



# 1 Physical Protection of Soil Carbon Stocks Under Regenerative 2 Agriculture

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9 Regenerative Agriculture, Aggregate Stability.

10

## 11 Abstract

12 Regenerative agriculture is emerging as a strategy for carbon sequestration and climate  
13 change mitigation. However, for sequestration efforts to be successful, long-term  
14 stabilisation of Soil Organic Carbon (SOC) is needed. This can be achieved either through the  
15 uplift in recalcitrant carbon stocks, and/or through physical protection and occlusion of  
16 carbon within stable soil aggregates. In this research, soils from blackcurrant fields under  
17 regenerative management (0 to 7 years) were analysed with respect to: soil bulk density  
18 (SBD), aggregate fractionation (water stable aggregates vs. non-water stable aggregates (WSA  
19 and NWSA respectively)), soil carbon content, and carbon stability (recalcitrant vs. labile  
20 carbon). From this, long term carbon sequestration potential was calculated from both  
21 recalcitrant and physically occluded carbon stocks (stabilised carbon). Results indicated  
22 favourable shifts in the proportion of NWSA:WSA with time. This ratio increasing from  
23 27.6% : 5.8% (control soil) to 12.6% : 16.0% (alley soil), and 16.1% : 14.4% (bush soil) after 7  
24 years. While no significant ( $p \geq 0.05$ ) changes in recalcitrant carbon stocks were observed  
25 after 7 years, labile carbon stocks increased significantly ( $p \leq 0.05$ ) from  $10.44 \text{ t C ha}^{-1}$  to  $13.87$   
26  $\text{t C ha}^{-1}$ . As a result, total sequesterable carbon (*stabilised carbon*) increased by  $1.7 \text{ t C ha}^{-1}$



27 over the 7 year period, due to the occlusion and protection of this labile carbon stock within  
28 WSA fraction. This research provides valuable insights into the mechanisms of soil carbon  
29 stabilisation under regenerative agriculture practices and highlights the importance of soil  
30 aggregates in physically protecting carbon net-gains.

### 31 **1. Introduction**

32 Land use change, conventional land management practice, and aggressive agricultural  
33 techniques remain key drivers of soil damage and degradation (Lal, 2001, Lambin et al., 2001,  
34 Foley et al., 2005, Pearson, 2007, Smith, 2008, Al-Kaisi and Lal, 2020). Without a shift to more  
35 sustainable approaches future agricultural productivity will be endangered, and with it the  
36 loss of food and economic security for many around the world (Zika and Erb, 2009, Tilman et  
37 al., 2011, Sundström et al., 2014).

38 The effects of soil degradation can greatly reduce environmental and ecosystem quality  
39 and function (IPBES, 2018). Soil erosion and loss of soil organic carbon (SOC), structural  
40 damage (destruction of soil aggregates and compaction), contamination, salinisation, and  
41 nutrient depletion all contribute to soil degradation (Lal, 2015, Montanarella et al., 2016,  
42 Sanderman et al., 2017); undermining the provision of key ecosystem services that underpin  
43 wider environmental health and function (Dominati et al., 2010, Power, 2010).

44 At landscape scales, soil degradation compounds and threatens desertification and  
45 biodiversity loss (Zika and Erb, 2009, Power, 2010, Orgiazzi and Panagos, 2018, Huang et al.,  
46 2020), while making significant contributions to greenhouse gas emissions and climate  
47 change (Lal, 2004, Smith et al., 2020). Globally, agriculture is associated with roughly a third  
48 of total land use and nearly a quarter of all global greenhouse gas emissions each year (Foley  
49 et al., 2011, Smith et al., 2014, Newton et al., 2020). To date it is estimated that more than  
50 176 Gt of soil carbon has been lost to the atmosphere (IPBES, 2018), with approximately 70-



51 80% of this (~130 - 140 Gt) as a direct consequence of anthropogenic land management and  
52 soil cultivation (Sanderman et al., 2017, Lal et al., 2018, Smith et al., 2020). Meanwhile the  
53 area of land affected by desertification globally has been reported to exceed 25% and is  
54 expanding each year (Huang et al., 2020).

55 A key mechanistic step in the degradation of soil, is the loss and destruction of stable soil  
56 aggregates and loss of SOC (Smith, 2008, Baveye et al., 2020). Soil aggregate formation, as  
57 facilitated by SOC, assists the stabilisation and storage of carbon and imparts resilience to  
58 soils against erosion and climate change while providing hydrological benefits and enhancing  
59 soil fertility (Lal, 1997, Abiven et al., 2009, Chaplot and Cooper, 2015, Veenstra et al., 2021).

60 In addition to mitigating the negative effects of soil degradation, the formation and  
61 persistence of stable soil aggregates is instrumental in soil carbon sequestration (Lal, 1997,  
62 Six et al., 1998, Abiven et al., 2009). Particularly due to physical protection of labile carbon  
63 within the soil aggregates which minimise biogenic and oxidative decay of SOC (Brodowski et  
64 al., 2006, Smith, 2008, Schmidt et al., 2011, Berhe and Kleber, 2013).

65 However, it is important, when viewed through the lens of carbon sequestration that we  
66 acknowledge not all carbon is equal. The potential for long-term carbon sequestration is  
67 governed by the resistance of the carbon to degradation. This resistance being conferred  
68 through i) inherent recalcitrance of the carbon, and ii) physical protection of the carbon and  
69 occlusion within soil aggregates. Thus, when considering carbon sequestration potentials as  
70 solutions to climate change it is imperative that we differentiate between soil carbon which  
71 is transient and soil carbon which endures.

72 By adopting of more sustainable management practices, agriculture can transition from a  
73 negative to a positive force for the environment; providing and enhancing a variety of key  
74 ecosystem services (*water regulation, soil property regulation, carbon sequestration and*



75 *biodiversity support* (de Groot et al., 2002, Dominati et al., 2010, Power, 2010, Baveye et al.,  
76 2016, Keenor et al., 2021)).

77 Regenerative agriculture offers opportunities to produce food and other agricultural  
78 products with minimal negative, or even net positive outcomes for society and the  
79 environment; potentially improving farm profitability, increasing food security and resilience,  
80 and helping to mitigate climate change (Al-Kaisi and Lal, 2020, Newton et al., 2020). Despite  
81 having no single definition or prescriptive set of criteria, regenerative agriculture is widely  
82 understood to include the key concepts of: (i) reducing/limiting soil disturbance; (ii)  
83 maintaining continuous soil cover (as vegetation, litter or mulches), (iii) increasing quantities  
84 of organic matter returned to the soil; (iv) maximising nutrient and water-use efficiency in  
85 crops; (v) integrating livestock; (vi) reducing or eliminating synthetic inputs (fertilisers and  
86 pesticides); and (vii) increasing and broadening stakeholder engagement and employment  
87 (Newton et al., 2020, Paustian et al., 2020, Giller et al., 2021).

88 Adoption of no/minimum-till techniques increases the extent of soil aggregation and  
89 improves long-term carbon storage potential (Lal, 1997, Gál et al., 2007, Ogle et al., 2012,  
90 Lehmann et al., 2020). Furthermore, in addition to providing physical protection to more  
91 labile forms of soil carbon, improved soil aggregation enhances resilience to the effects of  
92 drought and erosion, and provides better hydrological function and structure to the soil  
93 (Abiven et al., 2009, Bhogal et al., 2009, Baveye et al., 2020, Ferreira et al., 2020, Martin and  
94 Sprunger, 2022). No/minimum till techniques have been adopted worldwide and in a variety  
95 of agricultural contexts to help reduce soil erosion, increase crop yields and minimise input  
96 costs all while building soil organic matter (Sisti et al., 2004, Pittelkow et al., 2015, Ferreira et  
97 al., 2020). Adoption of minimum-till and no-till methods compared with conventional tillage  
98 has been reported to significantly increase SOC content within the top 30cm of a soil (Gál et



99 al., 2007, Ogle et al., 2012). However, these potential SOC increases depend on agricultural  
100 context, climate and soil type (Lal, 2004). Conversion from conventional to regenerative  
101 approaches may increase macro-aggregation and aggregate stability (Lal, 1997), and by  
102 extension, provide the means to protect labile soil carbon; thus, enhancing long-term soil  
103 carbon sequestration efforts (Six et al., 1998, Brodowski et al., 2006, Smith, 2008, Schmidt et  
104 al., 2011, Berhe and Kleber, 2013). Furthermore, adoption of regenerative methods such as  
105 no-till or reduced till can also lessen machinery costs, working hours and direct carbon  
106 emission (Kasper et al., 2009). Indeed, resulting from the adoption of no-till methods, it is  
107 estimated that emission reductions of approximately 241 Tg CO<sub>2</sub>e have been achieved  
108 globally since the 1970s (Al-Kaisi and Lal, 2020).

109 To evaluate the influence of transitioning to soft fruit production under regenerative  
110 principles, from a regime of conventional cropping and tillage, a field experiment was  
111 undertaken on a commercial blackcurrant farm in Norfolk, UK. The experiment evaluated 5  
112 blackcurrant fields managed under regenerative principles for increasing lengths of time, and  
113 a conventionally managed arable field evaluated as a datum. The research assessed carbon  
114 stocks across the regimes and thereafter the proportion of carbon stocks associated with the  
115 soil fractions: sand, water stable aggregates (WSA) and non-water stable aggregates (NWSA).  
116 Thermogravimetric Analysis (TGA) was used to differentiate labile and recalcitrant carbon  
117 pools, and their association to the respective soil fractions (Mao et al., 2022). The research  
118 sought to test the hypothesis that a switch from conventional arable farming to regenerative  
119 soft fruit production would increase total soil carbon stock with time and that this carbon  
120 stock would become increasingly stabilised, either associated with WSA (i.e. physically  
121 protected) and/or of greater resistance to degradation (i.e. recalcitrant).

## 122 **2. Methods**



123        **2.1 Field experiment**

124        This research was undertaken at Gorgate Farm, Norfolk, UK (52°41'58"N 0°54'01"E). The  
125        farm is part of the wider Wendling Beck Environment Project (WBNRP, 2024) a regenerative  
126        farming and landscape management program set in c. 750 ha. The field experiment  
127        comprised 5 blackcurrant fields established in 2019, 2017, 2015, and 2013 (1, 3, 5, and 7 years  
128        since soil disturbance) and a conventionally managed arable field as a datum (0 years since  
129        soil disturbance; field history in the arable regime (2014-2021) is shown in (Fig. SI 1. in the  
130        supplement).

131        The blackcurrant fields under regenerative management were planted using a conservation  
132        strip tillage approach, with the blackcurrant bushes planted as field length strips, leaving  
133        alleyways approximately 2m wide. Currants bushes occupied approximately 40% of the field  
134        and the alleyways between the crops approximately 60%. Once planted, the blackcurrant  
135        crop required minimal interventions beyond the yearly harvest, pruning, sowing of cover  
136        crops in the alleys and fertilisation. Fields remained covered year-round between the  
137        blackcurrant crop, with a diverse grazing cover crop through the autumn and winter months,  
138        and a summer fallow covering crop during the spring and summer months, both directly  
139        drilled, and are treated with sprays of compost tea and organic fertiliser. Comparatively the  
140        control comprised a conventionally managed arable field adjacent to the blackcurrant fields,  
141        cultivated yearly and drilled with winter wheat, with stubble re-incorporation. Samples were  
142        collected in late June, immediately prior to the harvest of both crops.

143        **2.2 Soil sampling**

144        Soil core samples (0 - 7.5cm; n = 5) were collected from beneath the blackcurrant bushes  
145        and at the centre of the alleyways using a soil Dent corer. Further soil core samples (n = 5)  
146        were randomly collected from a conventionally managed arable field. Soil samples were



147 sealed and retained in cold storage ( $\leq 4\text{ }^{\circ}\text{C}$ ) prior to laboratory analysis. Soil cores were  
148 subsequently oven dried ( $40\text{ }^{\circ}\text{C}$  for 24hrs) and soil bulk density calculated ( $n = 5$ ).

### 149 **2.3 Soil fractionation**

150 Soil fractionations, namely, Water Stable Aggregates (WSA), Non-Water Stable Aggregates  
151 (NWSA) and sand, were established using a capillary-wetting wet sieving method, adapted  
152 from Seybold and Herrick (2001): Briefly, the previously dried bulk density samples ( $n = 5$ )  
153 were dry sieved (2 mm) to remove all debris. Subsequently, 2mm sieved bulk soil (100 g) was  
154 placed on  $63\mu\text{m}$  sieves. Thereafter, soil was slowly wetted with de-ionised water. Once damp,  
155 samples were submerged and oscillated under de-ionised water (manually agitated at 30  
156 oscillations per minute in 1.5cm of water for 5 minutes). Material that passed through the  
157  $63\mu\text{m}$  sieve was collected and dried ( $40^{\circ}\text{C}$  for 24 hours) and then weighed, this fraction was  
158 defined as NWSA. The soil retained on the  $63\mu\text{m}$  sieve was further processed using in sodium  
159 hexametaphosphate solution (0.02 M), to disaggregate the WSA aggregates and separate  
160 from the sand fraction. The material remaining on the  $63\mu\text{m}$  sieve was then dried ( $40^{\circ}\text{C}$  for  
161 24 hours); and designated as the sand fraction. The WSA fraction (That which passed through  
162 the  $63\mu\text{m}$  sieve) was subsequently established by back calculation (**Eq. 1**):

163

164 **Eq.1**       $\% \text{ WSA} = \left( \frac{\text{Bulk Soil Mass}_{dry} - (\text{Sand Mass}_{dry} + \text{NWSA Mass}_{dry})}{\text{Bulk Soil Mass}_{dry}} \right) \times 100$

165

### 166 **2.4 Total C, and N content by elemental analysis**

167 Dry bulk soil, and soil fractions, were milled to produce a fine powder and samples (20 mg;  
168  $n = 4$ ) packed in  $8 \times 5$  mm tin capsules. An elemental analyser (Exeter CHNS analyser (CE440))  
169 was used to determine elemental abundance of C and N. Instruments were pre-treated within



170 conditioning samples (acetanilide 1900 $\mu$ g), a blank sample (empty capsule) and an organic  
171 blank sample (benzoic acid 1700 $\mu$ g) prior to sample analysis, and standard reference  
172 materials (acetanilide 1500 $\mu$ g) were run alongside samples (every 6<sup>th</sup> run) for QA/QC (a  
173 precision threshold of  $\pm$  1SD of the mean from the standard reference material) (Hemming,  
174 N.D.).

### 175 **2.5 Thermogravimetric assessment of SOC stability**

176 Thermal stability of the SOC in bulk soil, NWSA and sand fractions were assessed using a  
177 Thermo-gravimetric analyser (Mettler Toledo TGA/DSC 1). Samples (n=2) were contained in  
178 70  $\mu$ l platinum crucibles. Samples were heated, in an inert atmosphere, at a rate of 10 $^{\circ}$ C min<sup>-</sup>  
179 <sup>1</sup> from 25 $^{\circ}$ C to 1000 $^{\circ}$ C. TGA data was subsequently used to ascribe stable/not-stable carbon  
180 and inorganic carbon content of the bulk soil and soil fractions. Data was split into 3 distinct  
181 phases by temperature range according to organic matter attrition windows as stated in Mao  
182 *et al.* (2022): i) 25 $^{\circ}$ C – 125 $^{\circ}$ C (moisture evaporation), ii) 125 $^{\circ}$ C – 375 $^{\circ}$ C (labile components)  
183 and, iii) 375 $^{\circ}$ C – 700 $^{\circ}$ C (recalcitrant components).

### 184 **2.6 Carbon Assessment**

185 Soil carbon was assessed as total SOC, soil fraction C, total labile/recalcitrant C and  
186 physically protected/unstabilised C. In addition, C was further assessed on a total field carbon  
187 stock basis (in t ha<sup>-1</sup>). To calculate the total field carbon stock in t ha<sup>-1</sup> (for all carbon  
188 measures), the C content of both the alley and bush soils (or the sum of their relative  
189 fractions) was multiplied by the relevant soil bulk density measure and the depth of sampling  
190 (ca. 7.5cm) and subsequently added together with acknowledgment of their proportion of  
191 the field (60% and 40%, respectively), as set out in (Eq. 2):

$$192 \quad \text{Eq.2} \quad C \text{ tha}^{-1} = (0.6(C_{\text{Alley}} \times SBD_{\text{Alley}} \times \text{Depth})) + (0.4(C_{\text{Bush}} \times SBD_{\text{Bush}} \times \text{Depth}))$$

193





194        **2.7 Statistical analysis**

195        Significant differences between the field sites were determined using *post hoc* tests on  
196 one-way ANOVA with Tukey's HSD, data significance set to 95% ( $p \leq 0.05$ ) (ANOVA; IBM  
197 SPSS 28). Significant differences between the individual regimes within field sites (alley  
198 soil vs. bush soil) were determined using two tailed T-tests, with data significance set at  
199 two levels of confidence; 95% ( $p \leq 0.05$ ), and 99% ( $p \leq 0.01$ ) (independent samples T-test;  
200 IBM SPSS 28).

201        **3. Results and Discussion**

202        **3.1 Bulk Density**

203        Soil bulk density (SBD) provides insights into soil structures, arrangement of soil particles,  
204 and the extent of soil aggregation arising from the influence of physical, chemical, and  
205 biological edaphic factors (Al-Shammary et al., 2018). As SBD accounts for the total volume  
206 that soils occupy (including the mineral, organic and pore space components), they can act as  
207 a key soil condition indicator (Chaudhari et al., 2013, Allen et al., 2011). SBD maintains a close  
208 correlation to concentrations of organic matter and carbon within the soil, where soils  
209 become depleted in carbon SBD tends to increase, potentially leading to compaction of soil  
210 structures (Allen et al., 2011).

211        Land use management can have significant effect upon the physical condition of soils, and  
212 by extension the services provided by soils: management that culminates in soil compaction  
213 and structural damage reduces available pore space, greatly limiting the storage and  
214 infiltration capabilities of water, the depth to which roots can penetrate, and the movement  
215 of soil fauna; subsequently impairing the function and productivity of soils (Byrnes et al., 2018,  
216 Pagliai et al., 2004).



217 Soils may be considered compacted where soil resistance limits or inhibits the movement  
218 of roots through the soil (SBD between  $1.4 \text{ g cm}^{-3}$  (clay rich soils), and  $1.8 \text{ g cm}^{-3}$  (sand rich  
219 soils)), where SBD is found to exceed these limits negative effects to the growth and  
220 productivity of crops may be observed (Kaufmann et al., 2010, Shaheb et al., 2021).

221 SBD was observed to decrease significantly ( $p \leq 0.05$ ) in both the alley soils and bush soils  
222 in all regeneratively managed fields relative to the conventional control (**Fig. 1**). The highest  
223 overall SBD was measured in the control soil ( $1.75 \text{ g cm}^{-3}$ ) and the lowest SBD in the year 3  
224 bush soil ( $1.07 \text{ g cm}^{-3}$ ) (**Fig. 1**).

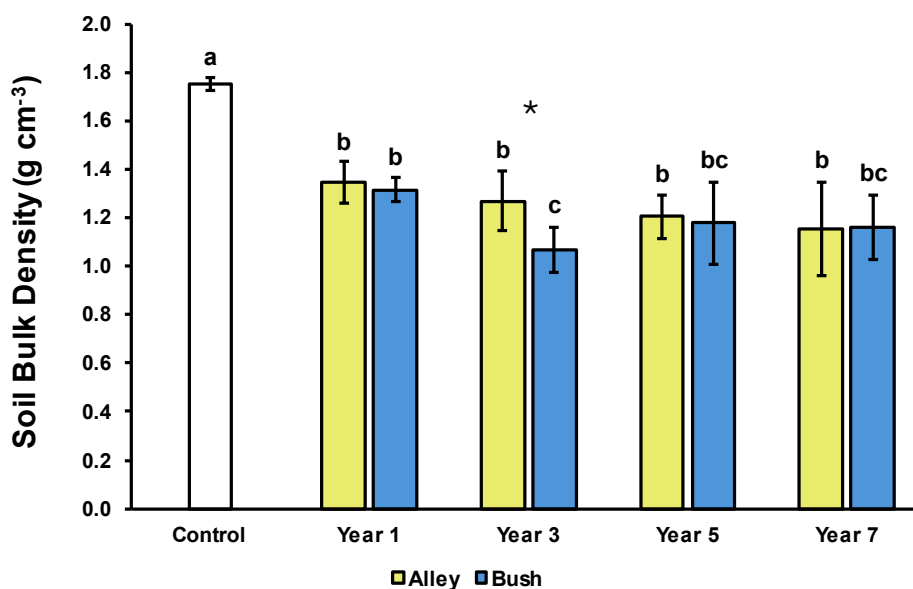
225 In the alley soils SBD was observed to decrease significantly ( $p \leq 0.05$ ) in all of the  
226 regeneratively managed soils compared to the conventional control (**Fig. 1**). Between the  
227 regeneratively managed soils SBD was observed to decrease (not significantly ( $p \geq 0.05$ ))  
228 successively with each additional year under regenerative management; from  $1.35 \text{ g cm}^{-3}$  in  
229 the year 1 alley soil, to  $1.15 \text{ g cm}^{-3}$  in the year 7 alley soil (relative to  $1.75 \text{ g cm}^{-3}$  in the  
230 conventional control soil) (**Fig. 1**).

231 In the bush soils SBD was also observed to decrease significantly ( $p \leq 0.05$ ) in all  
232 regeneratively managed soils relative to the conventional control (**Fig. 1**). Between the  
233 regeneratively managed soils SBD was observed to generally decrease with time, however  
234 this was not successive; the greatest decrease in SBD (significant ( $p \leq 0.05$ )) was observed  
235 between the year 1 and year 3 soils, reducing from  $1.32 \text{ g cm}^{-3}$  in to  $1.07 \text{ g cm}^{-3}$ , before  
236 increasing (not significantly ( $p \geq 0.05$ )) in years 5 and 7 (to  $1.18 \text{ g cm}^{-3}$  and  $1.16 \text{ g cm}^{-3}$   
237 respectively)(**Fig. 1**).

238 When compared pairwise, SBD in the alley soils and the bushes soils were observed to be  
239 broadly similar, with only one pair (*year 3*) showing a significant difference ( $p < 0.05$ ) between  
240 the alley and bush soils, measuring  $1.27 \text{ g cm}^{-3}$  and  $1.07 \text{ g cm}^{-3}$  respectively (**Fig. 1**).



241 None of the soils measured in this investigation were observed to exceed the root limiting  
 242 soil density factor of  $1.8 \text{ g cm}^{-3}$  suggesting no significant detriment to the growth of plants



**Figure 1:** Soil bulk density ( $n=5$ ) of alley (yellow) and bush (blue) regimes with increasing years of establishment. Error bars represent  $\pm 1SD$ . For a given regime (alley or bush) dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries. At a given timepoint, \* indicates a significant difference ( $p < 0.05$ ) between the alley and bush regimes.

243 from soil compaction. Furthermore, the overall trend of soil bulk density reduction seen over  
 244 the course of the 7-year period (**Fig. 1**) is likely a consequence of both increased aggregate  
 245 stability and quantity of stable aggregates (**Section 3.2**) alongside increases in soil carbon  
 246 stocks (**Section 3.3**), changes in which are shown to enhance soil physical properties, i.e.  
 247 optimising soil bulk density (Topa et al., 2021, Rieke et al., 2022, Kasper et al., 2009).

### 248 3.2 Soil Fractionation

249 Soil aggregates that remain stable and resist disaggregation when exposed to water (*water*  
 250 *stable aggregates*) are key determinants of soil structure and stability (Whalen et al., 2003).  
 251 Soil aggregates can be classified by their formation conditions; *biogenic* (decomposition of  
 252 organic matter and action of soil fauna), *physicogenic* (soil physical and chemical processes)



253 and *intermediate* (a combination of biogenic and physicogenic factors)(Ferreira et al., 2020).

254 Additionally, land management practice can further influence the formation and stability of

255 soil aggregates and can significantly alter their formation and destruction (Lal, 1997, Mikha

256 et al., 2021).

257 Stable soil aggregates act as an important indicator of overall soil quality due to their

258 influence on wider soil properties (Lehmann et al., 2020, Rieke et al., 2022). Aggregates exert

259 influence over soil bulk density and hydrology, due to the arrangement and make up of soil

260 structures and pore space (Rieke et al., 2022, Kasper et al., 2009) and can act as a physical

261 protection for organic matter and carbon (Smith, 2008, Brodowski et al., 2006, Abiven et al.,

262 2009).

263 Proportions of WSA and NWSA were seen to change significantly ( $p \leq 0.05$ ) in both the alley

264 and bush soils (**Fig. 2**). While the sand fraction also observed significant changes ( $p \leq 0.05$ )

265 between some of the alley and bush soils (**Fig. 2**), the overall change in sand fraction has been

266 discounted from further discussion as this fraction cannot be created or altered relative to

267 the NWSA or WSA fractions.

268 Soil WSA and NWSA fractions in both the alley soils and bush soils observed opposing trends

269 with age of establishment. With NWSA in both the regimes reducing in fractional share

270 significantly ( $p \leq 0.05$ ) over the 7 years of establishment, while the WSA fractional proportion

271 increased significantly over time ( $p \leq 0.05$ ) (**Fig. 2; Table SI 1** in the supplement). Such changes

272 were likely due to the effects of halting of soil tillage (*with a decrease in NWSA, and*

273 *commensurate increase in WSA in the first year of no-till adoption*) and increasing time since

274 soil disturbance. Furthermore, these shifts in NWSA vs WSA proportions were noted to be

275 commensurate with soil carbon increases (**Section 3.3**) and SBD decreases (**Section 3.1**),



276 Collectively these changes may enhance soil aggregate stability and cohesion (Abiven et al.,  
277 2009, Six et al., 2004, Kasper et al., 2009).

278 NWSA fractions in the alley soils decreased successively with time, from a total of 27.6% in  
279 the control soil to 12.6% in the year 7 soil, with significant reductions ( $p \leq 0.05$ ) measured  
280 between the control soil and all regeneratively managed soils (**Fig. 2; Table SI 1** in the  
281 supplement). Additionally, NWSA in the year 7 soil was measured to be significantly lower ( $p$   
282  $\leq 0.05$ ) than all other regeneratively managed soils (**Fig. 2; Table SI 1** in the supplement).

283 In the bush soil, NWSA fractions were also observed to decrease significantly ( $p \leq 0.05$ ) in  
284 all regeneratively managed soils relative to the control, ranging between 27.6% in the control  
285 to 15.2% in the year 1 soil (**Fig. 2; Table SI 1** in the supplement). However, this decrease was  
286 not successive, as the greatest reduction was measured in the year 1 soil and increased (not  
287 significantly ( $p \geq 0.05$ )) to then broadly plateau in subsequent years (**Fig. 2; Table SI 1** in the  
288 supplement). Furthermore, no significant differences ( $p \geq 0.05$ ) were observed between any  
289 of the regeneratively managed soils.

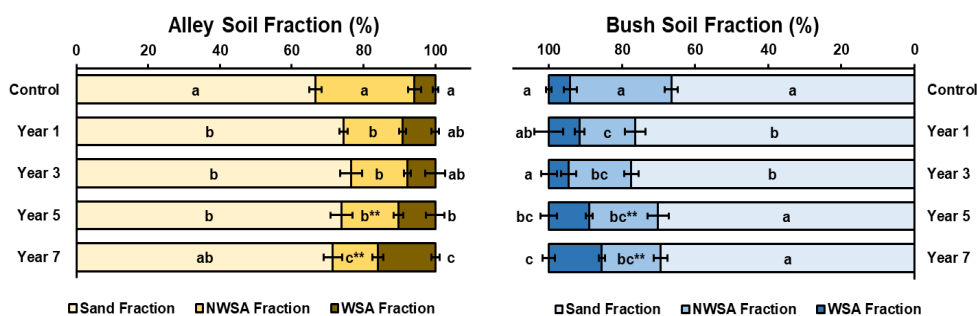
290 When compared pairwise significant differences ( $p \leq 0.01$ ) between the alley and bush soils  
291 were observed in the year 5 and year 7 soils (**Fig. 2; Table SI 1** in the supplement). NWSA  
292 content of the alley soils was measured to be significantly ( $P \leq 0.01$ ) lower than that of the  
293 bushes (15.9% vs. 18.8% in year 5; 12.6% vs. 16.1% in year 7, in the alley and bush soils  
294 respectively) (**Fig. 2; Table SI 1** in the supplement).

295 Conversely WSA fractions in the alley soils increased broadly with age of establishment,  
296 from 5.8% in the control soil to 16.0% in the year 7 soil, with significant increases ( $p \leq 0.05$ )  
297 measured between the control soil (5.8%) and both the year 5 and year 7 soils (10.3% and  
298 16.0% respectively), (**Fig. 2; Table SI 1** in the supplement). Additionally, the WSA fraction in



299 year 7 was observed to be significantly greater ( $p < 0.05$ ) than in all other regeneratively  
 300 managed soils (Fig. 2; Table SI 1 in the supplement).

301 In the bush soils, the WSA fraction was also observed to generally increase with time, from  
 302 5.8% in the control soil to 14.4% in the year 7 soil; with significant increases ( $p \leq 0.05$ )  
 303 measured in the year 5 and year 7 soils (11.0% and 14.4% respectively) (Fig. 2; Table SI 1 in  
 304 the supplement). Within the regeneratively managed soils, significant differences ( $p \leq 0.05$ )  
 305 were also observed between the year 5 soil and the year 3 soil, and between the year 7 soil  
 306 and years 1 and 2 soils (Fig. 2; Table SI 1 in the supplement). When compared pairwise no  
 307 significant differences ( $p \geq 0.05$ ) were observed for the WSA content of the alley and bush  
 308 soils in each year of regenerative management (Fig. 2; Table SI 1 in the supplement).



**Figure 2:** Sand, NWSA, WSA fractions (% total mass) ( $n=5$ ) of alley (left) and bush (right) regimes with increasing years of establishment. Error bars represent  $\pm 1SD$ . For a given regime (alley or bush) dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries. At a given timepoint, the \* indicates a significant difference ( $p \leq 0.05$ ) between the alley and bush regimes. \*\* indicates a significant difference ( $p \leq 0.01$ ), between the alley and bush regimes.

309

### 310 3.3 Soil Carbon and Thermal Stability

311 Soil organic carbon (SOC) underpins a wide range of ecosystem processes and functions  
 312 (Power, 2010, de Groot et al., 2002, Adhikari and Hartemink, 2016, Baveye et al., 2016,  
 313 Dominati et al., 2010). The relative stability of the carbon is an underlying feature of the  
 314 environmental value and utility of carbon. Indeed, biological function and soil biodiversity rely



315 heavily upon easily degradable carbon pools with short residence times, while services such  
316 as carbon sequestration and long-term storage rely upon the more stable recalcitrant carbon  
317 pools that can resist degradation (Dell'Abate et al., 2003, De Graaff et al., 2010, Kleber, 2010,  
318 Keenor et al., 2021, Martin and Sprunger, 2022).

319 SOC was observed to increase in both the alley and bush soils over time (**Fig. SI 2** in the  
320 supplement), with significant increases ( $p \leq 0.05$ ) in the year 5 bush soil ( $22.3 \text{ g kg}^{-1} \text{ C}$ ) and  
321 both the alley and bush soils of year 7 ( $29.9 \text{ g kg}^{-1} \text{ C}$  and  $23.8 \text{ g kg}^{-1} \text{ C}$  respectively) relative to  
322 the control soil ( $16.6 \text{ g kg}^{-1} \text{ C}$ ) (**Fig. SI 2** in the supplement). While increases in SOC were more  
323 pronounced in the alley soils than in the bush soils no significant ( $p \geq 0.05$ ) differences were  
324 observed when compared pairwise (**Fig. SI 2** in the supplement)

325 Thermal techniques such as thermogravimetric analysis can provide effective means of  
326 characterising organic matter pools in the soil, defining the profile of SOC stability (Plante et  
327 al., 2005, Dell'Abate et al., 2000, Dell'Abate et al., 2003, Plante et al., 2011, Mao et al., 2022).  
328 Furthermore, thermal stability can provide a proxy for biogenic decay and degradation of soil  
329 organic matter and carbon stocks (Plante et al., 2005, Nie et al., 2018, Gregorich et al., 2015,  
330 Plante et al., 2011, Mao et al., 2022).

331 Total labile and recalcitrant carbon pools were observed to increase in a broadly stepwise  
332 manner over the 7-year period, with marginally more labile carbon than recalcitrant carbon  
333 measured in both alley soils and bush soils and across all years (**Fig. 3**). Additionally, the  
334 content of labile carbon increased significantly ( $p \leq 0.05$ ) in both the alley and bush soils with  
335 time, while no significant differences ( $p \geq 0.05$ ) between recalcitrant carbon pools of either  
336 the alley or bush soils were observed (**Fig. 3**).

337 Labile soil carbon measured in the alley soils increased broadly stepwise with age of  
338 establishment, with labile carbon increasing in all regenerative managed soils relative to the



339 control soil (**Fig. 3**). These increases were significant ( $p \leq 0.05$ ) in both the year 5 and year 7  
340 soils relative to the control (increasing from  $7.9 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  (control) to  $13.6 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$ ,  
341  $17.6 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  respectively), i.e., an increase of  $9.7 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  (**Fig. 3**). Additionally, the  
342 labile carbon pool measured in the year 7 soil was observed to be significantly greater ( $p \leq$   
343  $0.05$ ) than that of the year 1 and 3 soils (**Fig. 3**).

344 In the bush soils, the labile soil carbon pool followed the same trend of broadly stepwise  
345 increase in all regeneratively managed soils relative to the control. Furthermore, significantly  
346 greater ( $p \leq 0.05$ ) carbon stocks were measured in the year 5 and year 7 soils relative to the  
347 control (increasing from  $7.9 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  to  $12.4 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  and  $13.9 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$ , respectively)  
348 i.e., an increase of  $4.0 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  (**Fig. 3**). Furthermore, significant differences ( $p \leq 0.05$ ) were  
349 measured between regeneratively managed soils (year 5 and 7 vs. year 3; and year 7 vs. year  
350 1) (**Fig. 3**).

351 When compared pairwise, labile carbon in the alley soil increased by a total of  
352  $9.7 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$ , vs. Increase of  $4.0 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  in the bush soil after 7 years of regenerative  
353 management, suggesting enhanced labile carbon stock growth in the alley soils relative to the  
354 bush soils. However, no significant differences ( $p > 0.05$ ) were observed in any given year)  
355 (**Fig. 3**).

356 Recalcitrant carbon measured in the alley soils increased broadly stepwise with increasing  
357 age of establishment, with all regeneratively managed soils increasing relative to the  
358 conventional control, however none of these increases were significant ( $p \geq 0.05$ ) (**Fig. 3**).

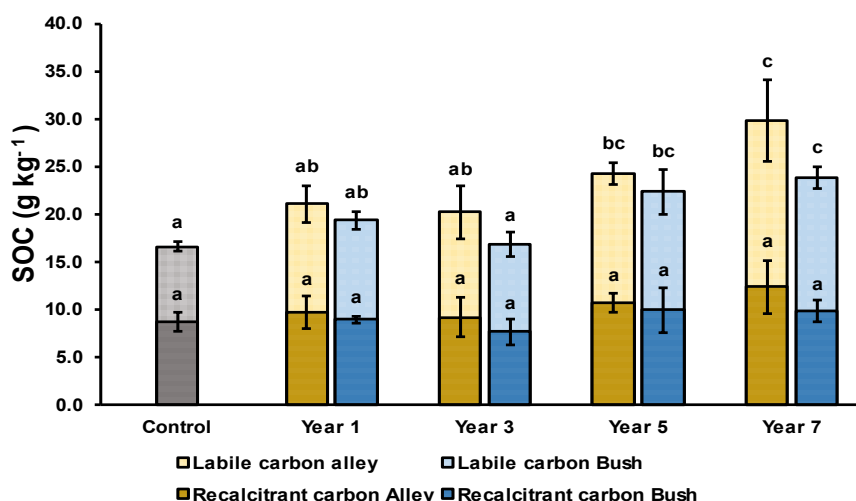
359 Over the 7 year period recalcitrant carbon in the alley soils increased (not significantly ( $p \geq$   
360  $0.05$ )) by  $3.6 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (from  $8.7 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (control) to  $12.3 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (year  
361 7 soils) (**Fig. 3**).





362 In the bush soils, recalcitrant carbon was also observed to generally increase with time (not  
 363 significantly ( $p \geq 0.05$ )). However these increases were smaller than those observed within  
 364 the alley soils (**Fig. 3**). Recalcitrant carbon in the bush soil increased (not significantly ( $p \geq$   
 365  $0.05$ ) from  $8.7 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (control) to  $9.9 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (year 7) i.e., a difference of  
 366  $1.2 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (**Fig. 3**).

367 When compared pairwise for labile and recalcitrant carbon stocks in the alley soils and bush  
 368 soils, no significant differences ( $p \geq 0.05$ ) were observed between any of the given years.  
 369 However, it was observed that both alley and bush soils followed the same trend, with a  
 370 greater proportion of both labile and recalcitrant carbon stored within the alley soils (**Fig. 3**).  
 371 By year 7, the alley soil was observed to contain a total carbon content of  $29.9 \text{ g kg}^{-1} \text{ C}$  (split  
 372 as  $17.6 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  and  $12.3 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$ ), while the bush soil contained a total carbon  
 373 content of  $23.8 \text{ g kg}^{-1} \text{ C}$  (split as  $13.9 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  and  $9.9 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$ ). In contrast, total  
 374 carbon content in the control soil was  $16.6 \text{ g kg}^{-1} \text{ C}$  (split as  $7.9 \text{ g kg}^{-1} \text{ C}_{\text{labile}}$  and  
 375  $8.7 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$ ) (**Fig. 3**).



**Figure 3:** SOC split by recalcitrant (hashed) and labile (plain) carbon pools ( $n=5$ ) in the alleyway (yellow) and bush (blue) regimes. Error bars represent  $\pm 1\text{SD}$ . For a given regime (alley or bush) dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries. At a given timepoint, \* indicates a significant difference ( $p < 0.05$ ) between the alleyway and bush regimes.

376



377 **3.4 Carbon Thermal Stability in Aggregate Fractions**

378 Total labile and recalcitrant carbon pools, when split by soil fraction, were found to diverge  
379 over the 7-year period, with greater proportions of carbon (*both labile and recalcitrant*)  
380 observed in the WSA fraction while diminishing in the NWSA fraction with time (**Fig. 4**). It is  
381 highlighted that despite their smaller fractional share (**Section 3.2**), WSA were substantially  
382 enriched in carbon relative to the NWSA fraction.

383 Labile carbon in the alley soils was observed to shift between dominance in the NWSA  
384 fraction to dominance of the WSA fraction with time, with significant decrease ( $p \leq 0.05$ ) in  
385 the NWSA fraction and a non-significant increase ( $p \geq 0.05$ ) in the WSA fraction (**Fig. 4A**).

386 When analysed by aggregate fraction, the labile carbon pool in the NWSA fraction was  
387 observed to significantly decrease ( $p \leq 0.05$ ) with increased time under regenerative  
388 management, from 33.7% (control) to 17.5% (year 7). However, no significant differences  
389 ( $p \geq 0.05$ ) were measured between the control and the other regeneratively managed soils  
390 (**Fig. 4A**).

391 Within the WSA fraction the labile carbon pool was observed to increase (not significantly  
392 ( $p \geq 0.05$ )) from 45.5% in the conventional control to 61.3% in the year 7 soil (**Fig. 4A**). Initial  
393 reductions in the labile carbon pool were observed in year 1 and year 3 relative to the control  
394 (reducing to 38.1% in the year 3 soil), before rebounding in years 5 and 7. However no  
395 significant differences ( $p \geq 0.05$ ) were observed between any of the soils (**Fig. 4A**).

396 Labile carbon in the bush soils was similarly observed to shift from dominance in the NWSA  
397 fraction to dominance in the WSA fraction with time under regenerative management,  
398 culminating in reduced NWSA and increased WSA fraction associated labile carbon by year 7.  
399 However, this trend was less pronounced within the alley soil, and no significant differences  
400 ( $p \geq 0.05$ ) were observed overall (**Fig. 4B**).



401 Within the NWSA fraction no significant differences ( $p \geq 0.05$ ) were observed between the  
402 control and any regeneratively managed soil (**Fig. 4B**). Labile carbon initially decreased in year  
403 1 relative to the control (from 33.7% to 24.8%) before converging with the control in years 3  
404 and 5 (33.6% and 33.8% respectively) and subsequently reducing again in year 7 (23.7%) (**Fig.**  
405 **4B**).

406 In the WSA fraction the labile carbon pool increased (not significantly ( $p \geq 0.05$ )) between  
407 the control and year 7 soil (45.5% to 54.8%). However these changes were not as substantial  
408 as those observed in the alley soils (**Fig. 4B**). WSA associated labile carbon decreased in the  
409 year 3 soil to 28.2%, while this decrease was not significant ( $p < 0.05$ ) relative to the control,  
410 labile carbon content was observed to rebound significantly ( $p \leq 0.05$ ) from year 3 to year 7  
411 (**Fig. 4B**).

412 When compared pairwise, a significant difference ( $p \leq 0.05$ ) was observed between the  
413 NWSA fraction of year 5 soil, with 23.7 % of the labile carbon pool contained within the NWSA  
414 fraction of the alley soil relative to 33.8 % in the bush soil; no further significant differences  
415 ( $p \geq 0.05$ ) were observed (**Fig. 4 A/B**).

416 Recalcitrant carbon in the alley soils was also observed to enrich in WSA relative to the  
417 NWSA fractions over time, with the decrease in NWSA being significant ( $p \leq 0.05$ ), while the  
418 increase in WSA was not significant ( $p \geq 0.05$ ) over the 7-year period (**Fig. 4C**).

419 When analysed by fraction, the recalcitrant carbon pool in the NWSA fraction was observed  
420 to decrease broadly stepwise, with a significant decrease ( $p \leq 0.05$ ) measured between the 7-  
421 year and control soils (from 33.2% to 18.9%) (**Fig. 4C**). Significant differences ( $p \leq 0.05$ ) were  
422 also observed between the year 3 and year 7 soils, where NWSA fraction proportion increased  
423 to converge with the control in the year 3 soil (32.2 %), thereafter decreasing in year 5 and  
424 year 7 (**Fig. 4C**).



425 In the WSA fraction the recalcitrant carbon pool was observed to increase (not significantly  
426 ( $p \geq 0.05$ )) with time, increasing from 50.1% in the control to 64.5% in the year 7 soil (**Fig. 4C**).

427 Initial decreases in recalcitrant carbon were observed in the year 1 soil relative to the control  
428 (decreasing (not significantly ( $p \geq 0.05$ )) to 41.0 %). Thereafter subsequent stepwise increases  
429 in all other regeneratively managed soils were observed (**Fig. 4C**).

430 Recalcitrant carbon in the bush soils was also observed to increase in the WSA fraction (not  
431 significantly ( $p \geq 0.05$ )) and decrease (not significantly ( $p \geq 0.05$ )) within the NWSA fraction  
432 from the control soil to the year 7 soil (**Fig. 4D**).

433 When analysed by fraction, the recalcitrant carbon pool in the NWSA fraction was observed  
434 to decrease overall by year 7 (from 33.2% in the control to 26.2%). However, no significant  
435 differences ( $p \geq 0.05$ ) were measured between any of the regeneratively managed soils and  
436 the control (**Fig. 4D**).

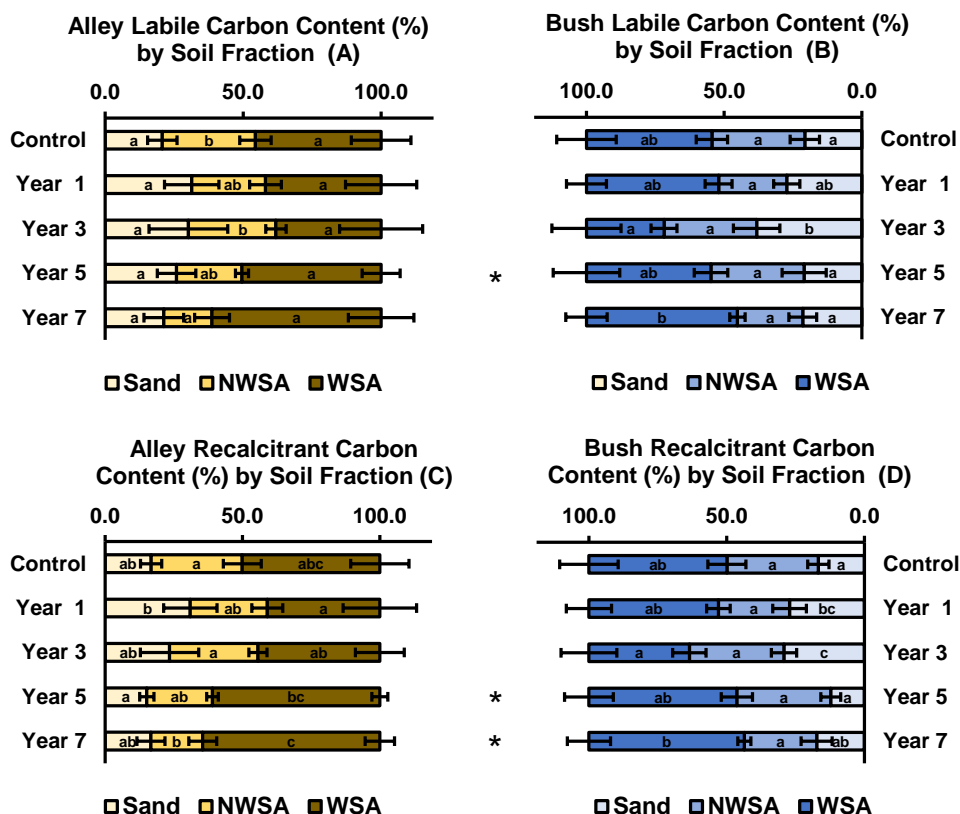
437 Within the WSA fraction, recalcitrant carbon was observed to increase overall from the  
438 control to year 7, with initial reductions (not significant ( $p \geq 0.05$ )) measured in year 1 and 3  
439 relative to the control soil, decreasing from 50.1% in the control to 36.4% in the year 3 soil  
440 (**Fig 4D**). WSA was subsequently observed to increase stepwise to a total of 56.4% in year 7  
441 (not significantly different ( $p \geq 0.05$ ) to the control) (**Fig. 4D**).

442 When compared pairwise significant differences ( $p \leq 0.05$ ) were observed between the in  
443 the recalcitrant carbon pools of the NWSA fraction in both year 5 and year 7 soils, with 23.9%  
444 and 18.9% stored in the alley soils, vs. 34.1% and 26.2% stored in the bush soils respectively  
445 (**Fig. 4 C/D**).

446

447

448



**Figure 4:** Labile (top) and recalcitrant (bottom) SOC split by soil aggregate fraction (Sand, NWSA and WSA) as a total % of soil mass (n=5), of alley (left) and bush (right) soils with increasing years of establishment. Error bars represent  $\pm 1$ SD. For a given soil fraction (sand, NWSA, WSA) dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries. At a given timepoint, the \* indicates a significant difference ( $p \leq 0.05$ ) between the alley and bush regimes. \*\* indicates a significant difference ( $p \leq 0.01$ ), between the alley and bush regimes.

449 **3.5 Aggregate Occlusion of Carbon**

450 Creation and stabilisation of soil aggregates depend on several key factors, including  
 451 climate, soil pH, mineralogy, land management practice, and the  
 452 incorporation/decomposition of organic matter content (Wagner et al., 2007, Lal, 1997).

453 Stable soil aggregates can also confer potentially long-term storage to soil carbon, through  
 454 stabilisation and occlusion, physically separating the carbon from its potential vectors of  
 455 degradation (Schrumpf et al., 2013, Gärdenäs et al., 2011, Six and Jastrow, 2002, Dungait et



456 al., 2012, Plante et al., 2011, McLauchlan and Hobbie, 2004, Smith, 2008). As such, stable  
457 aggregate associated labile carbon (occluded carbon) and non-aggregate/NWSA associated  
458 labile carbon (unprotected carbon) can be considered as separate pools where carbon  
459 stability is concerned, despite the inherent lability of both stocks (Six et al., 1998, McLauchlan  
460 and Hobbie, 2004); where decomposition rates of organic matter held within soil aggregates  
461 may be significantly less than non-aggregate associated organic matter, due to the exclusion  
462 of oxygen and soil biota which would otherwise catalyse decomposition (Smith, 2008, Berhe  
463 and Kleber, 2013, De Gryze et al., 2006, Six et al., 1998, Dungait et al., 2012). Additionally,  
464 aggregate size also plays an important role in stabilising carbon, where microaggregates  
465 better protect the soil carbon in the long term (the energy required to break a soil aggregate  
466 being inversely proportional to its size). However, this macroaggregate presence remains  
467 important to both soil structure and the formation mechanics of microaggregates (Six et al.,  
468 2004, McLauchlan and Hobbie, 2004, Dungait et al., 2012, Rabbi et al., 2013). Previous studies  
469 have shown that the carbon contained within soil aggregates may be relatively more labile  
470 than the broader soil environment as a whole, highlighting the efficacy of this physical  
471 protection granted by occlusion within soil aggregates (Six et al., 1998, Dungait et al., 2012,  
472 McLauchlan and Hobbie, 2004).

473 Stable aggregate occluded carbon considered the stabilised labile carbon stock held within  
474 the WSA fraction (**Section 3.4**), due to the physical protection offered by these aggregate  
475 structures inhibiting the breakdown and decomposition of the carbon stored within.  
476 Conversely unstabilised carbon considered the labile carbon that was not contained within  
477 the WSA fraction (**Section 3.4**), and thus with greater potential for degradation. Additionally,  
478 recalcitrant carbon (**Section 3.3**), was considered stabilised regardless of the soil aggregate  
479 pool in which it was contained due to the relative stability of this carbon fraction.

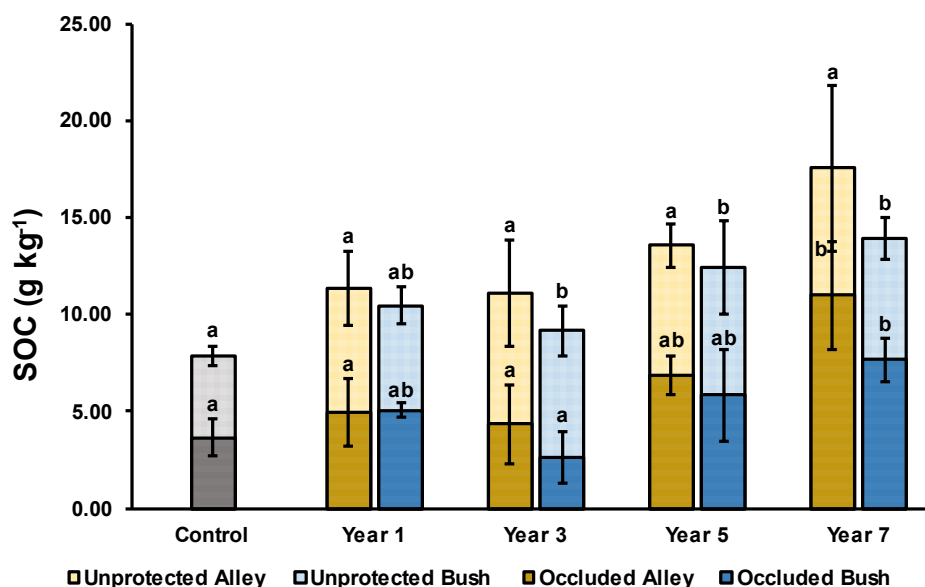


480 Ocluded carbon in the alley soils was observed to increase broadly stepwise with time,  
481 measuring increased occluded carbon content in all regeneratively managed soils relative to  
482 the conventional control. However, this increase was only significant ( $p \leq 0.05$ ) in the year 7  
483 soil, (increasing from  $3.64 \text{ g kg}^{-1} \text{ C}$  to  $10.99 \text{ g kg}^{-1} \text{ C}$  in the control and year 7 soil) (**Fig. 5**). In  
484 the bush soil, occluded carbon was observed to follow a similar trend to that in the alley,  
485 increasing significantly ( $p \leq 0.05$ ) from  $3.64 \text{ g kg}^{-1} \text{ C}$  in the control to  $7.66 \text{ g kg}^{-1}$  in the year 7  
486 soil (**Fig. 5**). However, a decrease (not significant ( $p \geq 0.05$ )) in the occluded carbon content  
487 of the year 3 soil was measured relative to the control soil, reducing to  $2.64 \text{ g kg}^{-1} \text{ C}$ , before  
488 rebounding in years 5 and 7 (**Fig. 5**). When compared pairwise, no significant differences ( $p \geq$   
489  $0.05$ ) were observed between the occluded carbon contents of either the alley soils or bush  
490 soils, with a greater quantity of occluded carbon stored within the alley soils than the bush  
491 soils in all but year 1 (**Fig. 5**).

492 Unprotected carbon in the alley soils was observed to increase (not significantly ( $p \geq 0.05$ ))  
493 in all of the regeneratively managed soils relative to the control soil. However, this increase  
494 remained broadly similar across all regeneratively managed soils, ranging between  $6.4 \text{ g kg}^{-1}$   
495  $\text{C}$  and  $6.7 \text{ g kg}^{-1} \text{ C}$ , compared with  $4.2 \text{ g kg}^{-1}$  in the control soil (**Fig. 5**). In the bush soil,  
496 unprotected carbon was observed to increase broadly stepwise, with significant increases ( $p$   
497  $\leq 0.05$ ) in the year 3, 5 and 7 soils relative to the control, and increasing to a maximum of  $6.6$   
498  $\text{g kg}^{-1}$  (in the year 5 soil) relative to  $4.2 \text{ g kg}^{-1}$  in the control soil (**Fig. 5**). When compared  
499 pairwise no significant differences ( $p \geq 0.05$ ) were observed between the regeneratively  
500 managed soils, with unprotected carbon measuring similarly in both the alley soils and bush



501 soils (Fig. 5).



**Figure 5:** Labile SOC split by occluded (hashed) and unprotected (plain) carbon pools ( $n=5$ ) in the alley (yellow) and bush (blue) regimes. Error bars represent  $\pm 1SD$ . For a given regime (alley or bush) dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries. At a given timepoint, \* indicates a significant difference ( $p < 0.05$ ) between the alley and bush regimes.

502

### 503 3.6 Carbon Stability at Field Scale

504 Acknowledging proportions of alley and bush soils (60% and 40% of field area, respectively)  
505 and accommodating the influence of SBD (Section 3.1; Fig. 1), soil carbon contents (in  $g C kg^{-1}$ )  
506 <sup>1)</sup> (Section 3.3; Fig. SI 2 in the supplement) were converted to carbon stocks ( $t ha^{-1}$ ). These  
507 field scale soil carbon stocks were observed to increase (not significantly ( $p \geq 0.05$ )) by  $1.74 t$   
508  $C ha^{-1}$  over the 7-year period relative to the control soil (from  $21.98 t C ha^{-1}$  to  $23.72 t C ha^{-1}$ )  
509 (Fig. SI 3 in the supplement).

510 When considering carbon stocks as split by labile and recalcitrant carbon pools, both were  
511 initially observed to decrease between the control and year 3 soil (Fig. 6A). The majority of  
512 this decrease occurred in the recalcitrant carbon stock, decreasing significantly ( $p \leq 0.05$ ) from  
513  $11.54 t C ha^{-1}$  to  $7.62 t C ha^{-1}$ , while labile carbon stock was observed to decrease gradually





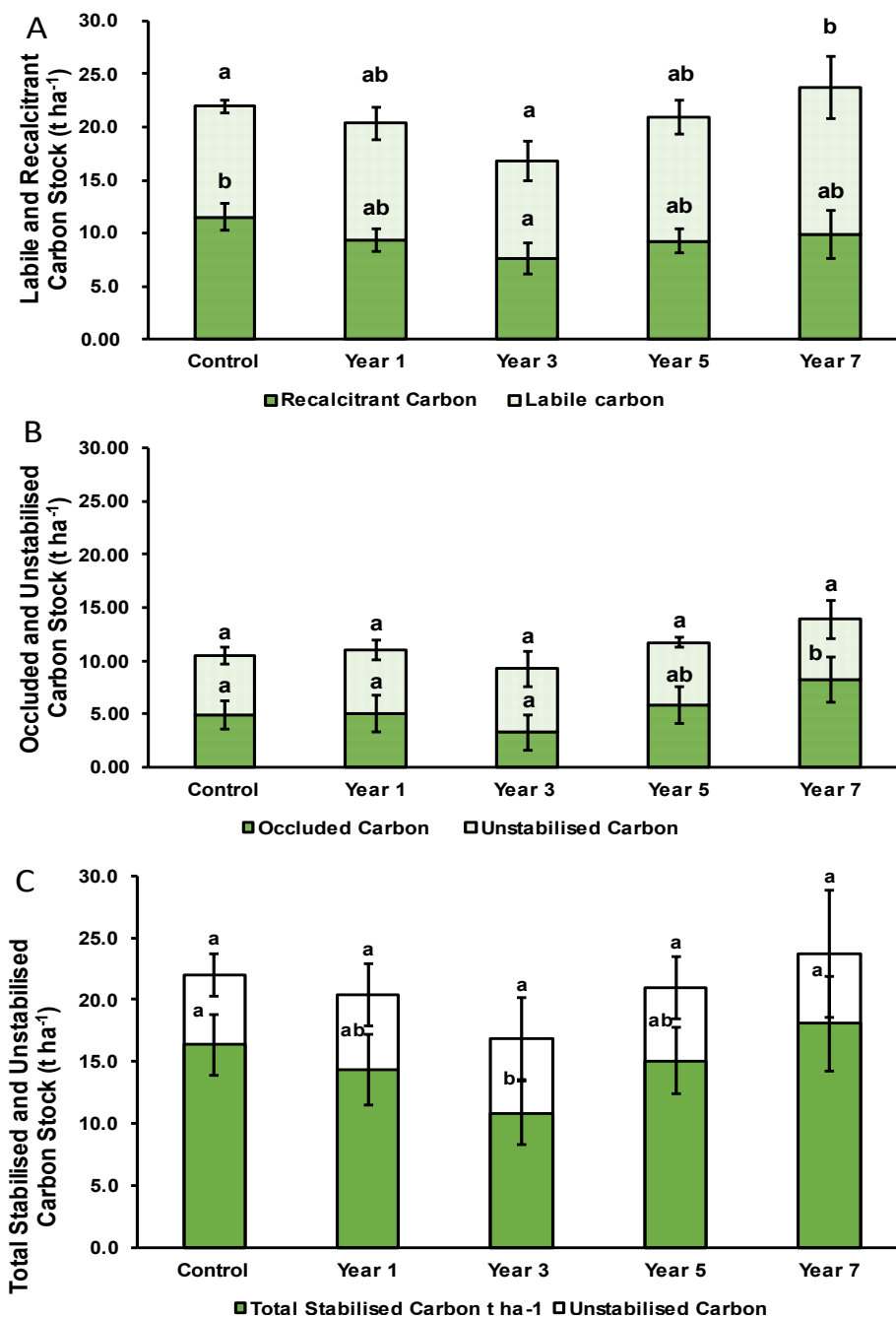
514 (not significantly ( $p \geq 0.05$ ) from  $10.44 \text{ t C ha}^{-1}$  to  $9.22 \text{ t C ha}^{-1}$  (**Fig. 6A**). Following this initial  
515 decrease in both labile and recalcitrant carbon stocks, subsequent yearly increases were  
516 observed in both years 5 and 7, by which point labile carbon stocks were observed to exceed  
517 those in the control (**Fig. 6A**).

518 Over the full 7-year period recalcitrant carbon stock was observed to decrease (not  
519 significantly ( $p \geq 0.05$ ) to  $9.85 \text{ t C ha}^{-1}$  (from  $11.54 \text{ t C ha}^{-1}$ ), while labile carbon stocks were  
520 observed to increase significantly ( $p \leq 0.05$ ) to  $13.87 \text{ t C ha}^{-1}$  (from  $10.44 \text{ t C ha}^{-1}$ ). Highlighting  
521 that the overall  $1.75 \text{ t C ha}^{-1}$  increase observed in soil carbon stock over the 7-year period was  
522 comprised entirely of labile carbon (**Fig. 6A** ; **Fig. SI 3** in the supplement). While recalcitrant  
523 carbon stocks were observed to increase in later years, this rate of increase was less than that  
524 of the labile carbon pool (**Fig. 6A**). However, it is likely that recalcitrant carbon stocks would  
525 recover to the level of the control and possibly increase further with additional time under  
526 regenerative management. Furthermore, It is likely that the initial decreases observed in both  
527 labile and recalcitrant carbon pools related to soil disturbance and changing inputs when  
528 transitioning from an arable to blackcurrant crop, alongside a soil priming effect from the  
529 increase in labile carbon content increasing the diversity and abundance of soil microbial  
530 communities that promote decomposition (De Graaff et al., 2010, Amin et al., 2021,  
531 Yazdanpanah et al., 2016, Lal et al., 2018). Additionally, it has been observed that significantly  
532 increasing labile carbon inputs to the soil can undermine the stability of recalcitrant carbon  
533 due to this enhanced priming effect (De Graaff et al., 2010), potentially causing the  
534 recalcitrant carbon loss initially observed.

535 Occluded carbon stocks were observed to increase mildly (not significant ( $p \geq 0.05$ ))  
536 between the control and year 1 soil (from  $4.81 \text{ t C ha}^{-1}$  to  $4.98 \text{ t C ha}^{-1}$ ), before decreasing  
537 relative to both in the year 3 soil (not significantly ( $p \geq 0.05$ )) (to  $3.23 \text{ t C ha}^{-1}$ ) (**Fig. 6B**).



538 Subsequently, occluded carbon stocks were observed to increase in the years 5 and 7 soils (to  
539 5.82 t C ha<sup>-1</sup> (not significantly ( $p \geq 0.05$ )), and 8.21 t C ha<sup>-1</sup> (significantly ( $p \leq 0.05$ ))  
540 respectively). An overall significant ( $p \leq 0.05$ ) increase in the occluded carbon pool between  
541 the control and year 7 soils, almost doubling from 4.81 t C ha<sup>-1</sup> to 8.21 t C ha<sup>-1</sup> (Fig. 6B). While  
542 unstabilised carbon was observed to remain broadly consistent across all soils with no  
543 significant differences ( $p \geq 0.05$ ) measured (Fig. 6B). Indeed, unstabilised carbon remained  
544 relatively unchanged between the control and year 7 soil (5.63 t C ha<sup>-1</sup> and 5.67 t C ha<sup>-1</sup>  
545 respectively). However, a small increase was observed in the year 1 soil following cultivation,  
546 increasing to 6.02 t C ha<sup>-1</sup>, before converging (Fig. 6B). It is highlighted that the significant ( $p$   
547  $\leq 0.05$ ) increase in occluded carbon corresponds to the almost identical increase in labile  
548 carbon measured in the same time period (3.40 t C ha<sup>-1</sup> and 3.42 t C ha<sup>-1</sup> respectively) (Fig.  
549 6A/B). As such, it can be concluded that virtually all of the uplift in labile carbon measured  
550 over the 7 year period had been physically protected within the stable aggregate fraction.  
551 This result is important as it confirms regenerative practices have been effective in cultivating  
552 aggregate stability capable of physically protecting what would otherwise be potentially  
553 degradable, labile, carbon. Thus, when viewed as total stabilised carbon (inclusive of  
554 recalcitrant carbon and occluded carbon) a total 1.7 t C ha<sup>-1</sup> increase (not significant ( $p \geq 0.05$ )  
555 of potentially sequesterable carbon observed after 7 years of regenerative management  
556 relative to the control (Fig. 6 C).



**Figure 6:** Carbon stock ( $n = 5$ ) split by recalcitrant carbon (hashed) and labile carbon (plain)(A) and occluded carbon (hashed) and unstabilised carbon (plain)(B); and total stabilised carbon (Green) and unstabilised carbon (plain). Total stabilised carbon considered both recalcitrant and occluded carbon stocks. Error bars represent  $\pm 1SD$ . Dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries.



558 **3.6 Carbon sequestration**

559 Efforts to increase soil carbon stocks, through methods such as regenerative agriculture, have  
560 become increasingly important strategies to support climate change mitigation (Lal et al.,  
561 2004, Smith, 2008, Smith et al., 2020, Soussana et al., 2019, Baveye et al., 2020, Keenor et al.,  
562 2021, Lal, 1997, Lal, 2004). However, it is important that we acknowledge not all carbon is  
563 equal in terms of its long-term sequestration potential. The results presented herein highlight  
564 the important nuances of both recalcitrant carbon pools and the physical protection of carbon  
565 (labile and/or recalcitrant) within soil aggregates. Given the physical protection conferred by  
566 stable soil aggregates even relatively labile carbon structures may be stabilised and physically  
567 protected in the long term as a result of their occlusion from degradative forces; with the  
568 aggregate stability governing the carbon residence time rather than its inherent stability  
569 (Schrumpf et al., 2013, Gärdenäs et al., 2011, Dungait et al., 2012, Six and Jastrow, 2002,  
570 Plante et al., 2011, McLauchlan and Hobbie, 2004)(**Section 3.4; Section 3.5**). While the  
571 average mean residence time (MRT) of aggregate stabilised carbon can range from decades  
572 to centuries, similarly to that of recalcitrant carbon, the permanence of this carbon can vary  
573 greatly between different land use types (as a result of soil management practice) (Six and  
574 Jastrow, 2002, Rabbi et al., 2013). As such It is highlighted that carbon protection is only  
575 conferred for as long as the carbon is occluded – i.e. activities that damage and destroy soil  
576 aggregates (*soil disturbance and ploughing*) can reverse these physical protections and allow  
577 for the entry of this carbon to the degradative labile carbon pool from which it had previously  
578 been isolated (Pandey et al., 2014, Six et al., 1998, McLauchlan and Hobbie, 2004). Within a  
579 no till rotational system, carbon storage within stable aggregates has been observed to range  
580 between 27 – 137 years (Six and Jastrow, 2002). Thus providing significant means of stabilising  
581 and sequestering carbon in the medium- to long-term, within regeneratively managed



582 systems (Lal, 1997, Abiven et al., 2009), and potentially on par with that of recalcitrant carbon  
583 stocks (Mao et al., 2022).

584 For accurate carbon sequestration accounting to be realised, focus must be placed on the  
585 role soil bulk density plays in carbon sequestration calculations; as changes in soil carbon  
586 content often culminate in commensurate changes to the bulk density of a soil (Ruehlmann  
587 and Körschens, 2009, Smith et al., 2020). Simply, as soil bulk density changes, the total volume  
588 that the soil occupies also changes (the total amount of soil remains the same, but its  
589 structure and arrangement in 3D space does not). Where soil bulk density decreases, the mass  
590 of soil per unit volume decreases. Consequently, to increase field-scale carbon stocks  
591 (assessed to a prescribed depth), SOC ( $\text{g kg}^{-1}$ ) must increase at a greater rate than bulk density  
592 decreases.

593 In this research, soil bulk density (**Section 3.1**), was observed to decrease with length of  
594 time under regenerative practices, meanwhile soil carbon content (**Section 3.2**) was observed  
595 to increase with time. However, when changes in carbon stocks were considered on a  $\text{t C ha}^{-1}$   
596 basis (with a prescribed soil depth of 7.5cm), carbon stocks did not increase incrementally  
597 with increasing time (**Section 3.6; Fig. SI 3** in the supplement). In effect there was a trade-off,  
598 as the rate of SBD decrease outpaced that of SOC increase. Consequentially, where soil carbon  
599 stocks are considered, while carbon content of the soil increased by ~65% between over the  
600 7 year period (increasing from  $16.6 \text{ g kg}^{-1}$  in the control to  $27.5 \text{ g kg}^{-1}$  after 7 years (alley and  
601 bush soil collectively)), the total field scale increase in carbon stock was only ~8% (increasing  
602 from  $21.98 \text{ t ha}^{-1}$  to  $23.72 \text{ t ha}^{-1}$ )(**Fig. SI 3** in the supplement).

603 Our results highlight the antagonism that exist between SBD and SOC where a prescribed  
604 soil depth is applied to soil carbon stock calculations. Thus, it is arguably more appropriate to  
605 acknowledge the depth of horizon transitions within a soil profile, and where SBD is increasing



606 (e.g. with time under regenerative practices) to in effect increase the volume of the original  
607 soil, this new soil depth of the horizon should be used in carbon stock calculation.

608 Yet it is often the case that soil analysis reports provided to farmers do not appreciate these  
609 changes in SBD; rather they present absolute soil carbon content (%). As a consequence, the  
610 credibility of both on-farm emissions reductions and creation of soil carbon credits is  
611 undermined, creating low integrity carbon sequestration and may lead to the abandonment  
612 of potentially significant transitional technologies due to a lack of trust. As such, the  
613 standardisation of accountancy methods, (alongside robust validation and verification) is  
614 imperative to restoring confidence and boosting the integrity of soil based carbon  
615 sequestration (Keenor et al., 2021).

616 Thus, accounting for recalcitrant carbon and total stabilised carbon with respect to SBD,  
617 potentially sequesterable soil carbon was measured to increase over the 7-year period by 1.7  
618 t C ha<sup>-1</sup> (**Section 3.6; Fig. 6 C**); offering significant benefit and potential to long term carbon  
619 storage at the farm and landscape scale. When calculated against the scale of regenerative  
620 blackcurrant production at Gorgate Farm (50.3 hectares) a total potential of 314 t CO<sub>2</sub>e could  
621 be sequestered with carbon residence on a decadal timescale.

622 As perennial plants, soft fruit and orchard crops offer significant opportunities for  
623 investment, engagement, and adoption of regenerative agriculture principles for soil  
624 enhancement and climate change mitigation, due to their low maintenance - long-term  
625 growing habits and the minimal need for soil disturbance. Were the same regenerative  
626 methods as practiced at Gorgate Farm to be applied to all UK soft fruit production (total of  
627 10,819 hectares (DEFRA, 2023)), this could provide a total UK wide sequestration potential of  
628 67,500 t CO<sub>2</sub>e after 7 years of continuous management, with the potential for further  
629 increases over a longer time period. Whilst this total sequestration after 7 years offers only a



630 small improvement at a nationwide scale, this could be achieved with minimal changes to  
631 current soft fruit production management practice. Furthermore,

#### 632 **4. Conclusion**

633 The results of this research highlight the potential for regenerative agriculture practices to  
634 increase SOC, increase the proportions of WSA, enrichment and physically protect labile  
635 carbon within these aggregates and thus afford opportunity for long-term carbon  
636 sequestration as stabilised carbon stocks. However, our results also bring to the fore  
637 important factors relating to soil carbon stock assessment. In particular, the antagonism  
638 between SBD decreasing at a rate greater than SOC increases; this creating a trade-off where  
639 soil carbon stocks are calculated to a standard prescribed depth. Further research and  
640 practical guidance is needed to enable more robust soil carbon stock assessment that  
641 acknowledges i) a full pedogenic soil horizon, ii) the inherent reactance of SOC, and iii) the  
642 proportion of SOC physically protected by association with soil aggregates.

#### 643 **Authorship contribution**

644 Reid was the Principal Investigator and Keenor the Senior Researcher for this research. Together  
645 Keenor, Reid and Lee undertook the investigation planning and fieldwork. Laboratory work was led by  
646 Keenor with assistance in preliminary laboratory study and WSA method development from Lee.  
647 Keenor undertook the soil data and carbon stability analysis, statistical analysis, literature review, and  
648 the drafting of the manuscript. Keenor and Reid undertook review and editing to deliver the final  
649 manuscript.

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#### 653 **Competing Interests**

654 The authors have no competing interests to declare.



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