grassland was not significant for the pore sizes equal to $3.56 \ \mu m$ . Beyond this pore
312	size, there was no significant difference between treatments (Fig. 5b). After 11ElevenTen
313	years <i>post</i> -conversion, pore surface density was greater ranking from grassland > arable >
314	bare-fallow, for all pore sizes smaller than 14.9 $\mu$ m, there was no significant difference

beyond this pore size (Fig. 5e). Years post-conversion and pore diameter were both used as
covariates in ANCOVA analysis of pore surface area. Accounting for these two covariates,

317 an equal slopes model identified a significant effect of land management upon pore surface

318 area ( $F_{2,2020} = 9.1, p < 0.001$ ). Post hoc pair-wise comparison of adjusted means indicated

319 that grassland supported a greater pore surface area  $(0.00784 \pm 0.000493 \ \mu m^2 \ \mu m^{-3})$  than

320 either arable  $(0.00617 \pm 0.000452 \ \mu\text{m}^2 \ \mu\text{m}^{-3}; \text{ difference} = 0.00167, t = 3.4, p = 0.001)$  or 321 bare-fallow  $(0.00593 \pm 0.000443 \ \mu\text{m}^2 \ \mu\text{m}^{-3}; \text{ difference} = 0.00191, t = 3.9, p < 0.001)$ . There 322 was no significant difference in pore surface area between arable and bare-fallow (difference 323 = 0.000240, t = 0.495, p = 0.621).

324

## 325 Discussion

326 The conversion plots studied here were derived from long-term bare-fallow management 327 converted to arable and grassland. A Llack of a significant treatment effect on porosity until 8 328 7-years post- conversion suggests that modification of micro-porosity at this scale takes 329 several years (Fig. 2). DespiteAnother study on the same soil realised after-2 and 4 years post 330 conversion found that thesome recovery of meso-faunal populations after 3-2-years of 331 conversion and an increase of in soil organic matter and microbial abundance after 54- and 3 332 2-years respectively (Hirsch et al., 2017). However, our study showed that, recovery 333 development of micron-scale porosity apparently takes longerchanges more slowly. This 334 might be related to carbon cycling processes which are modified by the microbial 335 communities and plants (via decomposition of organic matter and rhizodeposition). This is 336 likely to affect soil structure at the micro-scale, but not instantaneously. Greater Increased 337 pore formation under grassland compared to arable is consistent with a previous study, which 338 showed greater resistance to, and recovery-development from physical stresses of soil 339 structure from grassland (Gregory et al., 2009). They posited that the greater proportion of 340 organic matter enhanced the elastic recovery of the soil structure (Gregory et al., 2009). 341 Pore size distributions showed a more rapid response to altered management than total 342 porosity for the grassland treatment: after only 3-2-years of conversion (in 2010), a greater 343 diversity of pore sizes was observed-under the grassland treatment, and this trend was also 344 recorded in the data after 8-7- and 11-10-years (Fig. 3). The Gini-coefficient indicated that

345	soil converted to grassland established a more equitable even distribution of pore sizes than
346	the other treatments, meaning that grassland treatment had a greater diversity of pores after
347	32-, 8-7- and 44-10-years post-conversion (Supplementary Fig. 2) leading to enhanced
348	functionality. This increase in pore size diversity might be due to the increase of presence of
349	plants, active organisms and organic matter (Hirsch et al., 2017) as well as the eessation
350	absence of tillage. A study looking atfocused on the soil organic carbon on the same
351	experiment plot found that the conversion of the bare-fallow soil to grassland led to an
352	increase of soil organic carbon (+46 %) after 7-years post conversion (Jensen et al. 2020a).
353	There is no data regarding the conversion from bare-fallow to arable. Thus, the increase of
354	organic matter in the converted soil might have may play a role in the more homogenous
355	distribution of the pore sizes. Plants increase aggregation through root action and exudation
356	(Chan and Heenan, 1996; Haynes and Beare, 1997) and they can also break down aggregates
357	(Chan and Heenan, 1996; Materechera et al., 1994) by growing through existing pores in the
358	aggregates and disrupting them. However, plants enmesh soil particles forming large
359	aggregates (Tisdall and Oades, 1982), and release mucilage that binds soil particles with
360	organic matter which in turn stabilises new aggregates and pores (Bronick and Lal, 2005;
361	Chenu et al., 2000)Indeed, in a silty clay soil, The addition of organic matter can increase the
362	number of "transmission" (50 – 500 $\mu$ m) and "storage" (0.5 – 50 $\mu$ m) pores and decrease the
363	prevalence of macro-pores (> 500 µm) increasing overall functionality. Thus the action of
364	plants and the increase-greater of organic matter content increases the proportion of pores
365	between 0.5 to 500 $\mu$ m (Metzger and Yaron, 1987; Watts and Dexter, 1997) leading to a
366	more equitable distribution of pore sizes, i.e. a greater diversity of pore sizes. The greater
367	diversity of pore sizes under soil converted to grassland was consistent with a previous study
368	describing the long-term effect of grassland management on the same field experiment
369	(Bacq-Labreuil et al., 2018). After 5-4-years post-conversion, the pore size distribution did

370	not follow this trend (Fig. 3c), which could be due to the weather conditions prior sampling in
371	that year. Indeed, 2008 and 2012 ( <u>1-at the start and 5-4-years post-conversion respectively</u> )
372	were the wettest years during the experimental period (Supplementary Fig. 3). In the presence
373	of water, clay particles can swell, and the compression of entrapped air in capillary pores can
374	disrupt the pore architecture, and in turn, aggregation which might and affect the pore size
375	distribution (Denef et al., 2001; Grant and Dexter, 1990). Therefore, the pP ore networks
376	become are re-structured upon re-wetting due to the nature of soil particles. Changes in pore
377	size between $32_{-}$ , $54_{-}$ and $8-7_{-}$ years post-conversion raises the question of the dynamics of
378	this mechanism. The pore size distribution may have had a heterogeneous response over time
379	due to the impact of the wet year 5 4-years post-conversion, which shows the rapid recovery
380	regenerationdevelopment of the pore size distribution after a sustained wet period compared
381	to the impact of plant growthagricultural practices (Supplementary Fig. 3). For the plot
382	eonverted to arable treatment, this trend was not observed even after-10-years post-
383	conversion, which might be due to the associated tillage practices on the arable plots.
384	For pore connectivity, the magnitude of the plantconversion to grassland effect after 3 and 5
385	years was had a small effect after 2- and 4-years compared to after 41-10-years post-
386	conversion (Fig. 4 b, c, e). However, pore connectivity data after 44-10-years post-
387	conversion, for both arable and grassland converted soils (Fig. 4e), suggested the pore
388	network was less connected compared to a previous study (Bacq-Labreuil et al., (2018).; This
389	indicating-indicated that a longer time may be required to recover-develop the connectivity of
390	a pore network than total the overall porosity. Increased connectivity of pores promotes
391	water, gasses and nutrient flows within the pore structure (Dexter, 1988; Tisdall and Oades,
392	1982). Therefore, the subtle increases in the pore connectivity might increase the water, gas
393	and nutrient movement flux within the soil. As well as the pore connectivity, the pore surface
394	density was significantly influenced by the presence of plantsncreased in the grassland and
I	

395	arable 41-10-years post-conversion (Fig. 5e). Our results are congruent with Bacq-Labreuil et
396	al. (2018), which showed plants-grassland managed consistently for over 200 years has an
397	increased pore surface density compared to arable and bare-fallow soils, <i>i.e.</i> the pore-solid
398	interface which led to a greater surface of the pore where micro-organisms and plant roots
399	can colonise and water films can develop. A greater pore surface density in the converted plot
400	means that the grassland and the arable have a more complexed structure of pores than the
401	bare-fallow soil (Müller et al. 2019). The greater surface density for the grassland compared
402	to the arable might be induced by the greater SOC content and the absence of tillage for this
403	treatment (Hirsch et al. 2017; Jensen et al. 2020a). This can lead to the formation of new
404	habitats and niches which can be beneficial for microbial community diversity (Holden,
405	2011). The greater pore surface area might increase water and nutrient uptake by the
406	microbial community and plants.
407	This study suggests that the overall impact of the conversion from of degraded bare-fallow soil
408	to presence of plantsgrassland -requires at least 10-10-years after conversion before being
409	effective in terms of significant recovery development of soil structure at aggregate scale, as
410	assessed by total-the overall and connected porosity. Moreover, the conversion from bare-
411	fallow to arable had no significant effect on soil structural properties after a decade. In
412	general, the recovery of meso-fauna and organic matter (Griffiths et al., 2000; Hirsch et al.,
413	2017) were more rapid than the recovery of soil structure (Gregory et al., 2009). The pore
414	size distribution was the only characteristic which was more sensitive to changes changes
415	induced bysuch as wetting and drying cycles and ,-living organisms.
416	
417	Our first hypothesis was supported since porosity, pore size diversity, pore connectivity, and
418	pore surface area density were all enhanced in grassland soil. Moreover, the conversion to
410	

419 arable management did not affect soil structural development significantly. The conversion to

420	grassland increased the range of pore sizes after 2-years, consistent with our second
421	hypothesis. However, all other Minkowski functions (porosity, pore connectivity and pore
422	surface area density) responded to change more slowly. The mechanisms behind the
423	development of pore sizes appeared to be dynamic and possibly dependent upon weather
424	conditions before sampling. Apart from the pore size distribution, the magnitude of the
425	grassland effects on all other Minkowski functions was lower than the difference observed
426	after a minimum of 50 years of management (Bacq-Labreuil et al., 2018). In this study, the
427	effect of grassland upon porosity and pore connectivity were two-fold greater than bare-
428	fallow management. Here, the difference was significant but not as major. Bare-fallow soil
429	management for this long period (> 50 years) is detrimental to both physical and biological
430	soil properties and the development of the soil structure after this requires more than 10 years
431	after the conversion the grassland
432	
432	
432	Conclusions
	after the conversion the grassland         Conclusions <u>The Ss</u> oil structural recovery development of the a compromised degraded silt-clay loam soil,
433	Conclusions <u>The Ss</u> oil structural <u>recovery-development</u> of the <u>a compromised degraded silt-clay loam</u> soil, as quantified by <u>micro-scale</u> topological metrics-related to detail capture at 1.5 µm resolution,
433 434	<u>The son structural recovery-development</u> of the <u>a compromised degraded site cray toam</u> son,
433 434 435	as quantified by <u>micro-scale</u> topological metrics related to detail capture at 1.5 µm resolution,
433 434 435 436	as quantified by <u>micro-scale</u> topological metrics-related to detail capture at 1.5 µm resolution, requires at least 10-10-years of a new-grassland management before showing any significant
433 434 435 436 437	as quantified by <u>micro-scale</u> topological metrics-related to detail capture at 1.5 µm resolution, requires at least 10-10-years of a <u>new grassland</u> management before showing any significant effects of the presence of plants. Our first hypothesis was supported since porosity, <u>pore</u>
433 434 435 436 437 438	as quantified by <u>micro-scale</u> topological metrics-related to detail capture at 1.5 µm resolution, requires at least 10-10-years of a <u>new-grassland</u> management before showing any significant effects of the presence of plants. Our first hypothesis was supported since porosity, <u>pore</u> <u>size</u> the diversity of pore-sizes, pore connectivity, and pore-surface area density were all
433 434 435 436 437 438 439	as quantified by <u>micro-scale</u> topological metrics-related to detail capture at 1.5 µm resolution, requires at least 10-10-years of a <u>new-grassland</u> management before showing any significant effects of the presence of plants. Our first hypothesis was supported since porosity, <u>pore</u> <u>size</u> the diversity of pore sizes, pore connectivity, and pore surface area density were all enhanced by the presence of plants <u>in the grassland soil</u> . <u>Moreover</u> , the conversion to arable
433 434 435 436 437 438 439 440	as quantified by <u>micro-scale</u> topological metrics related to detail capture at 1.5 µm resolution, requires at least 10-10-years of a new-grassland management before showing any significant effects of the presence of plants. Our first hypothesis was supported since porosity, <u>pore</u> <u>size</u> the diversity of pore sizes, pore connectivity, and pore surface area density were all enhanced by the presence of plants in the grassland soil. Moreover, the conversion to arable management did not affect the soil soil structurale development significantly. The presence of
433 434 435 436 437 438 439 440 441	as quantified by <u>micro-scale</u> topological metrics related to detail capture at 1.5 µm resolution, requires at least 10-10-years of a new-grassland management before showing any significant effects of the presence of plants. Our first hypothesis was supported since porosity, <u>pore</u> <u>size</u> the diversity of pore sizes, pore connectivity, and pore surface area density were all enhanced by the presence of plants in the grassland soil. Moreover, the conversion to arable management did not affect the soil soil structurale development significantly. The presence of plants <u>The conversion to grassland</u> increased the range of pore sizes after only 2 years <sub>2</sub> post-

445	mechanisms behind the recovery development of pore sizes appeared to be dynamic and
446	possibly dependent upon weather conditions before sampling. Apart from the pore size
447	distribution, the magnitude of the plant the grassland effects on all other Minkowski functions
448	was lower than the difference observed after a minimum of 50 years of management (Bacq-
449	Labreuil et al., 2018). In this study, the effect of grassland upon porosity and pore
450	connectivity were two-fold greater than bare fallow management. Here, the difference was
451	significant but not as major., which means that soil structure requires more time to
452	completely establish micro-structure after being converted to grassland or arable.
453	Demonstrably, bBare fallow soil management for this extreme long period (> 50 years) is
454	detrimental to both physical and biological soil properties and the recovery development of
455	the soil structure after this requires more than 10 years after the conversion the grassland.
456	These is observations raises the question on the application to certain managements in
457	agricultural practices. For example, instead of applying a bare-fallow treatment in a crop
458	rotation, it would be beneficial for the soil characteristics to apply a vegetation cover <i>i.e.</i>
459	cover crops which increases the organic matter inputs and impact influence on soil structure,
460	leading to a 'conditioning' of soil physical and biological characteristics for the next crop.
461	This would prevent the further degradation of the soil and also help for its recovery
462	development if the soil characteristics were compromised. Moreover, the recovery
463	development of the soil structure is apparently a long process, process in the context of
464	current agricultural practices and perceived imperatives. ; thus Tthus, a modification of
465	cropping managements might should be anticipated to require some time before the
466	observation of beneficial impacts on soil structural dynamics. Therefore, this should be
467	
	accounted for the future research and conclusions.
468	accounted for the future research and conclusions.

469 Acknowledgements

470	This work was performed at the University of Nottingham Hounsfield facility which receives
471	funding from Biotechnology and Biological Science Research Council (Swindon, UK), and
472	The Wolfson Foundation (London, UK) Work at Rothamsted is supported by the BBSRC-
473	funded Soil to Nutrition strategic programme (BBS/E/C/00010310) and jointly by the Natural
474	Environment Research Council and BBSRC as part of the Achieving Sustainable Agricultural
475	Systems research programme (NE/N018125/1 LTS-M). Access to the Highfield Ley-Arable
476	experiments is supported by the UK's Long-Term Experiment National Capability, funded by
477	a Biotechnology and Biological Sciences Research Council Grant (BBS/E/C/000J0300). We
478	thank two anonymous reviewers for their insightful scrutiny of manuscripts and suggestions
479	for improvement of this paper. The data support the findings of this study are available from
480	the corresponding author upon reasonable request.
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630	Figure 1: Representative 2D X-ray attenuation images of soils subjected to different forms of
631	management over <u>10-10-years</u> after post-following conversion to these each treatments. Base
632	resolution is 40 µm; (P) pores are the darker shades and (S) soil matrix are the lighter shades
633	which relate to the attenuation of the X-ray (a sharpening algorithm has been passed over
634	these images to increase contrast of features); (a, d, g, j, m) bare-fallow; (b, e, h, k, n) arable;
635	and (c, f, i, l, o) grassland.
636	
637	Figure 2: Porosity (based on resolution of $1.5 \ \mu m$ ) in relation to bare fallow, arable and
638	grassland <u>soils</u> in regards to in the years post-conversion following: (a) 1-0-year; (b) 3-2-years;
639	(c) <u>5-4-years</u> ; (d) <u>8-7-years</u> ; (e) <u>11-10-years</u> . Bar-charts were <u>are</u> means ( $n = 9$ ) expressed as
640	the percentage of pores relative to the total volume, whiskers denote pooled standard errors.
641	(f) Porosity evolution from 10- to 1110-years post-conversion, with the points weredata
642	points represent means ( $n = 9$ ), whiskers denote pooled standard errors for clarity and trend
643	lines were are linear regressions.
644	
645	Figure 3: Cumulative pore size normalized to the total pore-volume in relation to bare-
646	fallow, arable and grassland in regards to the years post-following conversion: (a) 1-0-year;
647	(b) <u>3-2-years;</u> (c) <u>5-4-years;</u> (d) <u>8-7-years;</u> (e) <u>11-10-years</u> . <u>Data Ppoints</u> indicate means (n=9),
648	whiskers denote pooled standard errors. (f) Gini coefficient evolution development from 10-
649	to <u>1110-</u> -years postconversion, with the data points were represent means ( $n = 9$ ), whiskers
650	denote pooled standard errors for clarity and trend lines were are linear regressions.
651	
652	Figure 4: Pore connectivity normalized to total volume in continuous bare fallow, arable and
653	grassland <u>soils</u> in regards to the years post-following conversion: (a) 4-0-year; (b) 3-2-years;

654 (c) 5-4-years; (d) 8-7-years; (e) 11-10-years. Points-Data points indicate means (n=9),

655 whiskers denote pooled standard errors.

656

- 657 Figure 5: Pore Ssurface density in relation to continuous bare fallow, arable and grassland
- 658 soils in regards to the years post-following conversion: (a) 1-0-year; (b) 3-2-years; (c) 5-4-
- 659 years; (d) 8-7-years; (e) 11-10-years. Data Ppoints indicate means (n = 9), whiskers denote
- 660 pooled standard errors.

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