Physical Protection of Soil Carbon Stocks Under Regenerative Agriculture

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Keywords: Soil Carbon, Carbon Protection, Stabilised Carbon, Recalcitrant Carbon, Occluded Carbon, Regenerative Agriculture, Aggregate Stability.

Abstract

Regenerative agriculture is emerging as a strategy for carbon sequestration and climate change mitigation. However, for sequestration efforts to be successful, long-term stabilisation of Soil Organic Carbon (SOC) is needed. This can be achieved either through ~~the~~ uplift in recalcitrant carbon stocks, and/or through physical protection and occlusion of carbon within stable soil aggregates. In this research~~, the physical properties and carbon content and stability of~~ soils from blackcurrant fields under regenerative management (0 to 7 years) were assessed. ~~, split between the s~~Soils from under the blackcurrant bush crop (*bush* (ca. 40% of the field area)), and the alleyways between the blackcurrant crop rows (*alley* (ca. 60% of the field area) were ~~analysed~~considered. ~~Focus was placed upon with respect to:~~ ~~soil~~Soil bulk density (SBD), soil aggregate fractions ~~ation~~ (~~–~~proportions of water stable aggregates vs. non-water stable aggregates (WSA and NWSA, respectively)), soil carbon content, and carbon stability (recalcitrant carbon vs. labile carbon~~), and were discussed for the bush soils and the alley soils respectively~~) were assessed. From this, long term carbon sequestration potential was calculated from both recalcitrant and ~~physically~~ occluded carbon stocks (both defined as stabilised carbon). Results indicated favourable shifts in the proportion of NWSA : WSA with time, ~~. This ratio~~ increasing from 27.6 % : 5.8 % (control arable field soil) to 12.6 % : 16.0 % (alley soils), and 16.1 % : 14.4 % (bush soils) after 7 years. While no significant ($p \geq 0.05$)) changes in whole field (area weighted average of alley and bush soils ~~collectively~~) recalcitrant carbon stocks were observed after 7 years, labile carbon stocks increased significantly ($p \leq 0.05$) from 10.44 t C ha^{-1} to 13.87 t C ha^{-1}. Furthermore, ~~A~~as a result of~~,~~ the ~~due to the~~ occlusion ~~and protection~~ of labile carbon within the WSA fraction, total stabilised ~~sequesterable~~ carbon ~~(considered sequesterable*stabilised carbon*)~~ increased by 1.7 t C ha^{-1} over the 7 year period~~, due to the occlusion and protection of this labile carbon stock within WSA fraction~~. This research provides valuable insights into the potential for ~~mechanisms of soil~~ carbon stabilisation and long-term stability prognoses in soils managed under regenerative agriculture practices, ~~and highlights the importance of soil aggregates in physically protecting carbon net gains.~~

highlighting the important role in which soil aggregate stability plays in the physical protection of carbon, and potential therein to deliver long-term carbon sequestration.

1. Introduction

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

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

At landscape scales, soil degradation compounds and threatens desertification and biodiversity loss (Zika and Erb, 2009; Power, 2010; Orgiazzi and Panagos, 2018; Huang et al., 2020), while making significant contributions to greenhouse gas emissions and climate change (Lal, 2004; Smith et al., 2020). Globally, agriculture is associated with roughly a third of total land use and nearly a quarter of all global greenhouse gas emissions each year (Foley et al., 2011; Smith et al., 2014; Newton et al., 2020). To date it is estimated that more than 176 Gt of soil carbon has been lost to the atmosphere (IPBES, 2018), with approximately 70-80% of this *(~130 - 140 Gt*) as a direct consequence of anthropogenic land management and soil cultivation (Sanderman et al., 2017; Lal et al., 2018; Smith et al., 2020). Meanwhile the area of land affected by desertification globally has been reported to exceed 25% and is expanding each year (Huang et al., 2020).

A key mechanistic step in the wider degradation of soil and soil carbon loss, is through the loss and destruction of stable soil aggregates and associated SOC, mediated by conventional agricultural practice and soil disturbance (Smith, 2008; Baveye et al., 2020).

~~A key mechanistic step in the degradation of soil, is the loss and destruction of stable soil aggregates and loss of SOC (Smith, 2008; Baveye et al., 2020).~~ Soil aggregates that remain stable and resist disaggregation when exposed to water (*water stable aggregates*) are key determinants of soil structure and stability (Whalen et al., 2003), and act as an important indicator of overall soil quality due to their influence on wider soil properties (Lehmann et al., 2020; Rieke et al., 2022). Soil aggregate formation, as facilitated by SOC, assists the stabilisation and storage (through occlusion and physical protection) of carbon and imparts resilience to soils against erosion and climate change while providing hydrological benefits~~,~~ ~~influencing the arrangement of soil structures and pore space~~ and enhancing soil fertility (Lal, 1997; Abiven et al., 2009; Kasper et al., 2009; Chaplot and Cooper, 2015; Veenstra et al., 2021; Rieke et al., 2022).

~~In addition to mitigating the negative effects of soil degradation, t~~The formation and persistence of stable soil aggregates is instrumental in soil carbon sequestration (Lal, 1997; Six et al., 1998; Abiven et al., 2009). Particularly due to physical protection of labile carbon within ~~the~~ soil aggregates which minimise biogenic and oxidative decay of SOC (Brodowski et al., 2006; Smith, 2008; Schmidt et al., 2011; Berhe and Kleber, 2013). Soil aggregates can be classified by their formation conditions; *biogenic* (decomposition of organic matter and action of soil fauna), *physicogenic* (soil physical and chemical processes) and *intermediate* (a combination of biogenic and physicogenic factors)(Ferreira et al., 2020). Additionally, land management practice can further influence these formation conditions and the stability or destruction of soil aggregates (Lal, 1997; Mikha et al., 2021).

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

By adopting ~~of~~ more sustainable management practices, agriculture can transition from a negative to a positive force for the environment; providing and enhancing a variety of key ecosystem services (*water regulation, soil property regulation, carbon sequestration and biodiversity support*) (De Groot et al., 2002; Dominati et al., 2010; Power, 2010; Baveye et al., 2016; Keenor et al., 2021)~~)~~.

Therein, ~~R~~regenerative agriculture offers opportunities to produce food and other agricultural products with minimal negative, or even net positive outcomes for society and the environment; potentially improving farm profitability, increasing food security and resilience, and helping to mitigate climate change (Al-Kaisi and Lal, 2020; Newton et al., 2020).

Despite having no single definition or prescriptive set of criteria, regenerative agriculture is widely understood to include the key concepts of: (i) reducing/limiting soil disturbance; (ii) maintaining continuous soil cover (as vegetation, litter or mulches), (iii) increasing quantities of organic matter returned to the soil; (iv) maximising nutrient and water-use efficiency in crops; (v) integrating livestock; (vi) reducing or eliminating synthetic inputs (fertilisers and pesticides); and (vii) increasing and broadening stakeholder engagement and employment (Newton et al., 2020; Paustian et al., 2020; Giller et al., 2021).

Adoption of no/minimum-till techniques increases the extent of soil aggregation and improves long-term carbon storage potential (Lal, 1997; Gál et al., 2007; Ogle et al., 2012; Lehmann et al., 2020). Furthermore, in addition to providing physical protection to more labile forms of soil carbon, improved soil aggregation enhances resilience to the effects of drought and erosion, and provides better hydrological function and structure to the soil (Abiven et al., 2009; Bhogal et al., 2009; Baveye et al., 2020; Ferreira et al., 2020; Martin and Sprunger, 2022). No/minimum till techniques have been adopted worldwide and in a variety of agricultural contexts to help reduce soil erosion, increase crop yields and minimise input costs all while building soil organic matter (Sisti et al., 2004; Pittelkow et al., 2015; Ferreira et al., 2020). Adoption of minimum-till and no-till methods compared with conventional tillage has been reported to significantly increase SOC content within the top 30cm of a soil (Gál et al., 2007; Ogle et al., 2012). However, these potential SOC increases depend on agricultural context, climate and soil type (Lal, 2004). Conversion from conventional to regenerative approaches may increase macro-aggregation and aggregate stability (Lal, 1997), and by extension, provide the means to protect labile soil carbon; thus, enhancing long-term soil carbon sequestration efforts (Six et al., 1998; Brodowski et al., 2006; Smith, 2008; Schmidt et al., 2011; Berhe and Kleber, 2013). Furthermore, adoption of regenerative methods such as no-till or reduced till can also lessen machinery costs, working hours and direct carbon emission (Kasper et al., 2009). Indeed, resulting from the adoption of no-till methods, it is estimated that emission reductions of approximately 241 Tg CO_2e have been achieved globally since the 1970s (Al-Kaisi and Lal, 2020).

To evaluate the influence of transitioning to soft fruit production under regenerative principles, from a regime of conventional cropping and tillage, a field experiment was undertaken on a commercial blackcurrant farm in Norfolk, UK. The experiment evaluated 5 blackcurrant fields managed under regenerative principles for increasing lengths of time (0 – 7 years of establishment), and contrasted against a conventionally managed arable field, evaluated as a datum. The research assessed carbon stocks across the regimes and thereafter the proportion of carbon stocks associated with the soil fractions: sand, water stable aggregates (WSA) and non-water stable aggregates (NWSA), with respect to the soil under the blackcurrant bush crop (bush soil) and in between the rows of the blackcurrant crop (alley soils) respectively, and at the field scale (both alley and bush soils collectively) ~~as a whole~~. Thermogravimetric Analysis (TGA) was used to differentiate labile and recalcitrant carbon pools, and their association to the respective soil fractions (Mao et al., 2022). The research sought to test the hypothesis that a switch from a high soil disturbance conventional arable farming system to a no soil disturbance and perennial regenerative soft fruit production system would increase total soil carbon stock with time, and that this carbon stock would become increasingly stabilised, either as occluded carbon (held within WSA, conferring physical protection to these stocks), ~~associated with WSA in~~orwithin ~~(these~~i.e. ~~physically protected)~~ and/or with ~~of~~ greater resistance to degradation (i.e. recalcitrant carbon).

A glossary of terms defining different soil carbon pools and soil fractions considered in this research is provided in the supplementary information (**Table SI ~~2~~1**).

2. Methods

2.1 Field experiment

This research was undertaken at Gorgate Farm, Norfolk, UK (52º41'58"N 0º54'01"E). The farm is part of the wider Wendling Beck Environment Project (WBNRP, 2024) a regenerative farming and landscape management program set in approximately~~.~~ 750 ha ~~of farmland~~. The field experiment comprised 5 blackcurrant fields established on sandy-loam soils in 2019, 2017, 2015, and 2013 (these representing 1, 3, 5, and 7 years since soil disturbance, respectively) and ~~considered~~ a conventionally managed arable field as a datum (0 years since soil disturbance) drilled with winter wheat.~~;~~ Soil samples were collected in late June 2021, immediately prior to the harvest of the blackcurrant crops and a month prior to harvest of the winter wheat crop.

~~f~~Field cropping history in both the blackcurrant and the arable regimes (2014-2021) is shown in ~~(~~**Fig. 1**. ~~in the supplement).~~

<table>
<tr><th></th><th>2014/15</th><th>2015/16</th><th>2016/17</th><th>2017/18</th><th>2018/19</th><th>2019/20</th><th>2020/21</th></tr>
<tr><td>Control Field</td><td>Wheat</td><td>Barley</td><td>Wheat</td><td>Barley</td><td>Wheat</td><td>Barley</td><td>Wheat</td></tr>
<tr><td>Year 1 Field</td><td>Wheat</td><td>Barley</td><td>Wheat</td><td>Barley</td><td>Wheat</td><td>Barley</td><td>Blackcurrant</td></tr>
<tr><td>Year 3 Field</td><td colspan="3">Blackcurrant</td><td>Wheat</td><td colspan="3">Blackcurrant</td></tr>
<tr><td>Year 5 Field</td><td colspan="2">Blackcurrant</td><td colspan="5">Blackcurrant</td></tr>
<tr><td>Year 7 Field</td><td colspan="7">Blackcurrant</td></tr>
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Figure 1: *Field cropping history for the arable control, and regenerative blackcurrant fields (2014-2021). Discrete Boxes represent one full cropping cycle and where applicable re-planting of new bushes.*

The blackcurrant fields under regenerative management were planted using a conservation strip tillage approach, with the blackcurrant bushes planted as field length strips, leaving alleyways approximately 2m wide. Blackcurrant~~s~~ bushes occupied approximately 40% of the field and the alleyways between the crops, approximately 60%. Once planted, the blackcurrant crop required minimal interventions beyond the yearly harvest, pruning, sowing of cover crops in the alleys and fertilisation. Fields remained covered year-round between the blackcurrant crop, with a diverse grazing cover crop through the autumn and winter months, and a summer fallow covering crop during the spring and summer months, both directly drilled (**Table SI 2**). Furthermore, the blackcurrant crop was ~~and~~ treated with bi-yearly sprays of compost tea and urea fertiliser (78 kg ha^{-1} and 100 kg ha^{-1} pre and post flowering of the currants, respectively). Comparatively the control comprised a conventionally managed arable field adjacent to the blackcurrant fields and occupying the same sandy-loam soil type. This field was cultivated yearly to 30cm depth and had been drilled with winter wheat in a wheat/barley rotation for the preceding 6 years. Furthermore, during cultivation the previous crop~~,~~ ~~with~~ stubble was re-incorporated~~ion~~, and was subsequently treated with a urea-based fertiliser at a rate of 100 kg ha^{-1} post emergence.

In the case of blackcurrants being replanted into previously used fields (e.g., the year 5 field (**Fig. 1**)), these soils were no longer classified as under continuous regenerative management.

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The removal of previously established bushes and the subsequent soil disturbance caused through cultivation, and replanting constituted a clear disruption to ongoing regenerative practices, and goal of no soil disturbance. As such, this site was more accurately characterised by the initiation of a new cycle of regenerative management, reflecting this transition and the accompanied soil disturbance, rather than as a continuation of the previous management.

~~Samples were collected in late June, immediately prior to the harvest of crops.~~

2.2 Soil sampling

Soil core samples (0 - 7.5cm; n = 5) were collected from beneath the blackcurrant bushes and at the centre of the alleyways of each blackcurrant field using a ~~soil~~ Dent soil corer. Further soil core samples (n = 5) were randomly collected from a conventionally managed arable field. Soil samples were sealed and retained in cold storage (≤ 4 °C) prior to laboratory analysis. Soil cores were subsequently oven dried (40 °C for 24 hrs) and soil bulk density calculated (n = 5).

2.3 Soil fractionation

Soil fractionations, namely, Water Stable Aggregates (WSA), Non-Water Stable Aggregates (NWSA) and sand (**Table SI 1** ~~of the supplement~~), were established using a capillary-wetting wet sieving method, adapted from Seybold and Herrick (2001). ~~:~~ ~~Briefly,~~ To generate these different soil fractions, the previously dried bulk density samples (n = 5) were dry sieved (2 mm) to remove all debris and material ≥2mm, yielding the bulk soil fraction. Subsequently, this 2mm sieved bulk soil (100 g) was placed on 63 µm sieves and ~~.~~ ~~Thereafter, soil was~~ slowly wetted with de-ionised water. Once damp, samples were submerged and oscillated under de-ionised water (manually agitated at 30 oscillations per minute in 1.5 cm of water for 5 minutes). Material that passed through the 63 µm sieve was collected and dried (40 °C for 24 hours) and then weighed, yielding the ~~this fraction was defined as~~ NWSA. The soil retained on the 63 µm sieve was further processed using in sodium hexametaphosphate solution (0.02

M)~~,~~ to disaggregate any water stable aggregates from the remaining material, ~~the WSA aggregates~~ and separate these from the sand and inorganic ~~-~~material present in the sample~~fraction~~. The material remaining on the 63 µm sieve was then dried (40 ºC for 24 hours); and designated as the sand fraction. The WSA fraction (That which passed through the 63 µm sieve) was subsequently established by ~~back~~ calculation (**Eq. 1**):

Eq.1 $$\%\, \boldsymbol{WSA} = \left(\frac{\boldsymbol{Bulk\ Soil\ Mass_{dry} - (Sand\ Mass_{dry} + NWSA\ Mass_{dry})}}{\boldsymbol{Bulk\ Soil\ Mass_{dry}}}\right) \times \mathbf{100}$$

2.4 Total C, and N content by elemental analysis

Dry bulk soil, and the separated soil fractions (sand fraction and NWSA fraction), were milled to produce a fine powder, and subsequently samples (20 mg; n = 4) packed in 8 × 5 mm tin capsules. An elemental analyser (Exeter CHNS analyser (CE440)) was used to determine elemental abundance of C and N. Instruments were pre-treated within conditioning samples (acetanilide 1900 µg), a blank sample (empty capsule) and an organic blank sample (benzoic acid 1700 µg) prior to sample analysis, and standard reference materials (acetanilide 1500 µg) were run alongside samples (every 6^{th} run) for QA/QC (a precision threshold of ± 1SD of the mean from the standard reference material) (Hemming, N.D.). Subsequently, WSA fraction carbon contents were subsequently established by calculation (variation of **Eq 1**).

2.5 Thermogravimetric assessment of SOC stability

Thermal stability of the SOC in the bulk soil, and the separated soil fractions (sand fraction and NWSA fraction) were assessed using a thermo-gravimetric analyser (Mettler Toledo

TGA/DSC 1). Samples (n = 2) were contained in 70 µl platinum crucibles. Samples were heated, in an inert atmosphere, at a rate of 10 ºC min^{-1} from 25 ºC to 1000 ºC. TGA data was subsequently used to ascribe the labile and recalcitrant ~~stable/not stable~~ carbon contents ~~and inorganic carbon content~~ of the bulk soil and soil fractions, as well as any inorganic carbon within the samples. Data was split into 3 distinct phases by temperature range according to organic matter attrition windows as stated in Mao *et al.* (2022): i) 25 ºC – 125 ºC (moisture evaporation), ii) 125 ºC – 375 ºC (labile components) and, iii) 375 ºC – 700 ºC (recalcitrant components). Subsequently, WSA fraction carbon stabilities were subsequently established by calculation (variation of **Eq 1**).

2.6 Carbon Assessment

Soil carbon was assessed as total SOC, soil fraction C (NWSA associated carbon, and WSA associated carbon respectively), total labile~~/~~ and recalcitrant C, ~~and~~ occluded carbon (physically protected) ~~/~~ and unstabilised C (**Table SI 1** ~~of the supplement~~)**.** In addition, C was further assessed on a total field carbon stock basis (in t ha^{-1}). To calculate the total field carbon stock in t ha^{-1} (for all carbon measures), the C content of both the alley and bush soils (or the sum of their relative fractions) was multiplied by the relevant soil bulk density measure and the depth of sampling (ca. 7.5cm) and subsequently added together with acknowledgment of their proportion of the field (60% and 40%, respectively), as set out in (**Eq. 2**):

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

2.7 Statistical analysis

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

3. Results and Discussion

3.1 Bulk Density

When considering soil stability, ~~S~~soil bulk density (SBD) provides significant insights into ~~soil structures,~~ the arrangement and structure of soil particles, and the extent of soil aggregation~~,~~ ~~arising from the influenced of by physical, chemical, and biological edaphic factors~~ (Al-Shammary et al., 2018). As SBD accounts for the total volume that soils occupy (including the mineral, organic and pore space components), ~~they~~ it is~~can act as~~ a key ~~soil~~ indicator of soil condition ~~indicator~~ (Chaudhari et al., 2013; Allen et al., 2011). Furthermore, SBD maintains a close correlation to concentrations of organic matter and carbon within the soil, where soils become depleted in carbon, SBD tends to increase, potentially leading to compaction of soil structures (Allen et al., 2011).

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

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

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

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

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

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

None of the soils measured in this investigation were observed to exceed the root limiting soil density factor of 1.8 g cm^{-3} in sandy soil types, suggesting no significant detriment to the growth of plants from soil compaction (Kaufmann et al., 2010; Shaheb et al., 2021). Furthermore, the overall trend of soil bulk density reduction seen over the course of the 7-

year period (**Fig. 1**) is likely a consequence of both increased aggregate stability and quantity of stable aggregates (**Sect~~ion~~. 3.2**) alongside increases in soil carbon stocks (**Sect~~ion~~. 3.3**)~~,~~

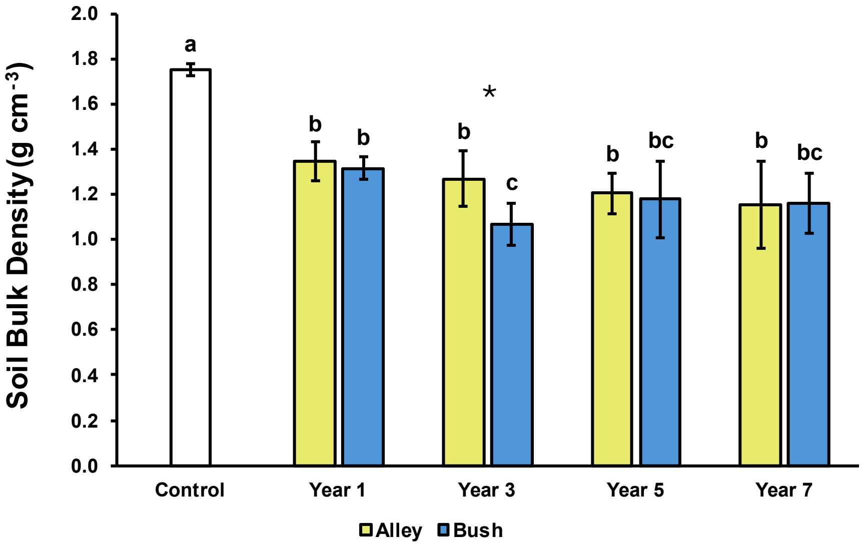

Figure 1: Soil bulk density (n=5) of alley (yellow) and bush (blue) regimes with increasing years of establishment. Error bars represent + 1SD. For a given regime (alley or bush) dissimilar lower-case letters indicate significant ($p \leq 0.05$) differences across the timeseries. At a given timepoint,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

~~changes in which are shown to enhance soil physical properties, i.e. optimising soil bulk density~~ (Topa et al., 2021; Rieke et al., 2022; Kasper et al., 2009).

3.2 Soil Fractionation

~~Soil aggregates that remain stable and resist disaggregation when exposed to water (*water stable aggregates*) are key determinants of soil structure and stability (Whalen et al., 2003). Soil aggregates can be classified by their formation conditions; *biogenic* (decomposition of organic matter and action of soil fauna), *physicogenic* (soil physical and chemical processes) and *intermediate* (a combination of biogenic and physicogenic factors)(Ferreira et al., 2020). Additionally, land management practice can further influence the formation and stability of~~

soil aggregates and can significantly alter their formation and destruction (Lal, 1997; Mikha et al., 2021).

Stable soil aggregates act as an important indicator of overall soil quality due to their influence on wider soil properties (Lehmann et al., 2020; Rieke et al., 2022). Aggregates exert influence over soil bulk density and hydrology, due to the arrangement and make up of soil structures and pore space (Rieke et al., 2022; Kasper et al., 2009) and can act as a physical protection for organic matter and carbon (Smith, 2008; Brodowski et al., 2006; Abiven et al., 2009).

Proportions of WSA and NWSA were seen to change significantly (p ≤ 0.05) in both the alley and bush soils (**Fig. 2**). While the sand fraction also observed significant changes (p ≤ 0.05) between some of the alley and bush soils (**Fig. 2**), the overall change in sand fraction has been discounted from further discussion as this fraction cannot be created or altered relative to theto focus reporting on NWSA or WSA fractions.

Soil WSA and NWSA fractions in both the alley soils and bush soils observed opposing trends with age of establishment. With NWSA in both the regimes reducing in fractional share significantly (p ≤ 0.05) over the 7 years of establishment, while the WSA fractional proportion increased significantly over time (p ≤ 0.05) (**Fig. 2; Table SI 13** in the supplement). Such changes were likely due to the effectsdue to of halting of soil tillage (*with a decrease in NWSA, and commensurate increase in WSA in the first year of no-till adoption*) and increasing time since soil disturbance. Furthermore, these shifts in NWSA vs WSA proportions were noted to be proportionate with soil carbon increases (**Section. 3.3**) and SBD decreases (**Section. 3.1**), Collectively these changes may enhance soil aggregate stability and cohesion (Abiven et al., 2009; Six et al., 2004; Kasper et al., 2009).

NWSA fractions in the alley soils decreased successively with time, from a total of 27.6% in the control soil to 12.6% in the year 7 soil, with significant reductions ($p \leq 0.05$) measured between the control soil and all regeneratively managed soils (**Fig. 2; Table SI 3~~1~~** ~~in the supplement~~). Additionally, NWSA in the year 7 soil was measured to be significantly lower ($p \leq 0.05$) than all other regeneratively managed soils (**Fig. 2; Table SI 3~~1~~** ~~in the supplement~~).

In the bush soil, NWSA fractions were also observed to decrease significantly ($p \leq 0.05$) in all regeneratively managed soils relative to the control, ranging between 27.6% in the control to 15.2% in the year 1 soil (**Fig. 2; Table SI 3~~1~~** ~~in the supplement~~). However, this decrease was not successive, as the greatest reduction was measured in the year 1 soil and increased (not significantly ($p \geq 0.05$)) to then broadly plateau in subsequent years (**Fig. 2; Table SI ~~1~~3** ~~in the supplement~~). Furthermore, no significant differences ($p \geq 0.05$) were observed between any of the regeneratively managed soils.

When compared pairwise significant differences ($p \leq 0.01$) between the alley and bush soils were observed in the year 5 and year 7 soils (**Fig. 2; Table SI 3~~1~~** ~~in the supplement~~). NWSA content of the alley soils was measured to be significantly ($P \leq 0.01$) lower than that of the bushes (15.9% vs. 18.8% in year 5; 12.6% vs. 16.1% in year 7, in the alley and bush soils respectively) (**Fig. 2; Table SI ~~1~~3** ~~in the supplement~~).

Conversely WSA fractions in the alley soils increased broadly with age of establishment, from 5.8% in the control soil to 16.0% in the year 7 soil, with significant increases ($p \leq 0.05$) measured between the control soil (5.8%) and both the year 5 and year 7 soils (10.3% and 16.0% respectively), (**Fig. 2; Table SI ~~1~~3** ~~in the supplement~~). Additionally, the WSA fraction in year 7 was observed to be significantly greater ($p < 0.05$) than in all other regeneratively managed soils (**Fig. 2; Table SI 3~~1~~** ~~in the supplement~~).

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

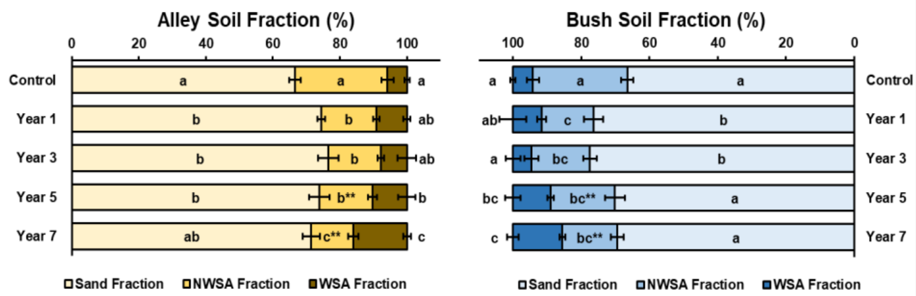

Figure 2: *Sand, NWSA, WSA fractions (% total mass)) (n=5) of alley (left) and bush (right) regimes with increasing years of establishment. Error bars represent ± 1SD. For a given regime (alley or bush) dissimilar lower-case letters indicate significant (p ≤ 0.05) differences across the timeseries. At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

3.3 Soil Carbon and Thermal Stability

~~Soil organic carbon (SOC) underpins a wide range of ecosystem processes and functions (Power, 2010; De Groot et al., 2002; Adhikari and Hartemink, 2016; Baveye et al., 2016; Dominati et al., 2010). The relative stability of the carbon is an underlying feature of the environmental value and utility of carbon. Indeed, biological function and soil biodiversity rely heavily upon easily degradable carbon pools with short residence times, while services such~~

~~as carbon sequestration and long-term storage rely upon the more stable recalcitrant carbon pools that can resist degradation (Dell'abate et al., 2003; De Graaff et al., 2010; Kleber, 2010; Keenor et al., 2021; Martin and Sprunger, 2022). r~~

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

The relative stability of soil carbon is an underlying feature of its inherent environmental value~~ and utility~~: ~~b~~Biological function and soil biodiversity rely heavily upon easily degradable carbon pools with short residence times, while services such as carbon sequestration and long term storage rely upon the more stable recalcitrant carbon pools that can resist degradation (Dell'abate et al., 2003; De Graaff et al., 2010; Kleber, 2010; Keenor et al., 2021; Martin and Sprunger, 2022). Thermal techniques such as thermogravimetric analysis can provide effective means of characterising these organic matter pools in the soil, defining the profile of SOC stability (Plante et al., 2005; Dell'abate et al., 2000; Dell'abate et al., 2003; Plante et al., 2011; Mao et al., 2022). Furthermore, this thermal stability can provide a proxy for biogenic decay and degradation of soil organic matter and carbon stocks (Plante et al., 2005; Nie et al., 2018; Gregorich et al., 2015; Plante et al., 2011; Mao et al., 2022).

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

Labile soil carbon measured in the alley soils increased broadly stepwise with ~~age~~ increasing period of establishment, with labile carbon increasing in all regenerative managed soils relative to the control soil (**Fig. 3**). These increases were significant ($p \leq 0.05$) in both the year 5 and year 7 soils relative to the control (increasing from 7.9 g kg^{-1} C_{labile} (control) to 13.6 g kg^{-1} C_{labile}, 17.6 g kg^{-1} C_{labile}~~-~~, respectively), i.e., an increase of 9.7 g kg^{-1} C_{labile} (**Fig. 3**). Additionally, the labile carbon pool measured in the year 7 soil was observed to be significantly greater ($p \leq 0.05$) than that of the year 1 and 3 soils (**Fig. 3**).

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

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

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

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

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

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

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8.7 g kg^{-1} $C_{recalcitrant}$) (**Fig. 3**).

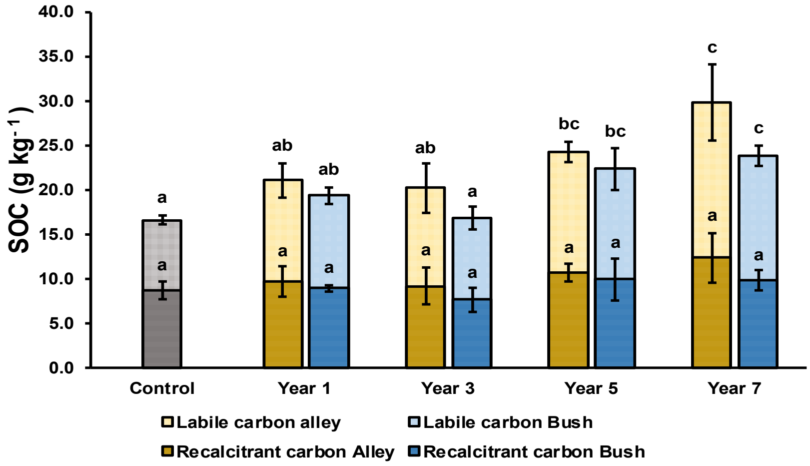

Figure 3: SOC split by recalcitrant (hashed) and labile (plain) carbon pools (n=5) in the alleyway (yellow) and bush (blue) regimes. Error bars represent ± 1SD. For a given regime (alley or bush) dissimilar lower-case letters indicate significant ($p \leq 0.05$) differences across the timeseries. At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

3.4 Carbon Thermal Stability in Aggregate Fractions

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

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

When analysed by aggregate fraction, the labile carbon pool in the NWSA fraction was observed to significantly decrease ($p \leq 0.05$) with increased time under regenerative management, from 33.7% (control) to 17.5% (year 7). However, no significant differences

($p \geq 0.05$) were measured between the control and the other regeneratively managed soils (**Fig. 4A**).

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

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

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

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

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

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

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

In the WSA fraction the recalcitrant carbon pool was observed to increase (not significantly ($p \geq 0.05$)) with time, increasing from 50.1% in the control to 64.5% in the year 7 soil (**Fig. 4C**). Initial decreases in recalcitrant carbon were observed in the year 1 soil relative to the control (decreasing (not significantly ($p \geq 0.05$)) to 41.0 %). Thereafter subsequent stepwise increases in all other regeneratively managed soils were observed (**Fig. 4C**).

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

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

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

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

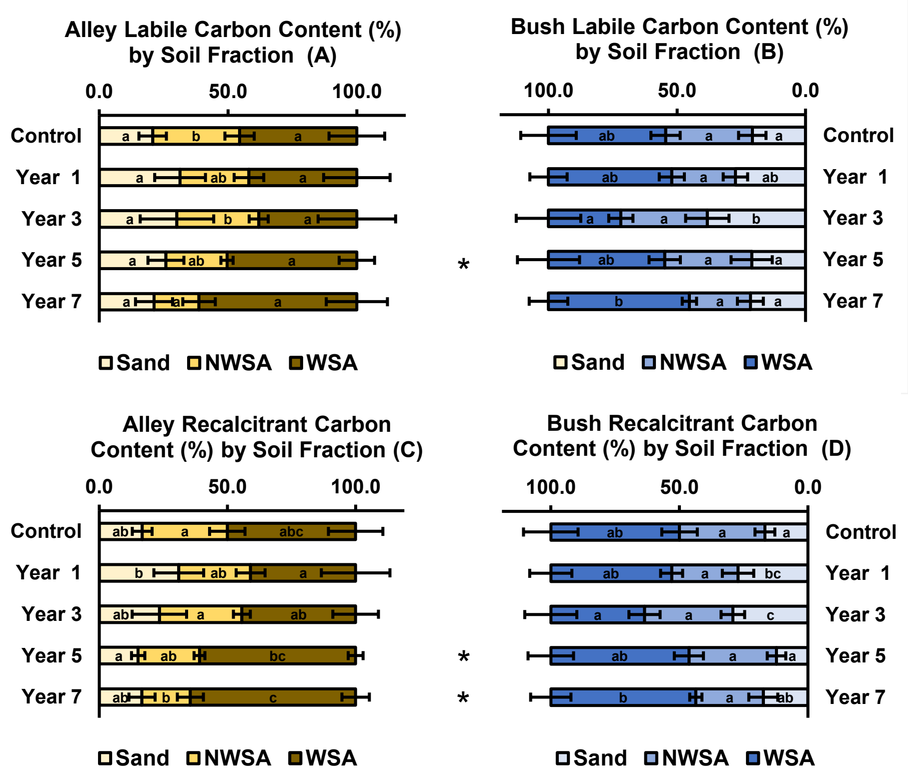

Figure 4: Labile (top) and recalcitrant (bottom) SOC split by soil aggregate fraction (Sand, NWSA and WSA) as a total % of soil mass (n=5), of alley (left) and bush (right) soils with increasing years of establishment. Error bars represent ± 1SD. For a given soil fraction (sand, NWSA, WSA) dissimilar 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.

3.5 Aggregate Occlusion of Carbon

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

Stable soil aggregates can also confer potentially long-term storage to soil carbon, through stabilisation and occlusion, physically separating the carbon from its potential vectors of

~~degradation (Schrumpf et al., 2013; Gärdenäs et al., 2011; Six and Jastrow, 2002; Dungait et al., 2012; Plante et al., 2011; Mclauchlan and Hobbie, 2004; Smith, 2008).~~

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

Despite the inherent degradability of the labile carbon stocks of in both NWSA and WSA aggregate structures, these can be considered as distinct carbon pools for the purpose of long-term carbon storage and stability (Six et al., 1998; Mclauchlan and Hobbie, 2004). ~~Stable aggregate o~~Occluded carbon considered the stabilised labile carbon stocks held within the WSA fraction (**Sect.~~ion~~ 3.4**), due to the long-term storage potential conferred by physical protection within the aggregate structures, physically separating the carbon from its potential vectors of degradation and inhibiting the breakdown and decomposition of the carbon stored within (Schrumpf et al., 2013; Gärdenäs et al., 2011; Six and Jastrow, 2002; Dungait et al., 2012; Plante et al., 2011; Mclauchlan and Hobbie, 2004; Smith, 2008). Conversely unstabilised carbon considered the labile carbon that ~~was not~~ contained within the NWSA fraction (**Sect~~ion~~. 3.4**), and thus with greater potential for degradation, due to the enhanced potential for carbon oxidation and decomposition by soil biota ~~-~~(Smith, 2008; Berhe and Kleber, 2013; De Gryze et al., 2006; Six et al., 1998; Dungait et al., 2012). Additionally, recalcitrant carbon (**Sect~~ion~~. 3.3**), was considered stabilised regardless of the soil aggregate pool in which it was contained (both WSA and NWSA) due to the relative stability of this carbon fraction.

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

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

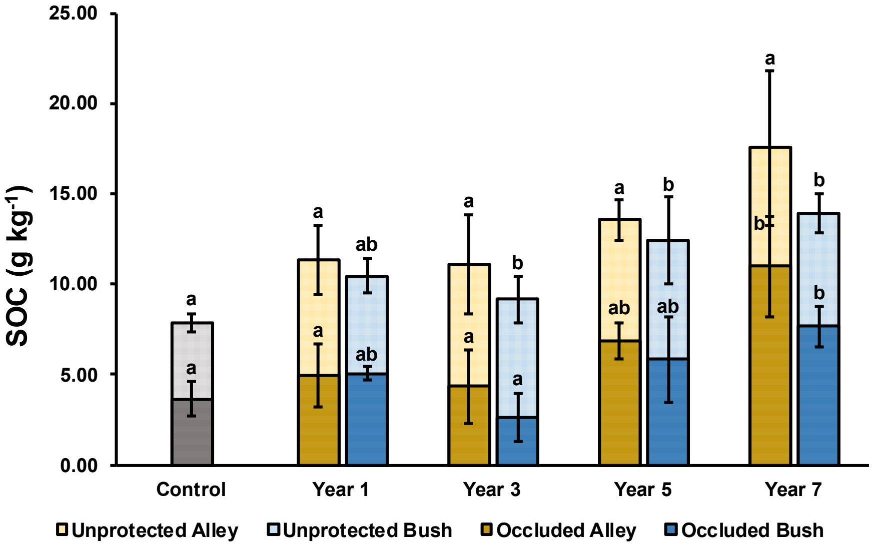

Figure 5: Labile SOC split by occluded (hashed) and unprotected (plain) carbon pools (n=5) in the alley (yellow) and bush (blue) regimes. Error bars represent ± 1SD. For a given regime (alley or bush) dissimilar lower-case letters indicate significant ($p \leq 0.05$) differences across the timeseries.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

3.6 Carbon Stability at Field Scale

Acknowledging proportions of alley and bush soils (60% and 40% of field area, respectively) and accommodating the influence of SBD (**Sect.i on 3.1; Fig. 1**), soil carbon contents (in g C kg^{-1}) (**Section. 3.3; Figg. SI 21** in the supplement) were converted to carbon stocks (t ha^{-1}).

These field scale soil carbon stocks were observed to increase (not significantly ($p \geq 0.05$)) by 1.74 t 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}) (**Fig. SI 2~~3~~** ~~in the supplement~~).

When considering carbon stocks as split by labile and recalcitrant carbon pools, both were initially observed to decrease between the control and year 3 soil (**Fig. 6A**), likely in response to lower soil carbon inputs, arising from small infrequent litter drop of the young plants compared with the yearly incorporation of crop ~~waste~~residues in the conventional system, and additionally soil disturbance during planting. The majority of this decrease occurred in the recalcitrant carbon stock, decreasing significantly ($p \leq 0.05$) from 11.54 t C ha^{-1} to 7.62 t C ha^{-1}, while labile carbon stock was observed to decrease gradually (not significantly ($p \geq 0.05$) from 10.44 t C ha^{-1} to 9.22 t C ha^{-1} (**Fig. 6A**). Following this initial decrease in both labile and recalcitrant carbon stocks, subsequent yearly increases were observed in both years 5 and 7, by which point labile carbon stocks were observed to exceed those in the control (**Fig. 6A**).

Over the ~~full~~ 7 -year period recalcitrant carbon stock was observed to decrease (not significantly ($p \geq 0.05$) to 9.85 t C ha^{-1} (from 11.54 t C ha^{-1}), while labile carbon stocks were observed to increase significantly ($p \leq 0.05$) to 13.87 t C ha^{-1} (from 10.44 t C ha^{-1}). Highlighting that the overall 1.75 t C ha^{-1} increase observed in soil carbon stock over the 7 -year period was comprised entirely of labile carbon (**Fig. 6A ; Fig. SI 2~~3~~** ~~in the supplement~~). While recalcitrant carbon stocks were observed to increase in later years, this rate of increase was less than that of the labile carbon pool (**Fig. 6A**). However, it is likely that recalcitrant carbon stocks would recover to the level of the control and possibly increase further with additional time under regenerative management. Furthermore, It is likely that the initial decreases observed in both labile and recalcitrant carbon pools related to soil disturbance and changing of organic input~~s~~ (crop residue) when transitioning from an arable to blackcurrant crop, alongside a soil priming effect from the increase in labile carbon content increasing the diversity and abundance of soil microbial communities that promote decomposition (De Graaff et al., 2010; Amin et al., 2021; Yazdanpanah et al., 2016; Lal et al., 2018). Additionally, it has been observed that significantly increasing labile carbon inputs to the soil can undermine the stability of recalcitrant carbon due to this enhanced priming effect (De Graaff et al., 2010), potentially causing the recalcitrant carbon loss initially observed.

Occluded carbon stocks were observed to increase ~~mildly~~ marginally (not significant ($p \geq 0.05$)) between the control and year 1 soil (from 4.81 t C ha^{-1} to 4.98 t C ha^{-1}), before decreasing relative to both in the year 3 soil (not significantly ($p \geq 0.05$)) (to 3.23 t C ha^{-1}) (**Fig. 6B**). Subsequently, occluded carbon stocks were observed to increase in the years 5 and 7 soils (to 5.82 t C ha^{-1} (not significantly ($p \geq 0.05$)), and 8.21 t C ha^{-1} (significantly ($p \leq 0.05$)) respectively). An overall significant ($p \leq 0.05$) increase in the occluded carbon pool between the control and year 7 soils, almost doubling from 4.81 t C ha^{-1} to 8.21 t C ha^{-1} (**Fig. 6B**). While unstabilised carbon was observed to remain broadly consistent across all soils with no significant differences ($p \geq 0.05$) measured (**Fig. 6B**). Indeed, unstabilised carbon remained relatively unchanged between the control and year 7 soil (5.63 t C ha^{-1} and 5.67 t C ha^{-1} respectively). However, a small increase was observed in the year 1 soil following cultivation, increasing to 6.02 t C ha^{-1}, before converging (**Fig. 6B**). It is highlighted that the significant ($p \leq 0.05$) increase in occluded carbon corresponds to the almost identical increase in labile carbon measured in the same time period (3.40 t C ha^{-1} and 3.42 t C ha^{-1} respectively) (**Fig. 6A/B**). As such, it can be concluded that virtually all ~~of~~ the uplift in labile carbon measured over the 7 ~~-~~year period had been physically protected within the stable aggregate fraction as occluded carbon. This result is important as it confirms regenerative practices have been effective in cultivating aggregate stability capable of physically protecting what would otherwise be potentially degradable, labile, carbon. Thus, when viewed as total stabilised carbon (inclusive of recalcitrant carbon and occluded carbon) a total 1.7 t C ha^{-1} increase (not significant ($p \geq 0.05$) of potentially sequesterable carbon was observed after 7 years of regenerative management relative to the control (**Fig. 6 C**).

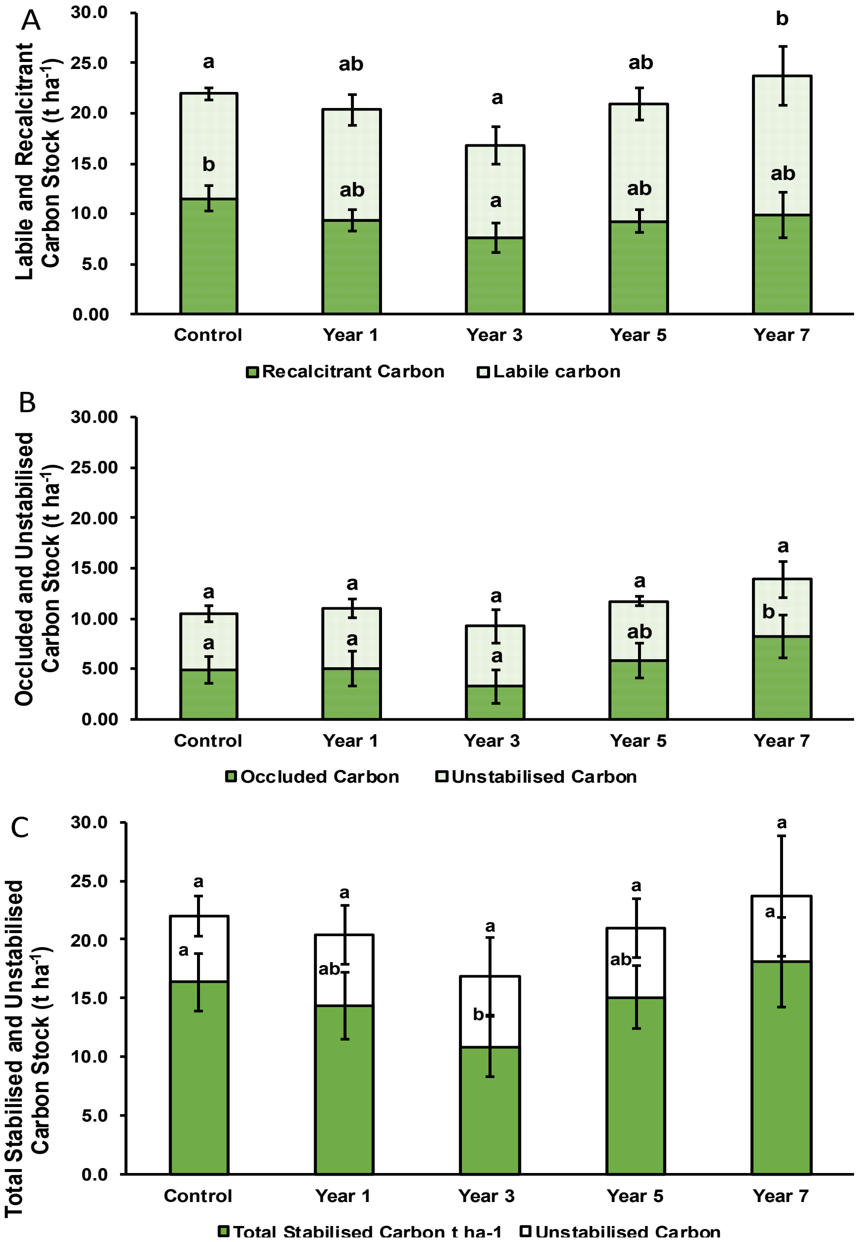

Figure 6: Carbon stock (n = 5) split by recalcitrant carbon (hashed) and labile carbon (plain)(A) and occluded carbon (hashed) and unstabilised carbon (plain)(B); and total stabilised carbon (Green) and unstabilised carbon (plain). Total stabilised carbon considered both recalcitrant and occluded carbon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

3.6 Carbon sequestration

Efforts to increase soil carbon stocks, through methods such as regenerative agriculture, have become increasingly important strategies to support climate change mitigation (Lal, 1997; Lal et al., 2004; Lal, 2004; Smith, 2008; Smith et al., 2020; Soussana et al., 2019; Baveye et al., 2020; Keenor et al., 2021). However, it is important that we acknowledge not all carbon is equal in terms of its long-term sequestration potential. The results presented herein highlight the important nuances of both recalcitrant carbon pools and the physical protection of carbon (labile and/or recalcitrant) within soil aggregates. Given the physical protection conferred by stable soil aggregates even relatively labile carbon structures may be stabilised and physically protected in the long term as a result of their occlusion from degradative forces; with the aggregate stability governing the carbon residence time rather than its inherent stability (Schrumpf et al., 2013; Gärdenäs et al., 2011; Dungait et al., 2012; Six and Jastrow, 2002; Plante et al., 2011; Mclauchlan and Hobbie, 2004)(**Sect~~ion~~. 3.4; Sec~~tion~~t. 3.5**). While the average mean residence time ~~(MRT)~~ of aggregate stabilised carbon can range from decades to centuries, similarly to that of recalcitrant carbon, the permanence of this carbon can vary greatly between different land use types (as a result of soil management practice) (Six and Jastrow, 2002; Rabbi et al., 2013). As such It is highlighted that carbon protection is only conferred for as long as the carbon is occluded – i.e. activities that damage and destroy soil aggregates (*soil disturbance and ploughing*) can reverse these physical protections and allow for the entry of this carbon to the degradative labile carbon pool from which it had previously been isolated (Pandey et al., 2014; Six et al., 1998; Mclauchlan and Hobbie, 2004). Within a no till rotational system, carbon storage within stable aggregates has been observed to range between 27 – 137 years (Six and Jastrow, 2002). Thus providing significant means of stabilising and sequestering carbon in the medium- to long-term, within regeneratively managed systems (Lal, 1997, Abiven et al., 2009), and potentially on par with that of recalcitrant carbon stocks (Mao et al., 2022).

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

In this research, soil bulk density (**Sect~~ion~~. 3.1**), was observed to decrease with ~~length~~ period of time under regenerative practices, meanwhile soil carbon content (**Sect~~ion~~. 3.2**) was observed to increase with time. However, when changes in carbon stocks were considered on a t C ha^{-1} basis (with a prescribed soil depth of 7.5cm), carbon stocks did not increase incrementally with increasing time (**Sect~~ion~~. 3.6; Fig. SI ~~3~~2** ~~in the supplement~~). In effect there was a trade-off, as the rate of SBD decrease outpaced that of SOC increase. Consequentially, where soil carbon stocks are considered, while carbon content of the soil increased by ~65% between over the 7 year period (increasing from 16.6 g kg^{-1} in the control to 27.5 g kg^{-1} after 7 years (alley and bush soil collectively)), the total field scale increase in carbon stock was only ~8% (increasing from 21.98 t ha^{-1} to 23.72 t ha^{-1}) over the 7.5cm depth measured (**Fig. SI ~~3~~2** ~~in the supplement~~).

Our results highlight the antagonism that exist between SBD and SOC where a prescribed soil depth is applied to soil carbon stock calculations. Thus, it is arguably more appropriate to acknowledge the depth of horizon transitions within a soil profile, and where SBD is increasing (e.g. with time under regenerative practices) to in effect increase the volume of the original soil, this new soil depth of the horizon should be used in carbon stock calculation.

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

Thus, accounting for recalcitrant carbon and total stabilised carbon with respect to the SBD measured, potentially sequesterable soil carbon was measured to increase over the 7~~-~~ year period by 1.7 t C ha^{-1} (**Sect~~ion~~. 3.6; Fig. 6 C**); offering significant benefit and potential to long term carbon storage at the farm and landscape scale. When calculated against the scale of regenerative blackcurrant production at Gorgate Farm (50.3 hectares) a total potential of 314 t CO_2e could be sequestered with carbon residence on a decadal timescale.

As perennial plants, soft fruit and orchard crops offer significant opportunities for investment, engagement, and adoption of regenerative agriculture principles for soil enhancement and climate change mitigation, due to their low maintenance - long-term growing ~~habits~~ cycle and the minimal need for soil disturbance. Were the same regenerative methods as practiced at Gorgate Farm to be applied to all UK soft fruit production (total of 10,819 hectares (Defra, 2023)), this could provide a total UK wide sequestration potential of 67,500 t CO_2e (after 7 years of continuous management). ~~, with the potential for further~~

increases over a longer time period. Additionally, changes to the measurement methodologies of soil bulk density determination, appreciating potential changes to soil depth may also increase the accuracy and validity of measurements – potentially increasing the sequestration values measured within this investigation. Ulitmately, Wwhilst this total sequestration potential measured after 7 years, offers only a small improvement at a nationwide scale, this is something that could be achieved presently with minimal changes to current soft fruit production management practice.

4. Conclusion

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

Authorship contribution

BJR Reid was the Principal Investigator and SGKeenor the Senior Researcher for this research. Together SGKeenor, BJReid and RLee undertook the investigation planning and fieldwork. Laboratory work was led by SGK~~eenor~~ with assistance in preliminary laboratory study and WSA method development from RL~~ee~~. SGK~~eenor~~ undertook the soil data and carbon stability analysis, statistical analysis, literature review, and the drafting of the manuscript. SGK~~eenor~~ and BJR~~eid~~ undertook review and editing to deliver the final manuscript.

Acknowledgments

This Research was supported by the Natural Environment Research Council and ARIES DTP [grant number NE/S007334/1] with additional support provided by Greenworld Sales Ltd, Norfolk.

Competing Interests

The authors have no competing interests to declare.

Data Availability Statement

Data can be made available from the corresponding author upon request.

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