

# **Soil Carbon Capture for Ecosystem Service Enhancement**

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# Abstract

This thesis considers potential methods, drivers, barriers, and outcomes relating to soil carbon sequestration with respect to climate change mitigation and the improvement of ecosystem services. Presented herein are four field experiments, split between two potential soil recarbonisation methods: i) direct addition of organic matter soil amendments (with a focus on paper crumble (PC)) for: improving soil properties and providing a measure of carbon prognosis (**Chapter 3**); and for improving hydrological and carbon outcomes in a drought prone sandy soil (**Chapter 4**); and ii) adoption of regenerative agriculture principles for: enhancing soil aggregate structures and physical carbon protection (**Chapter 5**); and impacts upon soil microbial biodiversity, abundance and community structure (**Chapter 6**). Review and analysis of these methods, policy, economic drivers, potential environmental outcomes, data validity, robust methodology and surety of soil carbon metrics is provided in the introductory literature review and perspectives chapter (**Chapters 1 and 2**) respectively. Furthermore, the details of two additional experiments are included as a record of research translation to wider stakeholders.

Applications of the PC soil amendment significantly ( $p \leq 0.05$ ) increased SOC in both clay rich and sand rich soils. Long term carbon prognosis measured significant ( $p \leq 0.05$ ) quotients of carbon would persist for the long term ( $\geq 50$  years). PC applications were also observed to regulate soil physical properties and bulk density, significantly ( $p \leq 0.05$ ) improve water holding capacity and infiltration rates, and provided a source of essential nutrients. Regenerative agriculture principles significantly ( $p \leq 0.05$ ) increased SOC stocks and enhanced soil aggregation, mediating the transition of

carbon from non-stabilised to stabilised aggregates, conferring physical protection to carbon. Additionally, regenerative agriculture significantly ( $p \leq 0.05$ ) influenced soil microbial biodiversity through shifts in community composition facilitated by changes in soil properties with respect to time under regenerative management.

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**“Despite our artistic pretensions, our sophistication, and our many accomplishments — owe our existence to a six-inch layer of topsoil and the fact that it rains.”**

**Paul Harvey 1978**

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# **Chapter 1:**

## **Introduction and Key Concepts**

## 1.1 The Importance of Soil: Carbon and Ecosystem Services

*'[We owe our] existence to a six-inch layer of topsoil and the fact it rains' (Paul Harvey 1978).*

Soils are found in every terrestrial ecosystem around the world, ranging from temperate and tropical regions to the deserts and tundra. Comprised of dynamic and complex mixtures of organic and inorganic components, soils play a pivotal role in shaping the overall health and function of our global ecosystem and provide a large variety of goods and services to the environment and society on which we rely (Dominati et al., 2010, Adhikari and Hartemink, 2016, Lal, 2016).

Despite the importance of these ecosystem services, and indeed of soils themselves, there still persist many gaps in our understanding of the role soils play in providing these services and how our interactions with the environment, especially agricultural land management, may improve or diminish ecosystem service provision (Dominati et al., 2010, Adhikari and Hartemink, 2016). Owing to this, we have and continue to view soil as an inert substance from which our food, fuels and fibres are grown, upon which we build our lives and homes, and where we store our waste – yet this does not appreciate the living and dynamic nature of soil, and the value of all the services supplied to us (*and all living things*) by the soil (Janvier et al., 2007). Soils do not just provide resources; they also manage many of the less tangible ecosystem

services, essential to life on land and critical to the continued function of our environments (Smith et al., 2013, Pereira et al., 2018).

Of primary importance to soil, is the quantity and quality organic matter and carbon stocks - this carbon underpins and mediates the processes of water filtration and storage, nutrient and carbon cycling, biodiversity support, fertility and production, as well as providing the structural and formation conditions of soil (Milne et al., 2015, Lal, 2016, Masciandaro et al., 2018, Vereecken et al., 2022). Furthermore, the carbon stored within soil represents a significant global carbon stock, accounting for approximately 2400 Gt C, or the third largest carbon store on earth, with the quotient of carbon contained within soil being more than 3 times that held in the atmosphere (Paustian et al., 2016, Smith et al., 2020). Consequently, the management of soils (such that they sequester or release this carbon), presents significant potential to either mitigate, or exasperate global climate change.

Within temperate environments, soils with larger carbon stocks generally provide a greater level of ecosystem service provision and are more resilient and resistant to environmental stress relative to soils with less carbon (Powlson et al., 2011a, Orwin et al., 2015, Adhikari and Hartemink, 2016). Despite significant progress and focus placed upon global soil resource management in recent years, a historically myopic view of soil, coupled with the effects of climate change, and pressure exerted by increased global population and wealth, have catalysed soil carbon loss and put our key soil resources under extreme strain (Milne et al., 2015). Actions, both biogenic and anthropogenic, which lead to soil erosion, the loss of soil carbon stocks and declines in soil biodiversity culminate in overall soil degradation - posing significant challenges to climate change, food security, ecosystem service delivery and environmental

sustainability worldwide (Lal, 2004a, Lal, 2004b, Power, 2010, Adhikari and Hartemink, 2016). Furthermore, the impacts of soil degradation are often self-perpetuating, with further damage and functional impairment occurring as a result of the continued degradation process.

Yet soil degradation and its impacts can be mitigated and even reversed: Adopting methods which seek to recarbonise soils can abate much of this damage to the soil environment, redressing and improving the provision of ecosystem services (Power, 2010, Powlson et al., 2011a, Adhikari and Hartemink, 2016, Lal, 2023): Furthermore, doing so may significantly boost soil resilience to climate change (minimising the impacts of droughts, and floods and slowing or reversing the spread of desertification), enhance global food security, and assist in wider environmental goals such as biodiversity net-gain (Doran, 2002, Power, 2010, Smith et al., 2013, Adhikari and Hartemink, 2016, Latawiec et al., 2020). Where this can be achieved alongside improvement to long-term carbon storage, significant opportunity also arises for emissions abatement and climate change mitigation (Smith et al., 2020). However, for this to be achieved, interventions need to be appropriate to their agri-environmental context and tailored to fulfil the desired outcome (e.g., increasing soil carbon content to improve soil hydrological properties in drought prone soils).

The ability to enhance soils by adapting and improving land management practice, while not a panacea, offers a very significant opportunity to enhance wider environmental function and combat climate change. Achieving these aims will require a re-framing of the way we view soils and our environment, such that we better appreciate how the decisions we make and effect upon the agricultural landscape in turn have effects that stretch beyond agricultural production (Costanza et al., 2014).

Thus, several key challenges must be addressed:

- i) Adopting a more holistic view of agricultural land management that values and supports both tangible and intangible ecosystem services;
- ii) Implementing evidence-based transition towards techniques which enhance soil properties and improve ecosystem service provision;
- iii) Placing adequate value upon the goods and services provided by soils;
- iv) Acknowledging the potential to apply these interventions economically at scale;
- v) Creating and adopting robust and reproducible soil measurement methodologies, which acknowledges carbon stability and the influence of soil physical properties upon long term carbon storage;
- vi) Including additional ecosystem service metrics such as soil biodiversity;
- vii) Improving confidence in soil carbon offset potential with accurate modelling and prognosis of carbon residence time in soil. In doing so, we can highlight the potential of a soil-centric solution to the environmental challenges of climate change and ecosystem disfunction, while also improving sustainability, resilience and food security.

This thesis provides an evaluation and synthesis of some of the key challenges that presently limit the adoption, efficacy and impact of recarbonisation and soil management for improved ecosystem service delivery. Through both the review of literature and analysis of experimental data this thesis helps contribute to the growing body of knowledge linking soil science, sustainable land management and climate change mitigation for improved environmental outcomes.

## **1.2 The Challenges: Climate Change, Soil Degradation, Food Security and Ecosystem Service Disfunction**

Globally, we face an increasingly changing and challenging climate due to the accumulation of greenhouse gas emissions in the atmosphere and globally endemic environmental degradation. Since the onset of the great acceleration (*from approximately 1750 to the present day*), these direct and indirect anthropogenic impacts and their knock-on effects upon earth systems have become increasingly observable and impactful (Steffen et al., 2015). Indeed, concentrations of greenhouse gasses have increased to approximately 420 ppm and continue to rise in the order of 2 ppm yr<sup>-1</sup>. As a result, anthropogenically induced warming surpassed average +1°C globally in 2017 (Kell, 2012, Marotta et al., 2023). The consequences of these climatic changes are already of great detriment to the environment: Increasingly severe and erratic weather causing flash floods and drought events, increased rates of soil erosion, desertification and salinisation, biodiversity and habitat loss, and impacts to global food and water security (Kopittke et al., 2019, Lal, 2023). Yet the damage exerted upon the environment – specifically the pedosphere – is not limited purely to environmental and climatic changes, with much of the degradation and damage of soils relates to agriculture, land use and historic soil management (Khaledian et al., 2017). Indeed, population growth and industrialisation has led to the exponential exploitation of soil resources (Sanderman et al., 2017). This (mis)-management has in large part been a direct driver of climate change and carbon emissions, creating a self-perpetuating cycle of environmental degradation (Smith et al., 2016, Kopittke et al., 2019). Conventional agricultural practices particularly have led to the gradual but continued degradation of soil resource stocks globally, through aggressive soil

cultivation, increased agrochemical input (pesticides, herbicides and fertilizers), and the widespread expansion of farming and agricultural mechanisation (Lal, 1993, Janvier et al., 2007). These processes have been shown to significantly reduce many aspects of soil quality and therefore impact the delivery of key ecosystem services (Pagliai et al., 2004, Power, 2010). Yet, due to the negative impact upon ecosystem services, damage done to soil is not confined purely to the soil, it has wide reaching negative consequences to the entire planetary system. Broadly, the provision of ecosystem services, regulation of the environment and even the wealth and economic prosperity of individuals and nations is inexplicably linked to the soil (Daily, 1997, Dominati et al., 2010).

Destruction of soil carbon stock is the primary driver of soil degradation, impacting myriad soil and ecosystem services (Lal, 2023). To date soils are estimated to have lost in between 130 - 180 Gt C to the atmosphere as a result of cultivation mediated destabilisation and decomposition (inclusive of biogenic, erosive and oxidative decomposition), with the potential for further losses of 36 Gt C by 2050 if emission drivers are not redressed (Lal, 2004b, Pimentel, 2006, Bhogal et al., 2009, Sanderman et al., 2017, IPBES, 2018, Lal, 2018, Smith et al., 2020). This damage has accelerated with the adoption of more intensive conventional agricultural techniques since the industrial revolution, culminating in the degradation of global soil stocks (estimated as approximately 20% of the total global soil resource, or 50% of all agriculturally managed soils) (Lal, 2001, Stavi and Lal, 2015, Steffen et al., 2015, Bateman and Muñoz-Rojas, 2019). While abundant and long lasting, soils (*if not managed sustainably*), represent a non-renewable resource on an anthropogenic scale (Rojas et al., 2016, Kopittke et al., 2019); as the rate of replacement from new soil creation is

often too slow compared with current rates of topsoil loss (Papendick and Parr, 1992, Pulleman et al., 2012). Yet, in many regions the rate of soil degradation outpaces natural replacement by a factor of 10 – 40 times; thus, it follows that soil degradation will continue at a rate of 5 – 10 million hectares per year (Pimentel, 2006, Stavi and Lal, 2015, Bateman and Muñoz-Rojas, 2019). This loss has contributed to significant reductions of soil carbon stocks (up to 50% in many soils) (Lal, 2001). Indeed, largely as a result of degradative soil carbon loss, agriculture and land use have been identified as a major contributing source of greenhouse gas emissions globally (Rehberger et al., 2023), estimated at approximately 24% of all anthropogenic greenhouse gas emissions each year (Smith et al., 2014, Soussana et al., 2019).

Beyond the emission of carbon to the atmosphere, soil degradation also severely limits the provision of essential ecosystem services that underpin environmental functions (Power, 2010); including flood mitigation, water holding and conservation, soil carbon storage and sequestration, and food/resource production (Power, 2010, Adhikari and Hartemink, 2016, Latawiec et al., 2020). Consequential reductions in soil carbon have been linked to decreased crop yield (Bauer and Black, 1994, Follett, 2001), loss of soil biodiversity and soil fertility and altered soil hydrology and nutrient provision (Lal, 2001, Lal, 2006, Kimetu et al., 2008). Furthermore, management which impairs provision of ecosystem services and drives loss of soil carbon have significant knock-on impacts to global food security - requiring increased water and nutrient input to maintain adequate yields, significantly raising barriers to food security in a variety of geographical, climatic and economic contexts (Lal et al., 2004, Schmidhuber and Tubiello, 2007, Lal, 2009, Rojas et al., 2016).

Food insecurity is a growing threat in the 21st century mediated not just by climate change and soil degradation, but also as a function of significant population increases and socio-economic improvements in the developing world (Lal, 2006, Lal, 2009, Sposito, 2013, Milne et al., 2015). Global population is expected to grow from the present 8 billion, to nearly 10 billion by 2050; compounding impacts, much of this projected growth expected in regions with degraded soil and limited water resources, and most at risk to changes in climate (Lal, 2006, Lal, 2009, Sposito, 2013, United Nations, 2022). To provide adequate food for this population an estimated increase in production of 50 – 100% is required, yet as a result of soil degradation and changes to regional climates (specifically affecting water availability) agricultural productivity is at risk of stagnation and decline (Foley et al., 2011, Tilman et al., 2011, Sposito, 2013, Rojas et al., 2016, FAO, 2021, O'Donoghue et al., 2022, United Nations, 2022).

The destabilisation of soils has resulted in significant reductions to net primary production and has catalysed the loss of fertile land to desertification (Yong-Zhong et al., 2005, Zika and Erb, 2009). As a consequence, global crop yields measured since 1960 have been found to vary greatly by region, ranging from improvements of +20% as a result of efficiency gains, to reductions of -17% per year as a result of climate, soil degradation and poor policy decisions (Brisson et al., 2010, Lanz et al., 2018, Abd-Elmabod et al., 2020). Furthermore, global crop yield estimates suggest an aggregate annual decrease of 0.3% per year, to a total decline of ~10% by 2050 if the issues pertaining to soil degradation are not addressed (FAO, 2015).

Currently, agricultural land use occupies more than a third of the total ice-free land area globally, totalling more than 4.7 billion hectares (Foley et al., 2011, Sposito, 2013, FAO, 2022). Thus, addressing the potential requirement of 50 – 100% more food

production raises a significant issue to global land use. However, given the present scale of global agricultural land use, a large proportion of the required increase in agricultural productivity must come from existing agricultural land in order to contain runaway degradation and limit further loss of important and ecologically sensitive environments, (Lal, 2009, Foley et al., 2011, Sposito, 2013). Changes in land use have significant negative knock-on effects to other terrestrial systems, both directly and indirectly affecting the climate and ecosystem, either through changes to albedo, hydrology and the water cycle, and carbon sequestration/emission, as well as biodiversity (Foley et al., 2005, Geisen et al., 2019, Azadi et al., 2021). Compounding this, land use change can catalyse soil degradation, desertification and soil contamination as a function of management (Sanderman et al., 2017, Smith et al., 2016).

Thus, the restoration of degraded soils is needed to improve the productivity and ecosystem service delivery of currently underutilised or damaged soil resources. Such enhancements offer significant potential to boost crop production efficiency, and thus bolster food security (Chalise et al., 2019, Jägermeyr et al., 2016). Furthermore, focus on improving these soils may offer substantial opportunities to help deliver ancillary environmental benefits such carbon sequestration and increases in soil biodiversity diversity and abundance (Lal, 2015, Lal, 2016, Bünemann et al., 2018, Geisen et al., 2019). It is here that global policy recommendations and development assistance programmes for holistic soil resource management – such as UN FAO climate smart agriculture, and RECSOIL offer significant potential (FAO, 2017, UNFAO, 2020). As a broad example, sandy soils occupy approximately 5 billion hectares worldwide, yet due to issues of soil-water availability only 4% of sandy soils are currently used for

crop cultivation compared to 12% cultivation for other soil types (Huang and Hartemink, 2020). These soils offer significantly lower production values than other soil types as a result of water deficiencies and drought, owing to high permeability, low water holding capacity and yearly/seasonal evapotranspiration outpacing precipitation (Yost and Hartemink, 2019, Huang and Hartemink, 2020). Improvement of soil properties linked to hydrological capacities in sandy textured soils can help mitigate the negative effects of water scarcity, stress and low crop yields (Dekker and Ritsema, 1994, Huang and Hartemink, 2020, Adhikari et al., 2022). Hence, adoption of methods which redress soil degradation, increase soil carbon stocks, improve soil hydrology and enhance wider ecosystem service provision - may improve the productivity and quality of soil resources, achieving food security goals and providing agricultural and environmental resilience (Lal, 2009, Garbowski et al., 2023).

### **1.3 Ecosystem Services**

An ecosystem service encompasses any good or benefit granted to the wider environment or to humans, that has in some way originated from the natural world (Costanza et al., 1997, Adhikari and Hartemink, 2016). Our ecosystems and the diversity of our landscapes offer many benefits to human society in the form of goods and services, each in their own way with an inherent value (de Groot et al., 2002, Costanza et al., 2017). Soils provide and support a vast array of these ecosystem services, fundamental to the structure and function of our terrestrial environment; providing goods such as food, fuel and fibres – and services, such as, water and climate regulation and support for biodiversity (de Groot et al., 2002, Dominati et al., 2010, Lal, 2013, Adhikari and Hartemink, 2016, Latawiec et al., 2020). These soil ecosystem services can be broken down into four main groups: i) provisioning services - including

all resource and food production; ii) regulating services – including water management and climate regulation; iii) supporting services – including carbon sequestration and storage, and biodiversity habitat provision, and; iv) cultural services – providing recreation and aesthetic value (MA, 2005, Adhikari and Hartemink, 2016, Costanza et al., 2017, Latawiec et al., 2020, Keenor et al., 2021). Some of these goods provided by soils are defined as private goods (those whose value is economic and tangible) and others public goods (those that provide an essential service but are not tangible and thus hold no direct economic value) (Chee, 2004, Kubiszewski et al., 2020).

Whilst there is a great wealth of research on the topic of ecosystem services in general, there still remains a distinct disconnect between the provision of these services and the soil which underpins them (Adhikari and Hartemink, 2016). A key determinant in the adequacy and provision of ecosystem services relies upon the relative health and quality of the soil underpinning them (Doran, 2002, Lal, 2016, Bünemann et al., 2018). Indeed, up to 80% of all ecosystem services rely upon soils, mediated broadly by their management, carbon content and the wider climatic context (Dominati et al., 2010, Lal, 2013, Adhikari and Hartemink, 2016, Bai et al., 2019, Bateman and Muñoz-Rojas, 2019). Healthy soils show greater resilience, adaption and recovery from environmental stress along with an enhanced ability to support wider ecosystem function relative to poor quality or degraded soils (Lehman et al., 2015, Lal, 2016). By extension, soil health and quality also influence agricultural and environmental sustainability more broadly, as well as cascading down to effect individual plant, animal, and human health and wellbeing (Papendick and Parr, 1992, Acton and Gregorich, 1995, Doran, 2002, Lal, 2016). Largely governing this soil health and quality is soil carbon content. Soil carbon exerts a shaping influence upon the

physical, chemical, biological and hydrological properties of soil; thus, activities which influence soil carbon content directly impact ecosystem service provision, soil health and quality (Doran, 2002, Lal, 2016, Vereecken et al., 2022). Hence, taking actions which increase soil carbon stocks may correspond to an increase in soil functional ability, and thus improved capacity to deliver ecosystem services (Bünemann et al., 2018).

Managing the environment to better provide ecosystem services has been a central topic of research and policy discussion since the inception of the ecosystem services research field (Dominati et al., 2010, Braat and de Groot, 2012, Costanza et al., 2014). However, as a result of increasing environmental damage and degradation, the need for better understanding of the value and role ecosystem service provision and sustainability plays within the environment has come into increased focus.

Yet, despite their importance, ecosystem services at large are often greatly undervalued. As a result of this lack in perceived value there often exists little incentive for sustainable management or improvement of ecosystem services, in favour of extracting the tangible value for short term gain (Dasgupta et al., 2000, Pearce, 2007, Turner and Daily, 2008). The consequences of undervaluing ecosystem services extend beyond their direct exploitation however, as this view fails to address the potential benefits/disbenefits affected upon ecosystem service provision by removing them from the decision-making process (Costanza et al., 1997). Therefore, by ascribing an explicit value to the goods and services provided by the environment, we can acknowledge the cost of their exploitation and ensure that they are adequately accounted for in policy and land management decisions (Costanza et al., 1997, Chee, 2004, Costanza et al., 2017).

Collectively the natural capital and ecosystem services contained within the Earth's biosphere were estimated at an approximate value of \$125 - \$145 trillion USD each year in 2011, a corrected yearly reduction of \$20 trillion USD relative to the previous assessment in 1997 due to the effective reduction in ecosystem service provision as a consequence of environmental degradation (Costanza et al., 1997, Costanza et al., 2014). By 2050 it is expected that the value of ecosystem services and natural capital will range between \$71.3 trillion USD and \$152 trillion USD, as a result of either environmental degradation or enhancement – with such outcomes dependent upon a wide range of societal, demographic, environmental and technological variables and the perceived change in ecosystem service values themselves (Kubiszewski et al., 2020). Reductions in ecosystem service provision of this scale would have catastrophic consequences for food and water quality and security, greatly exasperate climate change, and culminate in biodiversity loss and ecosystem disfunction in many regions of the world (Pimentel, 2006, Smith, 2008, Wagg et al., 2014, Pereira et al., 2018, Bateman and Muñoz-Rojas, 2019, Geisen et al., 2019, Chinedu et al., 2020). Conversely, improvements in ecosystem service provision would improve resilience to climate change (for both humans and the environment), improve food and water security and help repair the damage of environmental degradation accrued to date (Lal, 2006, Smith et al., 2016). Thus, if we wish to maintain our current level of ecosystem service provision, let alone improve upon this, we must adopt a set of policies and land management strategies that seek to preserve and improve environmental health and quality – herein soil recarbonisation provides significant opportunities.

#### **1.4 The Potential for Soil Recarbonisation**

The agricultural sector at large has the potential to transition from a net source of emissions to a significant carbon sink by adopting methods which reduce, mitigate or ameliorate damage to soils, through adoption of carbon farming techniques (Powlson et al., 2012, Smith et al., 2014, Paustian et al., 2016, Soussana et al., 2019, Lal, 2023). Taking this more soil-centric approach to soil management offers substantial opportunity to reverse soil degradation and sequester significant quantities of atmospheric carbon (Paustian et al., 2006, Horowitz and Gottlieb, 2010, Latawiec et al., 2020, Mao et al., 2022). Restoring and protecting the carbon content of soils is key to sustaining agricultural productivity and food security, as well as biodiversity conservation and enhancing the provision of the myriad ecosystem services that soils underpin (Doran, 2002, Power, 2010, Smith et al., 2013, Adhikari and Hartemink, 2016, Latawiec et al., 2020). Globally, agricultural land has the potential to sequester and store large quantities of carbon, especially within the top 30 – 40 cm of soil (Lal, 2004b, Soussana et al., 2019). Soil focussed initiatives, such as the 4p1000 and RECSOIL demonstrate how soil recarbonisation and management can be central to global climate change mitigation policy and practice (Lal, 2018, Soussana et al., 2019, Amelung et al., 2020, FAO, 2020, Smith et al., 2020). Additionally, potential to improve agricultural production while changing the system to redress deficits in ecosystem service provision, such that both can be delivered on the same land without further damage and degradation, may be possible (O'Donoghue et al., 2022). This may be achieved through the implementation of more sympathetic and holistic land management practices, e.g. adopting regenerative agriculture principles, and/or through the direct incorporation of carbon rich soil amendments.

Carbon sequestration amounting to a 10% increase in the soil carbon stock offers technical potential to reduce atmospheric CO<sub>2</sub> concentrations by around 20% (Kell, 2012, Dubey, 2022). With more stringent adherence to agricultural best practices potentially delivering a sequestration of up to 65 Gt C, or the equivalent of offsetting approximately 35% of total historical agricultural soil carbon emissions (Padarian et al., 2022, Lal, 2023). Furthermore, adjusting practices to include activities such as conservation tillage, reduced agrochemical input, cover-crop rotations, and the application of carbon rich soil amendments, raises further opportunity to rejuvenate soils and capture carbon (Gál et al., 2007, Lal, 2004a, Ogle et al., 2012, Powlson et al., 2012, Soussana et al., 2019). Indeed, in so far as total adherence to initiatives such as the 4p1000 initiative, perhaps as much as 3.4 – 5 Gt C yr<sup>-1</sup> may be sequestered with the utilisation of all land use types and sequestration methods – offsetting potentially a third of total yearly anthropogenic emissions in 2030 (Soussana et al., 2019, Lozano-García et al., 2020, Smith et al., 2020).

Thus, soil recarbonisation is of key importance as this represents a potentially significant negative emissions technology, achievable both economically and geographically at scale with current technology (Fuss et al., 2018). Furthermore, soil recarbonisation offers the opportunity to provide an additional stream of income to farmers and land managers: This commodification of soil carbon (and therefore to a lesser extent ecosystem services) can help drive the adoption of specific land management strategies that deliver carbon sequestration through financial incentives, while disincentivising practices which cause environmental harm (Keenor et al., 2021, Lal, 2023).

Given the potential to improve environmental and ecosystem service functions, alongside meaningful carbon sequestration and potential economic benefit, soil recarbonisation can offer a multiple-win opportunity for agricultural management and the environment. Yet, to do so we must adopt a view to farm carbon as well as crops, with significant consideration given over as to what aim is most appropriate in each given environmental, climatic, agricultural and soil context (Moinet et al., 2023). Furthermore, it is important that we consider the forms in which this carbon stock is stored (i.e. not all carbon is equal), and that the methods of carbon measurement/determination used are accurate and appreciative of the impact soil properties exert over carbon storage. Some soil carbon stocks are transient in nature (labile carbon), conferring benefit to soil biodiversity (through its value as a food source, priming soil life (de Graaff et al., 2010, Amin et al., 2021, Yazdanpanah et al., 2016, Lal et al., 2018); while other forms of carbon are more resistant to the forces of degradation (recalcitrant carbon) and can provide long term carbon storage potential for decades to come (Mao et al., 2022, Smith et al., 2020, Campbell and Paustian, 2015, Dungait et al., 2012). Additionally, some carbon can be contained and physically protected within stable soil aggregates (occluded carbon), potentially able to store even relatively labile carbon long term, while providing additional benefit to soil structure and function (Schrumpf et al., 2013, Gärdenäs et al., 2011, Dungait et al., 2012, Six and Jastrow, 2002, Plante et al., 2011, McLauchlan and Hobbie, 2004).

Experimental methods such as thermogravimetric analysis (TGA), can offer significant insight as to the proportions of carbon considered labile or recalcitrant, as the thermal stability of organic materials is related to their relative biodegradability (Plante et al., 2005, Mao et al., 2022, Capel et al., 2006). Additionally, the influence of

soil bulk density upon in-situ soil stocks must also be appreciated, as changes in soil carbon stock correspond to changes in soil bulk density, and hence changes to the total volume of the soil (Ruehlmann and Körschens, 2009, Smith et al., 2020, Powlson et al., 2012). Finally, propagating this data forward into carbon prognosis models offers a cost effective and scalable solution that can additionally consider agricultural and climatic information and provide an accurate and full prediction of soil carbon stocks into the future (Smith et al., 2020, Mao et al., 2022).

### **1.5 Soil Amendments**

The use and application of organic matter rich soil amendments can be an effective means of delivering agricultural sustainability and improving soil health and quality (Ansari et al., 2019). Organic matter amendments improve soil properties and increase soil carbon stocks, mitigating the negative effects of soil degradation and improving ecosystem service delivery (Powlson et al., 2012, Smith, 2016, Garbowski et al., 2023). Soil amendments offer a powerful, fast acting tool in the arsenal of agricultural sustainability and soil recarbonisation, as applications of organic matter rich soil amendments result in the immediate uplift of soil carbon, generally in proportion to the quantity of amendment applied (Larney and Angers, 2012).

Organic matter rich soil amendments can include a variety of different products from a variety of different sources, and include; farm waste (i.e. manures, crop residues, straw), Municipal wastes (i.e. sewage sludge, biosolids, anaerobic digestate) and organic waste (i.e. paper mill residues, biochar, compost) (Chantigny et al., 1999, Cooperband, 2002, Lal, 2004a, Lima et al., 2009, Powlson et al., 2011a, Powlson et al., 2011b, Powlson et al., 2012, Smith, 2016).

Using soil amendments affords the opportunity to sustainably adapt conventional agriculture practices, reducing inorganic fertiliser inputs (and thus cutting the emissions and soil damage associated with their production and use), and by recycling/re-using waste or by-products for soil improvement (Ogle et al., 2005, Rehberger et al., 2023). Adoption of these materials as soil amendments diverts these feedstocks away from incineration or landfill where they would contribute towards climate change, and into a useful material that can be used to mitigate climate change while additionally leading toward a more holistic, sustainable and circular agricultural economy (Cooperband, 2002, Lima et al., 2009, Tejada et al., 2009, Ansari et al., 2019).

While sometimes viewed as a regenerative agriculture method, soil amendment application should be considered a distinct and separate practice; given that many soil amendments do not originate wholly or in part from an agricultural setting (or indeed the one to which they are applied), often relying on the import of exogenous products (Paustian et al., 2016, Rehberger et al., 2023). Furthermore, the use of organic matter soil amendments does not preclude the use of conventional farming practices.

In general, soil amendments help improve the regulation of soil properties, enhancing soil aggregation and hydrological function, boosting fertility and crop yields, increasing soil microbial diversity and abundance, storing significant quantities of carbon and improving ecosystem service delivery as a result (Cooperband, 2002, Tejada et al., 2009, Powlson et al., 2012, Angelova et al., 2013, Mao et al., 2022, Garbowski et al., 2023). Additionally, amendment use can provide benefit to crop-pathogen suppression and minimise the toxicity of heavy metals (Angelova et al., 2013, Ansari et al., 2019).

Using soil amendments to augment soil carbon stocks can provide significant opportunity to sequester carbon in the long term (Paustian et al., 2016, Soussana et al., 2019, Mao et al., 2022). Furthermore, soils treated with organic matter soil amendments can sequester carbon at a faster rate than untreated soils or those treated with other inorganic fertiliser products, observed to store up to an additional 9.4 t C ha<sup>-1</sup> (Rehberger et al., 2023).

The technical potential for carbon sequestration using soil amendments varies widely as influenced by amendment types, their dosage and the soil/environmental contexts in which they are amended: As a result, measurements in-situ must be taken regularly to maintain and verify a proper account of the changes to soil carbon stocks. Subsequently coupling these measurements to soil carbon prognoses can determine the potential residence time of this carbon and provide assurance of long-term sequestration potential (Mao et al., 2022). In general however, the average sequestration capacities of some of the more common soil amendments have been reviewed and reported for their potential uplift (t C ha<sup>-1</sup> t<sup>-1</sup> of dried solids added): farmyard manures (0.06 ± 0.02); biosolids (digestate) (0.18 ± 0.02); sewage sludge (0.13 ± 0.2); compost (0.06 ± 0.01); paper crumble (0.06); straw (0.05 ± 0.015) (Powlson et al., 2012). However, the influence and effects of the different soil amendments upon soil properties can be substantial and vary greatly from site to site - and soil to soil - often as a result of the products origin, constituents and management/manufacture processes (Ansari et al., 2019). Thus, it is important that soil amendments are further scrutinised in the different agricultural uses and environmental contexts in which they are used to accurately determine the influence their use has upon soil properties, carbon sequestration and ecosystem service.

## **1.6 Regenerative Agriculture**

Regenerative agriculture offers an alternative approach to farming across a wide range of environmental contexts; with an overall focus on improving the sustainability of agriculture through the adoption of more holistic and ecologically accommodating management (Schreefel et al., 2020). Broadly, regenerative agriculture brings together a set of land management practices and principals which seek to rehabilitate and rejuvenate the environment, improving the overall quality and increasing the availability of natural resources and environmental services, rather than depleting them, and which are suitable to the agricultural and environmental contexts in which they are applied (Moyer et al., 2020). This may be achieved by adopting practices in agriculture which closely mimic natural ecosystems that, in general, maintain higher soil carbon contents and improved ecosystem service capacity compared to conventional croplands (Paustian et al., 2020, Schreefel et al., 2020).

Transitions towards more holistic forms of agriculture offer significant opportunities to support ecosystem adaptation to and mitigation of climate change, alongside potential for soil carbon sequestration and soil biodiversity enhancement (Paustian et al., 2016, Geisen et al., 2019, Gosnell et al., 2019, O'Donoghue et al., 2022). Regenerative agriculture can additionally offer opportunities beyond environmental improvements per se, with potential to improve profitability (through reduced input costs) and increase food security (Kasper et al., 2009, Al-Kaisi and Lal, 2020, Newton et al., 2020)

During the 1970s and 1980s the concept of shifting the approach of agriculture, towards a method that can both provide for the direct resource needs (i.e. food and fibres), and improvement to the many ecosystem services provided by agricultural

land, has gained significant traction (Sampson, 1982, Newton et al., 2020, Giller et al., 2021, O'Donoghue et al., 2022). Despite a lack of agreed consensus as to what practices specifically regenerative agriculture farming entails (Newton et al., 2020, Giller et al., 2021, Rehberger et al., 2023), it is widely understood to include the concepts and principles of: (i) limiting soil disturbance and cultivation; (ii) maintaining a continuous ground cover (using cover crops, or organic litters); (iii) increasing the carbon content of soils; (iv) improved water and nutrient use-efficiency; (v) (re)integration of livestock; (vi) reducing synthetic agrochemical and exogenous inputs; (vii) increasing crop (and wider plant) diversity; (viii) encouraging environmental engagement at all levels (i.e. individual, farmer, policy), (ix) integration of perennial crops (Newton et al., 2020, Paustian et al., 2020, Giller et al., 2021, Rehberger et al., 2023).

Uptake in regenerative practice has increased substantially in the past decade, with broad support and promotion by a variety of stakeholders, including food companies, farmers, NGOs and the general public (Gosnell et al., 2019, Newton et al., 2020, Giller et al., 2021). Globally it is estimated that more than 180 M ha of farmland are presently managed in line with regenerative agriculture principles (Al-Kaisi and Lal, 2020).

Adoption of regenerative agriculture principles has led to some significant beneficial outcomes, including increased soil carbon content, enhanced soil structural and aggregate integrity (reducing erosion, degradation and carbon loss), improved water retention and infiltration, gains in biodiversity, and improvement in ecosystem service provision (Gosnell et al., 2019, O'Donoghue et al., 2022).

One of the most commonly adopted regenerative agriculture practices, reduced-till or no-till soil management, has been observed to substantially increase soil carbon content in the top 30cm of soil, relative to a conventional-till management style (Gál et al., 2007, Ogle et al., 2012). Globally, as a result of no-till management alone an estimated 0.24 Gt CO<sub>2</sub>e emissions have been avoided since the 1970s due to decreased soil cultivation (Kasper et al., 2009, Al-Kaisi and Lal, 2020).

The aggregated technical potential for regenerative agriculture principles to sequester and recarbonise soil is estimated to range between 0.9 – 8.3 t C ha<sup>-1</sup> yr<sup>-1</sup> dependent upon the specific practices used and soil/environmental contexts; with the greatest recarbonisation potential through practices such as crop rotation, perennial cropping and managed grazing (Rehberger et al., 2023). Such practices can promote synergistic effects: enhancement of soil carbon stocks and reduced soil cultivation culminate in improved soil aggregation and structure, in turn improving physical protection to soil carbon, significantly enhancing soil sequestration potential (Six et al., 2004, Kasper et al., 2009, Ogle et al., 2012, Lehmann et al., 2020).

As a result of its recarbonisation potential and the many benefits extended to the environment and ecosystem service provision, regenerative agriculture is seen as a potentially powerful solution for climate change adaptation and mitigation as well as environmental and ecosystem enhancement with the greatest potential in historically degraded soils (Gosnell et al., 2019, Paustian et al., 2020, O'Donoghue et al., 2022, Rehberger et al., 2023). However, it is important that the potential for regenerative agriculture mediated soil/ecosystem enhancement is not overstated, and adoption of these methods is based on sound knowledge and evidence, that appreciates the influence of agricultural and environmental contexts upon outcomes – as it is

highlighted that what is effective in some conditions may not be in others (Ogle et al., 2005, Giller et al., 2021). Thus, further research must consider context alongside the overall aims of a regenerative project (e.g. focus on soil health, soil carbon sequestration, biodiversity net gain etc.) to deliver fit for purpose projects that offer defined environmental enhancement rather than detriment. Instrumental to this is improving our understanding of the effects that transitioning to regenerative agriculture methods has upon soil biology, soil properties and soil carbon stocks with time (Szoboszlay et al., 2017, Wagg et al., 2014).

## 1.7 Aims, Objectives and Hypotheses

The aim of this research was to evidence the potential for enhancing provision of soil ecosystem service through the recarbonisation of soils. To achieve soil recarbonisation *direct* amendment of carbon via paper crumble (PC) and *ancillary* increases in soil carbon as a result of regenerative agricultural practices were investigated.

While there is considerable research on the topics of both organic matter soil amendment application and regenerative agricultural method adoption; this research seeks to fill gaps in current understanding, especially those relating to:

- i) The specific use of paper crumble, and its impacts upon a range of soil properties in contrasting soil types (clay vs. sand).
- ii) Regenerative agriculture relating to long-term perennial crop production and its influence upon soil properties.
- iii) How the changes to soil properties and carbon stocks, as a result of altering land management, influences the delivery of a range of ecosystem services, including: carbon storage, carbon stabilisation, water storage, soil microbial biodiversity and soil health.
- iv) The barriers and facilitators linked to soil carbon markets.

The results chapters in this thesis provide evidence of the influence certain soil management practices exert upon soil properties and ecosystem service provision, in addition to policy and economic drivers as supported by the literature (**Chapters 1 and 2**) or through experimentation and data collection (**Chapters 3-6**). These chapters have either been published (**Chapters 2 and 3**), are in draft for publication submission

(**Chapter 4**), are submitted for publication and under review (**Chapter 5**), or were produced solely for this thesis (**Chapters 1 and 6**). Appended, are two reports, 1) a report on the influence of PC for improving agricultural productivity, submitted to the Environment Agency, and 2) a baselining report on soil physical properties, carbon stability and biodiversity data, submitted to the Wendling Beck Nature Recovery Project. These are included as a record of research translation to wider stakeholders.

**Table 1: Soil management regimes and soil ecosystem services investigated in each chapter**

Chapter	Regime or management		Ecosystem Service						Publication Status
	PC amendment	Regenerative Agriculture	Soil Carbon sequestration	Soil Hydrology	Soil Health and Quality	Soil fertility Agronomic function and food security	Soil Biodiversity	Soil Aggregation and stability	
1. Background and introduction	<b>Literature Review</b>								Thesis
2. Capturing a soil carbon economy	<b>Perspectives Paper</b>								Published
3. Recycling paper to recarbonise soils	<b>X</b>		<b>X</b>	<b>X</b>		<b>X</b>			Published
4. Influence of Paper Crumble on Soil Hydrology and Soil Carbon Stocks	<b>X</b>		<b>X</b>	<b>X</b>		<b>X</b>			To be submitted
5. Physical Protection of Soil Carbon Stocks Under Regenerative Agriculture		<b>X</b>	<b>X</b>					<b>X</b>	Submitted in Review
6. Changes to soil bacterial and fungal diversity and abundance under a transition to regenerative agriculture		<b>X</b>	<b>X</b>		<b>X</b>			<b>X</b>	Thesis

## **1.8 Overview and Chapter Summary**

### **1.8.1 Overview of Chapter 2: Capturing a Soil Carbon Economy**

This chapter analyses the value of soil carbon with respect to ecosystem service provision and climate change mitigation through the lens of policy and economics. Soils are highlighted for their value in underpinning a broad spectrum of natural capital stocks and ecosystem services, and the detrimental effects that are observed from soil carbon loss, including the exacerbation of climate change. Financial disincentives (taxes and fees) which encourage emissions reductions have proven to be successful in a variety of contexts, yet while financial incentives to sequester carbon emissions are readily available, their efficacy is often lower. This chapter discusses the obstacles preventing wider adoption of carbon sequestration practices and highlights the disparity between implementation and remuneration, likely stemming from a lack in confidence in the soil sequestration methods, as result of lacking quantification and verification methods that show long term soil carbon storage and additionality can be achieved. Furthermore, the chapter emphasizes the role of a soil carbon economy as a viable climate change mitigation strategy but highlights the need for further research to develop reliable measurement and verification systems, as well as the importance of supportive policies to incentivise farmer participation.

### **1.8.2 Overview of Chapter 3: Recycling Paper to Recarbonise Soil**

This chapter reports the potential of using PC to improve soil physical, chemical and hydrological properties while improving the long-term carbon storage potential for climate change mitigation. Paper crumble was deployed in a field experiment using PC doses of 0, 50, 100 and 200 t ha<sup>-1</sup> and was analysed in relation to its impact on the

properties of a clay rich soil. Carbon stability and hence sequestration potential was assessed using a thermogravimetric analysis and carbon fate modelling approach. PC applications were observed to significantly ( $p \leq 0.05$ ) increase SOC and water holding capacity, and significantly decrease ( $p \leq 0.05$ ) soil bulk density, overall enhancing soil structure and function. PC applications were also observed to influence the concentration of essential and trace elements within the soil with no detriment to crop yields. This chapter highlights the potential of PC to increase soil carbon stocks by up to  $6.65 \text{ t C ha}^{-1}$  over a 50 year period assuming a rotational (4 yearly) amendment of PC, in heavy clay dominated soil types across the UK.

### **1.8.3 Overview of Chapter 4: Influence of Paper Crumble on Soil Hydrology and Soil Carbon Stocks**

This chapter reports on the potential of PC soil amendments to improve the hydrological outcomes on a drought prone sandy soil through increased soil carbon content. Additionally, this chapter provides an appreciation for the long-term stability of the carbon stored within the soil and the potential for PC soil amendments to enhance soil water availability. While similar in scope to chapter 3, this chapter offers a counterpoise to the influence paper crumble exerts upon soil properties (sandy soil vs clay soil), but with a targeted focus on upon soil hydrology and carbon storage as ecosystem services. This study was conducted over a period of 3 years to determine the potential influence of time (and by extension PC persistence) upon the influence of PC on soil property changes. Applications of PC were observed to significantly increase ( $p \leq 0.05$ ) water holding capacity, infiltration rates and SOC stocks, with the effects being more pronounced 1 year after amendment, and in higher doses (200 t

ha<sup>-1</sup>). Additionally, increases in the total available water holding capacity, and potential for soil carbon sequestration were calculated from the data of the 200 t ha<sup>-1</sup> treatment, 3 years post amendment, highlighting significant scope hydrological and carbon sequestration net gains.

#### **1.8.4 Overview of Chapter 5: Physical Protection of Soil Carbon Stocks Under Regenerative Agriculture**

This Chapter reports the effects of regenerative agricultural management in its potential to deliver carbon sequestration and physical protection of carbon stock (occlusion within stabilised soil aggregates). This chapter highlights the important role that stable soil aggregates play in physical soil stabilisation, the delivery of ecosystem services and the separation and protection of soil carbon stocks. Adoption of regenerative agriculture practice was observed to increase soil carbon content over time – additionally, increases in the proportion of water stable aggregates relative to non-water stable aggregates were also observed. As a result, increased carbon content was associated with the stable aggregate fraction after 7 years. When analysed for carbon stability (using a thermogravimetric analysis approach), recalcitrant carbon was observed to decrease (not significantly ( $p \geq 0.05$ )) with time, while labile carbon increased significantly ( $p \leq 0.05$ ), suggesting the majority of the carbon stored within the stable aggregates was from the labile carbon fraction. Taken together, the recalcitrant carbon fraction and the occluded carbon fraction could be used to calculate total carbon sequestration potential over the 7 year period.

### **1.8.5 Overview of Chapter 6: Changes to Soil Bacterial/Fungal Diversity and Abundance Under a Transition to Regenerative Agriculture**

This chapter reports the effects of a preliminary study of regenerative agriculture management for its potential to alter soil properties, and how these changes to soil properties influence soil microbial biodiversity and abundance (of bacterial and fungal communities). This chapter highlights the importance of soil biodiversity for catalysing and underpinning soil health and a range of ecosystem services. Soil biodiversity metrics were analysed and assessed through metagenomic sequencing (16s and ITS) and subsequent statistical analyses to compare against physical soil measurements. Transition from conventional agricultural management towards a regenerative model was found to impact soil properties, with significant changes ( $p \leq 0.05$ ) to soil moisture, soil pH, and SOM, alongside observable increases (not significant ( $p \geq 0.05$ )) in soil carbon contents (including both labile and recalcitrant carbon stocks). These changes in soil properties were observed to significantly ( $p \leq 0.05$ ) shift bacterial and fungal community compositions over increased time under regenerative management. Furthermore, changes to soil properties were observed to influence the community structure and abundance of bacteria and fungi at family level upregulating or downregulating their abundance, highlighting the influence land management exerts upon soil microbial diversity.

**Table 2: Chapter Aims and Hypotheses**

<b>Chapter</b>	<b>Aims</b>	<b>Hypotheses</b>
1	Literature Review	Literature review
2	<p>a) Define and discuss the potential for and economic viability of soil carbon sequestration and what benefit soil recarbonisation will offer for climate change mitigation and ecosystem service provision.</p> <p>b) Investigate the current issues preventing adoption of soil recarbonisation initiatives at scale.</p>	Perspectives paper
3	<p>a) Determine the influence variable application doses of PC, to a clayey soil, exert upon soil properties and the provision of ecosystem services.</p> <p>b) Through a modelling approach to determine a prognosis for carbon sequestration in soil as delivered by PC.</p>	<p>i) Amending a clay rich soil with PC will significantly increase soil carbon content commensurate with increased treatment dose.</p> <p>ii) PC application will significantly increase long term carbon storage potential and hence deliver climate change mitigation potential.</p> <p>iii) Treatment with PC will reduce soil bulk density and regulate soil hydrology.</p> <p>iv) Treatment with PC will increase nutrient availability, and soil fertility, increasing crop yields.</p>
4	<p>a) Determine the influence variable application doses of PC, to a sandy soil, upon soil properties and the provision of ecosystem services.</p> <p>b) Investigate the extent to which paper crumble application can improve soil hydrological function.</p>	<p>i) Amending a sand rich soil with PC will significantly increase soil carbon content commensurate with increased treatment dose.</p> <p>ii) Paper crumble application will significantly enhance soil hydrological function with increased effects at higher doses.</p> <p>iii) Paper crumble application will act as an effective means of increasing tolerance to drought through improved water holding capacity.</p>
5	<p>a) Determine the influence of regenerative agriculture practices upon soil properties and the provision of ecosystem services.</p> <p>b) Determine the extent to which regenerative agriculture can increase carbon stocks, stabilise soil aggregates and protect soil carbon from loss.</p>	<p>i) Increased time under regenerative agriculture management will increase soil carbon content.</p> <p>ii) Increased time under regenerative agricultural management will increase the fraction of stable soil aggregates.</p> <p>iii) Stable soil aggregates will be fractionally enriched in carbon and provide physical protection to soil carbon stocks.</p> <p>iv) Regenerative agriculture methods will provide a significant means of long term soil carbon storage.</p>
6	<p>a) Determine the influence of regenerative agriculture practice upon soil microbiological biodiversity.</p> <p>b) Investigate the linkages between soil biodiversity and soil property change as a result of regenerative agriculture adoption.</p>	<p>i) Increased time under regenerative agriculture management will increase soil bacterial and fungal diversity and abundance.</p> <p>ii) Adoption of regenerative agriculture practice will lead to changes in soil properties over time</p> <p>iii) Such changes to soil properties will have an influence over microbial populations, catalysing the upregulation and downregulation of specific microbes</p>

## 1.9 Authorship Statement – Sam G Keenor

The following table outlines the involvement of Sam G. Keenor in the production of research: experimentation, data analysis, and drafting of manuscripts and chapters contained within this thesis.

<b>Soil Carbon Capture for Ecosystem Service Enhancement</b>			
<b>Chapter</b>	<b>Chapter title</b>	<b>Contribution to experimental work and data analysis</b>	<b>Contribution to writing</b>
<b>1</b>	Literature review	Literature review and discussion was solely undertaken by Keenor.	First Author. Keenor led the drafting of the manuscript with review provided by supervisor Reid.
<b>2</b>	Capturing a Soil Carbon Economy*	Literature review and discussion was led by Keenor, with co-authors providing text in sections aligned to their expertise.	First author. Keenor led the drafting of the manuscript with assistance from Reid and subsequent review from other co-authors (Reid, Rodrigues, Latawiec, Harwood, Mao).
<b>3</b>	Recycling Paper to Recarbonise Soil**	Field work data collection and laboratory analysis was carried out jointly by Keenor and Mao. Keenor led the interpretation of soil property data and statistical analysis, while Mao led the carbon fate modelling.	Second Author. Keenor led the review of the literature, and manuscript sections relating to soil properties. Mao led sections relating to the carbon fate modelling. All authors were involved in review and editing to deliver the final manuscript (Keenor, Mao, Reid, Cai, Killham, Murfitt).
<b>4</b>	Influence of Paper Crumble on Soil Hydrology and Soil Carbon Stocks	Fieldwork data collection, laboratory analysis, interpretation and statistical analysis was solely undertaken by Keenor.	First Author. Keenor led the drafting of the manuscript with review provided by supervisor Reid.
<b>5</b>	Physical Protection of Soil Carbon Stocks Under Regenerative Agriculture***	Fieldwork data collection, and laboratory analysis, were carried out by Keenor with Assistance from Lee. Data interpretation and statistical analysis was solely undertaken by Keenor.	First Author. Keenor led the drafting of the manuscript with review provided by supervisor Reid.
<b>6</b>	Changes to soil bacterial and fungal diversity and abundance under a transition to regenerative agriculture	Fieldwork data collection and laboratory analysis of soil was carried out solely by Keenor. 16S and ITS data was generated by collaborators at the Earlham Institute (Falk Hildebrand and Ezgi Ozkurt). All data analysis, interpretation and statistical analysis was undertaken solely by Keenor	First author. Keenor led the drafting of the manuscript with review provided by supervisor Reid.

\* Keenor, S.G., Rodrigues, A.F., Mao, L., Latawiec, A.E., Harwood, A.R. and Reid, B.J., 2021. Capturing a soil carbon economy. *Royal Society open science*, 8(4), p.202305.

\*\* Mao, L., Keenor, S.G., Cai, C., Kilham, S., Murfitt, J. and Reid, B.J., 2022. Recycling paper to recarbonise soil. *Science of the Total Environment*, 847, p.157473

\*\*\* Keenor, S.G., Lee, R. and Reid, B.J., 2025. Physical Protection of Soil Carbon Stocks Under Regenerative Agriculture. *EGUsphere*, 2025, pp.1-36. (Pre-print)



## **Chapter 2:**

# **Capturing a Soil Carbon Economy**

## **2.1 Abstract**

Current carbon pricing and trading mechanisms, despite their efficacy in reducing GHG emissions from industry, will not be sufficient to achieve Net Zero targets. Current mechanisms that redress emissions are largely economic *disincentives*, in effect financial penalties for emitters. In order to attain Net Zero futures, financial *incentives* for activities that sequester carbon from the atmosphere are needed. Herein, we present the environmental and economic co-benefits of soil re-carbonisation and justify support for soil carbon remuneration. With increasing momentum to develop green-economies, and projected increases in carbon price, growth in the global carbon market is inevitable. The establishment of a soil-based carbon economy, within this emerging financial space, has the potential to deliver a paradigm shift that will accelerate climate change mitigation, and concurrently realise net-gains for soil health and the delivery of soil ecosystem services. Pivotal to the emergence of a global soil carbon economy will be a consensus on certification instruments used for long-term soil carbon storage, and the development of robust institutional agreements and processes to facilitate soil carbon trading.

## **2.2 Introduction**

Soils support all life on Earth. They provide a primary source of food and resources, filter water, regulate climate, and provide the strata on which terrestrial life is supported (Pereira et al., 2018). The prosperity and economic status of nations are inextricably linked to the health of soils (Daily, 1997, Dominati et al., 2010). Yet to many, soil is dirt, a nuisance and unclean. This mentality of ‘inconvenience’ that has contributed to the damage and degradation of one of the most precious, largely non-

renewable resources on Earth (FAO, 2015, Rojas et al., 2016). Furthermore, this myopia precludes appreciation that soils are, in fact, living, dynamic, and essential ecosystems, providing not only tangible ‘goods’, but also services that support, regulate and sustain the global system (Dominati et al., 2010, Baveye et al., 2016a, Vicente-Vicente et al., 2019).

Overt linkages connect the climate system to soil-mediated regulation of climate-relevant atmospheric gasses. In particular, soils play a fundamental role in the two-way exchange of carbon (as CO<sub>2</sub> and CH<sub>4</sub>) and nitrogen (as N<sub>2</sub>, N<sub>2</sub>O and NH<sub>3</sub>) (Sisti et al., 2004a, Lal, 2008). Soil carbon is central to shaping edaphic soil factors (**Section 2.3**) (Schimel et al., 1994). This carbon facilitates soil aggregation, development of soil structure; and thus, the physical flows of water and gases (Sisti et al., 2004a). Loss of soil carbon, via mineralisation to CO<sub>2</sub> and/or erosion, results in a reduction of the soil carbon stock, thereby increasing atmospheric concentrations of carbon (primarily CO<sub>2</sub>), and/or undermining the integrity of the soil across its inextricably linked chemical, biological and physical attributes (Van Gestel et al., 1991). Soil degradation has wide-reaching consequences for biodiversity, food security, freshwater provision, and wider ecosystem service delivery (Lal, 2004d, Lal, 2004a, Power, 2010, Sanderman et al., 2017). It is emphasised that damage done to soil is not confined to soil; it has negative impacts on the entire planetary system (**Section 2.4**).

Strategies and tools are urgently needed to combat both soil degradation and climate change (Latawiec et al., 2020a). Facilitating a method of economic remuneration for re-carbonisation of soils has potential to act beneficially on both counts (**Section 2.5**). In this paper, we explain the pivotal importance of soil carbon and the fundamental role it plays in sustaining the delivery of key ecosystem services.

We explain the premise and operation of carbon markets (**Section 2.6**), evaluate how these may be aligned to realise policy (**Section 2.7**) and propose a platform/mechanism that will allow payments to be collected and divested to re-carbonise soils (**Section 2.8**). Thereafter, we discuss issues pertaining to carbon permanence, and the barriers that must be overcome to deliver a trading platform that supports a soil carbon economy (**Section 2.9**).

### **2.3 The Indispensability of Soil Carbon**

Due to differences in climate, parent material and formation conditions, soils vary greatly across the surface of the Earth. Soils are dynamic and complex matrices; composed of organic and inorganic materials, water, air, and organisms (Dominati et al., 2010) with each constituent contributing to the effective functioning of the wider soil system. Soils provide many valuable ecosystem services (De Groot et al., 2002a, MA, 2005, Power, 2010). Specifically, soils facilitate ‘*goods*’ and service provisions such as; resource and food productions (*provisioning services*), water filtration, flood mitigation and climate regulation (*regulating services*), carbon sequestration and carbon storage (*supporting services*), and aesthetics and recreation (*cultural services*) (Dominati et al., 2010, Baveye et al., 2016a, Adhikari and Hartemink, 2016a, Latawiec et al., 2020a). The health of a soil is categorised by its capacity to sustain life, and the extent to which it may enhance or maintain the provision of ecosystem services (Doran, 2002, Bünemann et al., 2018).

Healthy soils show greater resistance to stress (Lehman et al., 2015, Lal, 2016), providing greater resilience to the negative impacts of drought, flood, and erosion (Bhogal et al., 2009a). Of primary importance to soil health is soil carbon (Lal, 2016, Lal, 2018b), and the ecosystem services it sustains (Papendick and Parr, 1992,

Acton and Gregorich, 1995, Doran, 2002, Adhikari and Hartemink, 2016a, Masciandaro et al., 2018). Soil carbon exerts influence over a variety of soil attributes, including physical, chemical, hydrological, and biological properties (Abiven et al., 2009a, Brevik, 2010, Powlson et al., 2012a, Lehmann and Kleber, 2015, Lehman et al., 2015). Thus, soil carbon is a robust proxy with which to gauge soil health and quality.

Soil organic matter (SOM); comprised of organic forms of carbon and other bioactive elements (nitrogen, phosphorous, sulfur) is derived from the remnants of plant, animal, and microbial material. SOM contains both labile and recalcitrant fractions, in different stages of decomposition and decay (Bot and Benites, 2005, Lal, 2016, Brady et al., 2008, Lal, 2018b). Soils naturally sequester carbon through the accumulation of dead and decaying organic matter that is slowly incorporated and stored (Lal, 2008, Lal, 2016, Lal, 2018b). These different forms of SOM provide the resource to prime soil life (via the labile carbon pool that can be utilised relatively easily), and the means to deliver long-term carbon storage (via the recalcitrance carbon pool that resists degradation) (De Graaff et al., 2010, Kleber, 2010).

Soils with high organic matter have more developed soil structure, with greater aggregation and cohesion (Abiven et al., 2009a, Baveye et al., 2020a). These structures are more resistant to drought and erosion, due to improved porosity and reduced compaction (Bhogal et al., 2009a). Well-aggregated and well-structured soils are more accommodating to rainfall (Rai et al., 2017). Thus, improving water infiltration, water storage and buffering of the hydrological cycle (Franzluebbers, 2002). In addition, more developed soil aggregates provide stronger physical protection to SOM stocks (dos Reis Ferreira et al., 2020).

Globally, soils contain 2000 – 2500 Pg C; thus, soils hold approximately three times more carbon than the atmosphere (Janzen, 2004, Lal, 2004a, Smith et al., 2020b). This soil carbon store is not fixed or permanent; in reality, it is in dynamic equilibrium with other Earth systems (Lal, 2004a, Friedlingstein et al., 2019). Changes in land use (e.g. forest vs. pasture vs. arable) greatly alter the balance of carbon stored in soil and in the atmosphere (Doran, 2002, Smith, 2008). Consequently, actions that alter land use also alter soil carbon stocks, influence atmospheric carbon levels and, thus by extension, the global climate system (Friedlingstein et al., 2019).

#### **2.4 De-carbonisation of Soil**

Damage caused to the soil system through anthropogenic action has occurred at an unprecedented rate. In the last 150 years more than half of all soils have been damaged (WWF, 2018). Degradation of soil has been accompanied by the attrition of >50% of the soil organic carbon (SOC) stock in some cultivated soils, with over 2 billion hectares affected globally (Lal, 2001, Lal, 2004d, Lal, 2004a). Soils subjected to degradation become a significant emission source of CO<sub>2</sub> to the atmosphere (Reicosky, 1997, Lal, 2004d, FAO, 2015). Soil degradation has liberated an estimated 176 Gt of soil carbon globally (IPBES, 2018b); a significant quotient when contextualised against the 890 Gt C held in the atmosphere by 2023 (Friedlingstein et al., 2025). Averaged over the last 150 years, the soil carbon loss rate equates to  $1.6 \pm 0.8 \text{ Gt C yr}^{-1}$  (Smith, 2008). In context, anthropogenic global carbon emissions in 2000 were estimated to be  $7.5 \text{ Gt C yr}^{-1}$  (Van Vuuren et al., 2011) (i.e. the rate of annual SOC loss is ~20% of this value). Agriculture, forestry, and land use change is reported to be directly responsible for ~18-24% of total

anthropogenic GHG emission each year (Smith et al., 2014b, Friedlingstein et al., 2019). This conversion of natural ecosystems to managed systems is reported to deplete SOC stocks by an average of 60% in temperate regions, and up to 75% in the worst affected regions of the tropics, accounting losses of up to 80 t C ha<sup>-1</sup> (Lal, 2004a).

Inadequate SOC stocks have been linked to impaired soil function, reduced nutrient provision and water availability, and loss of below and above ground biodiversity (Lal, 2001, Lal, 2006, Kimetu et al., 2008a). SOM degradation increases the vulnerability of soils to erosion and accelerates the desertification process (Yong-Zhong et al., 2005, Zika and Erb, 2009). It is important to appreciate that soil resources, although abundant and long lasting, are non-renewable on an anthropogenic timescale (Rojas et al., 2016). Where rates of soil loss/degradation outpace rates of biogenic and geological soil replacement/recovery, the sustainability balance is tipped (Papendick and Parr, 1992, Pulleman et al., 2012, IPBES, 2018b). Globally, poor soil management and loss of SOC have exacerbated topsoil losses to a point where they are 10-40 times greater than natural replacement rates: In the US, topsoil loss rates are roughly 10 times that of replacement; while in India and China, loss rates exceed 30-40 times natural replacement (Lang, 2006).

Failures in soil management decrease crop yields (Bauer and Black, 1994, Follett, 2001a) and impair society's ability to grow sufficient crops (Schmidhuber and Tubiello, 2007, Rojas et al., 2016). Degraded SOC stocks have been reported to underpin decreases in crop productivity of 0.3% per year; a decrease which if not arrested, may aggregate to an average of 10% reduction in yields by 2050 (with the worst affected regions experiencing up to 50% yield reductions) (FAO, 2015,

IPBES, 2018b). Across the European Union 45% of agricultural soils are considered impaired or very impaired in SOM content (SOER, 2010).

## **2.5 Re-carbonisation of Soil**

The agricultural sector has potential to transition from a significant net source of GHG emissions to a net carbon sink (Paustian et al., 2006, Horowitz and Gottlieb, 2010). By altering land/soil management practices, the negative effects of agriculture upon soils and the environment may be substantially abated (Smith et al., 2001, Kragt et al., 2012, Powlson et al., 2012a, Lal, 2018b). Agricultural soils have potential to make significant contributions to carbon capture and storage in both the long and short term (Lal, 1993, Lal, 2004d, Lal, 2004a, Powlson et al., 2012a, Soussana et al., 2019a).

Taking the UK as an example, emissions of GHG from agricultural sources in 2017, were 45.6 million tonnes CO<sub>2</sub>e (CO<sub>2</sub>e = total global warming potential of all emissions normalised to CO<sub>2</sub> temperature forcing potential (Kragt et al., 2012)), delivering one-tenth of the total UK emission (435.2 Mt CO<sub>2</sub>e (2019) (DBEIS, 2018)). It is highlighted that agricultural GHG emissions differ from those associated with industries such as fossil fuel energy. In contrast to these industries (that emit predominantly CO<sub>2</sub>), agricultural sector emissions are, for the most part, associated with CH<sub>4</sub> and N<sub>2</sub>O, accounting for up to 80% of total agricultural emissions (Solazzo et al., 2016, Jantke et al., 2020). In the UK, total agricultural emissions are split: 40% CH<sub>4</sub> and 50% N<sub>2</sub>O and 10% CO<sub>2</sub> (NFU, 2019, Yue et al., 2017). The UK National Farmers' Union (NFU), the largest farmers' organisation, has suggested three pillars of intervention to offset the majority of agricultural GHG (NFU, 2019). These pillars relate to: 1) improving farming productive efficiency; 2) farmland carbon storage; and 3) boosting renewable

energy and the wider bio-economy. Under pillar 2, the NFU Aspiration seeks to sequester 9 Mt CO<sub>2</sub>e per year. Most of this carbon capture is linked with interventions that enhance soil carbon storage (5 Mt CO<sub>2</sub>e per year), and peatland and wetland restoration (3 Mt CO<sub>2</sub>e per year). Taken together, these interventions are projected to deliver ~20% offset against agricultural sector GHG emission in the UK by 2040.

*Carbon farming* (**Box 2.1**) describes the holistic approach of using agricultural methods to reduce or offset GHG emission from agriculture; through the capture and storage of carbon in soils and vegetation (Brady et al., 2019). Increasing the carbon stock of soils, on a global scale, has an estimated sequestration potential of 3.4-5 Gt C per year (Fuss et al., 2018, Soussana et al., 2019a, Smith et al., 2020b).

Effective methods for significantly increasing SOC stocks, within a short time frame, include lower impact tillage approaches and the use of soil amendments, such as compost, paper crumble, manure, and biochar (Lal, 2004a, Lehmann et al., 2006a, Powlson et al., 2012a, Smith, 2016). A shift away from aggressive soil tillage regimes (that promote disaggregation of soil, and soil carbon oxidation/mineralisation (Lal, 2004d, Lal, 2004a, Mehra et al., 2018), to minimum or no-tillage alternatives have reported capacity to rebuild farmland carbon stocks by 0.09-0.12 Gt C in Western Europe yearly (Smith, 2004). While the use of high carbon soil amendments may improve soil health and deliver long-term sequestration (Lehmann et al., 2006a, Powlson et al., 2012a, Soussana et al., 2019a). Adoption of such methods to optimise soil management practices could realise annual soil carbon uplifts of 0.6-1.2 Gt C (Lal, 2004a, Karhu et al., 2012).

Soil centric programmes, such as '4p1000' and FAO 'RECSOIL (Re-carbonisation of global soils)' Initiatives, have highlighted the opportunity for soils to be at the

forefront of global climate change abatement practice and policy (Lal et al., 2018b, Soussana et al., 2019a, Smith et al., 2020b, FAO, 2020, Amelung et al., 2020). By increasing soil carbon stocks in line with methods proposed by '4p1000' (i.e., yearly increases in the carbon content of agricultural soils by 0.4% in the top 40cm) there is capacity to sequester up to 3.4 Gt C yr<sup>-1</sup>. Such a level of sequestration would provide effective carbon offset for approximately a third predicted yearly emission from the fossil fuel and cement sectors in 2030 (estimated 10.9Gt C) (Soussana et al., 2019a, Smith et al., 2020b).

### **Box 2.1 Carbon Farming – Case Study Australia**

To reduce emissions and meet government commitments (80% emission reductions from 2000 levels by 2050), Australia adopted a national carbon pricing mechanism (CPM) in 2011. This was facilitated through the creation of an Australian ETS (that covered approximately 50% of national emissions from a range of sectors (excluding agriculture)), and increases in fuel duties (Verschuuren, 2017, Maraseni and Reardon-Smith, 2019, Guglyuvatyy and Stoianoff, 2020). To run concurrently with this ETS, the carbon farming initiative (CFI) was adopted to provide offsets that could be used within, and promote emissions reduction within the agricultural sector (2011a, Verschuuren, 2017, Guglyuvatyy and Stoianoff, 2020). The CFI was supported by the Australian Carbon Pricing Scheme and issued carbon credit units for each tonne of CO<sub>2</sub>e abated or sequestered (2011b, Macintosh and Waugh, 2012, Murray, 2012, Verschuuren, 2017, Copland, 2020). The CFI was the first nationwide example of carbon credit creation and trade by the agriculture and forestry sectors to a wider market (Macintosh and Waugh, 2012, Evans, 2018). Carbon farming methods pertained to activities that increase soil carbon stocks and/or store carbon within

vegetation, or facilitated emissions avoidance (Kragt et al., 2017, Verschuuren, 2017). Accepted methods of carbon sequestration under the CFI included: limiting inputs of agrochemicals (e.g. inorganic fertilisers) to the soil, limiting the use of aggressive tillage regimes (transition to minimal/no till), implementing cover-cropping rotations, increasing permanent and semi-permanent pasture land, adoption of silvicultural and silvopastoral systems, expanding riparian zones, afforestation, and by '*feeding*' soil with carbon rich amendments (Kragt et al., 2012, Kragt et al., 2017, Lal et al., 2018b).

It is estimated, that if properly managed, carbon farming in Australia could have the potential to remove  $\sim 497$  Mt CO<sub>2</sub>e yr<sup>-1</sup>; with contributions of  $\sim 68$  Mt CO<sub>2</sub>e yr<sup>-1</sup> from arable land,  $\sim 286$  Mt CO<sub>2</sub>e yr<sup>-1</sup> from high volume grazing rangeland and  $\sim 143$  Mt CO<sub>2</sub>e yr<sup>-1</sup> from forestry (Garnaut, 2008, Kragt et al., 2012). Australia's annual GHG emission has been reported to be 528 Mt CO<sub>2</sub>e yr<sup>-1</sup> (2020d) with agricultural sources contributing 13% of the total GHG emission (Verschuuren, 2017). Thus, carbon farming in Australia has the potential to completely absolve agricultural GHG emission and, in reality, offset virtually all of Australia's present-day GHG emissions.

The first iteration of the CFI (through the CPM) was a voluntary baseline and credit offset scheme (Macintosh and Waugh, 2012). Where offsets were determined relative to a predefined baseline/reference value, and verified credits were sold or auctioned to ETS regulated industry, or internationally where recognised as Kyoto Protocol CDM compatible offsets (Kragt et al., 2012, Kragt et al., 2016, Crowley, 2017, Verschuuren, 2017). Payments were initially made at a carbon floor price of \$23 AUD/t CO<sub>2</sub>e. To provide an economic disincentive to industry, and encourage divestment from high emission activities, this price was projected to increase by between 2.5-5% p.a. (Crowley, 2017, Verschuuren, 2017, Evans, 2018). In its first 2 years of operation

(2012-2014) national emissions reduced (2020d, Grudnoff, 2020), and total emission from the energy generation sector (accounting for approximately 37% of national GHG emission), dropped from 199.1Mt CO<sub>2</sub>e yr<sup>-1</sup> (2012) to 180.8Mt CO<sub>2</sub>e yr<sup>-1</sup> (2014) (Maraseni and Reardon-Smith, 2019). However, in late 2014, the CPM (that underpinned offset ETS trading of CFI credits) was repealed (Kragt et al., 2017, Verschuuren, 2017, Evans, 2018). The repeal and subsequent withdrawal of the CPM was politically motivated by a change in government that negatively framed the CPM as a 'carbon tax' to secure votes (Crowley, 2017, Copland, 2020). Following the withdrawal, Australia's GHG emissions rebounded to exceed 2014 emissions levels (and have done so subsequently each year) (2020d, Grudnoff, 2020). Energy sector specific emissions increased to 187Mt CO<sub>2</sub>e yr<sup>-1</sup> the year following the repeal (2015), and further to 189MtCO<sub>2</sub>e yr<sup>-1</sup> in 2016 (increasing towards similar levels of emissions from prior to CPM adoption) (Maraseni and Reardon-Smith, 2019).

In November 2014, the Emissions Reduction Fund (ERF) was established as a successor scheme and granted a budget of \$2.55bn AUD for the following 4 years (2015-2019), and CFI methods were continued (Authority, 2014, Burke, 2016, Crowley, 2017, Kragt et al., 2017, Verschuuren, 2017, Evans, 2018). The ERF operated on the basis of reverse auctioning (Verschuuren, 2017), wherein, CFI projects bid their mitigation/emission-avoidance (i.e. expected quantity of CO<sub>2</sub>e) and the total operational cost. The most cost-effective schemes are subsequently purchased at auction (in majority by the government, but some by private entities) (Kragt et al., 2017, Verschuuren, 2017). Although transition to the ERF has led to substantial decreases in the price of carbon (from ~\$23 AUD to ~\$12 AUD t CO<sub>2</sub>e (Evans, 2018), contracts granted to farmers have been found more economically stable and

favourable, providing steady incomes over time (Verschuuren, 2017). As of October 2020, a total of 866 projects had been registered through the ERF; and more than 85 million credits issued (Regulator, 2020).

## 2.6 Putting a Price on Carbon

By assigning a tangible value to a unit of carbon (or more broadly, a unit of CO<sub>2</sub>e), a mechanism is established that enables charges to be applied to GHG emitters. At present, there are several different carbon valuation metrics, each seeking to place a direct financial, or wider commodified value upon carbon (**Table 1**) (Stiglitz et al., 2017, Tvinnereim and Mehling, 2018, Weber et al., 2018, Skovgaard et al., 2019). Under a regime where carbon emission has a 'cost' that can be recovered from a polluter, an economic lever exists to discourage polluting activity and/or encourage operational efficiency and divest from sources of high emission (Gaines, 1991, Tvinnereim and Mehling, 2018). Such a financial instrument provides an economic *disincentive* to continue with current practices, especially in cases where mitigation measures are more financially favourable than business as usual (Stiglitz et al., 2017, Haites, 2018). Such a philosophy has its roots in the '*polluter pays principle*' that emerged in the 1980s (Gaines, 1991, Hepburn et al., 2006).

**Table 2.1:** Disincentive and incentive carbon trading/payment mechanisms

	Disincentive		Incentive
	Emissions trading schemes <sup>d e</sup>	Carbon pricing (taxation) <sup>b c d</sup>	Carbon offsetting <sup>a</sup>
<b>Summary</b>	<p>Pre-defined sectors/industries that emit over a certain threshold (of CO<sub>2</sub>e) must acquire permits in order to operate (1 permit equates to 1 t CO<sub>2</sub>e). Once obtained the installation may then operate and emit CO<sub>2</sub>e up to the defined permit limit. Additional permits or carbon offsets must be purchased in instances where emissions exceed permit allowance (or fines will be applied). Surplus permits (from under emission) may be sold or auctioned on the ETS market.</p> <p>Direct supply of emissions permits are decreased each year to promote scarcity of permits and raise prices, encouraging sustainable development and efficiency.</p>	<p>Ascribes a price for carbon that may directly tax (all relevant) sources of carbon emission. Payments may be based on the total potential economic, environmental, and social cost of emissions or coupled to the carbon market price.</p> <p>High price of carbon tax disincentivises and reduces emissions through increased operating costs.</p>	<p>Voluntary market-based solution that encourages net emitters of CO<sub>2</sub> to buy 'offsets' which may include emission reduction technologies or payment for activities that sequester carbon, thus lowering their emissions by proxy.</p> <p>Voluntary carbon offsetting can be coupled to ETS schemes or can be paid as standalone offsets by individuals or companies.</p>
<b>Valuation mechanism</b>	Market price with minimum/maximum boundaries	Fixed or market-coupled price	Market based / Cost of implementation
<b>Carbon prices</b>	Variable	Fixed / Variable	Variable
<b>Scale</b>	Large companies and industries with emission that exceed the emission threshold	Individual - large scale business and industry	Individual – large scale business and industry (ETS partners)
<b>Direct reductions in emissions (t CO<sub>2</sub>e)</b>	Yes	Yes	No
<b>Direct payment of sequestration activities</b>	No	No	Yes

<sup>a</sup>Taiyab, 2006 (Taiyab, 2006)

<sup>b</sup>Marron, Toder and Austin, 2015 (Marron et al., 2015)

<sup>c</sup>Boyce, 2018 (Boyce, 2018)

<sup>d</sup>Tvinnereim and Mehling, 2018 (Tvinnereim and Mehling, 2018)

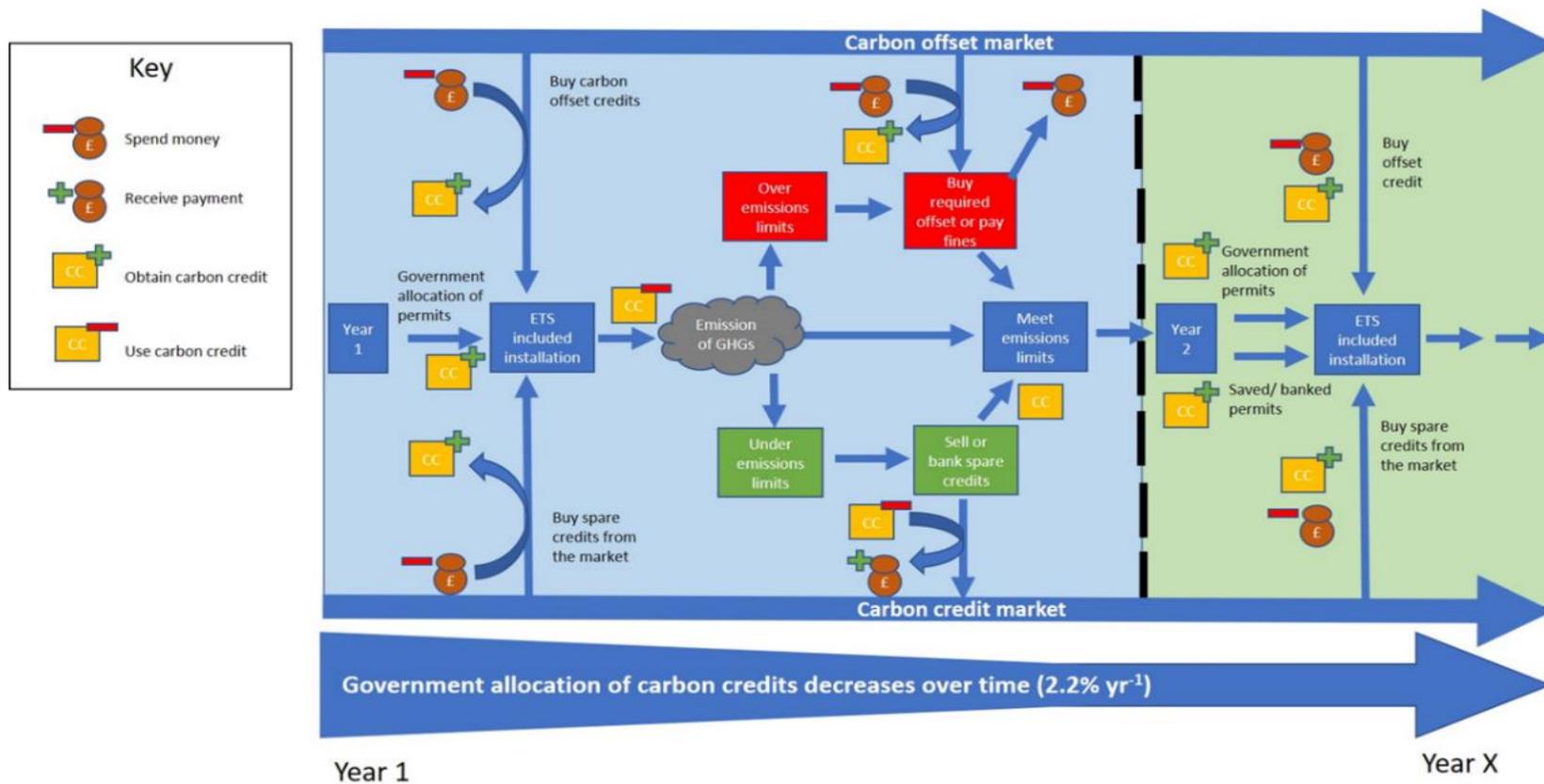
<sup>e</sup>World Bank, 2020 (WorldBank, 2020)

Globally, different regions/nations have taken contrasting approaches to carbon-pricing policies, carbon offsetting, and carbon trading (**Table SI2.1**) (Stiglitz et al., 2017). Current carbon valuation metrics focus heavily upon the aforementioned

economic *disincentives*: Levying carbon taxes and adoption of emissions trading schemes (ETS) - with yearly reductions in allocated credits (cap-and-trade) (Brunner et al., 2012, Aldy, 2017). These disincentive instruments, although successful at reducing emissions (through fiscal squeeze on emitters), do not ease the burden of carbon already emitted (Stiglitz et al., 2017). In many instances, carbon taxes, and carbon trading platforms, have been effective in leveraging business engagement and reducing emissions, while promoting development in low carbon alternative technologies (Tvinnereim and Mehling, 2018). Many of these net-gains have been associated with ETS (**Table SI2.1**), primarily targeting industry and energy generation sectors that emit large quantities of GHGs (Zhang and Zhang, 2019).

ETS (**Table 2.1, Table SI2.1, Figure 2.1**) allow for emission of GHGs to pre-defined levels, through the allocation or auction of permits that must be 'paid' to the governing body when used (*upon emission of the specified amount of GHG*, generally; 1 permit = 1t CO<sub>2</sub>e (Haites, 2018)). The EU currently operates the world's largest ETS (Kossov et al., 2015, Hirst and Keep, 2018). Established in 2005, the EU ETS (based on a cap-and-trade mechanism) functions in all EU countries, Iceland, Norway, and Lichtenstein. The EU ETS limits emissions from over 11,000 factories, power stations and commercial flights operating between EU member states; and collectively covers around 45% of all EU GHG emissions (EuropeanComission, 2015). The EU ETS has been instrumental in delivering a total reduction of 21% in emissions between 1990 and 2013 (Vollebergh and Brink, 2020). Within the EU ETS, a limited number of emissions permits are directly allocated (based upon the individuals share of sector emissions, assumed emission from business as usual, and calculated sector emission benchmarks (Kruger and Pizer, 2004, Ellerman and Buchner, 2007, Sartor et

al., 2014)). Allocations are reduced yearly by 2.2% (post 2021), further encouraging transition and investment into energy efficiency via reduced emission limits and permit scarcity. By extension, permit reductions also lead to increased permit trade in the marketplace and increased permit value, further driving efficiency due to raised operating cost (European Commission, 2015, Hirst and Keep, 2018). Thus, yearly increases in carbon prices lever increased investment in efficiency and environmentally friendly practice (Brink et al., 2016). Remaining permit requirements (where allocations are exceeded), are met through auction and trade at market prices (Burtraw et al., 2001, Schmalensee and Stavins, 2017, Stuhlmacher M et al., 2019), or through purchase of equivalent and verified carbon offsets (Kruger and Pizer, 2004). Emissions permits (and by proxy carbon) have typically been traded between €3 t<sup>-1</sup> CO<sub>2</sub>e and €25 t<sup>-1</sup> CO<sub>2</sub>e (Brink et al., 2016).



**Figure 2.1:** Mechanism for increased carbon offset and permit trading within emissions trading schemes (ETS) through increased availability and acceptance of verified carbon offsets.

At present, valid carbon offsets include investment in sustainable or high efficiency energy generation programmes, and credible certified emissions reductions, primarily sourced internationally (facilitated by the clean development mechanism (CDM) criteria of the Kyoto protocol) (Hepburn, 2007, Pearse and Böhm, 2014, Naegele, 2018). Offsets were limited to 1600 Mt CO<sub>2</sub>e between 2008-2020, due to offset costs being substantially lower than ETS trading prices (and often in third party countries (*not ETS members*)), thus undermining emissions reductions in favour of paying for cheaper offsets with no direct benefit granted to member states (Hu et al., 2015, Naegele, 2018).

Installations subjected to ETS quotas that exceed yearly emissions caps, and without sufficient additional permits (from verified offsets or purchased in the marketplace), are fined by the ETS governing body. The cost of the fine (~€100 t CO<sub>2</sub>e) exceeds market price; thus, an emitter is dissuaded from overrunning their quota (Hintermann, 2010, EuropeanComission, 2015, Hirst and Keep, 2018). It is highlighted that, a minimum of 50% of the revenue generated through permit auctions and emissions fines, is subsequently invested in low carbon technologies, green-energy projects, environmental protections, and sustainable innovation within ETS member states (EuropeanComission, 2015, Velten et al., 2016).

In the UK, carbon trading has historically been operated under the EU ETS umbrella (following guidelines and rules) and affects primarily the energy generation sector (Kirat and Ahamada, 2011, Hirst and Keep, 2018), however, this has since diverged into a separate UK ETS from January 2021. In addition to ETS market coupled pricing, a pre-defined minimum value for which carbon can be traded (a carbon support price) was set in 2013 (Edenhofer et al., 2017); an approach that has since been adopted by

the EU ETS (Flachsland et al., 2020). Implementation of support prices mitigates issues of permit oversupply or fluctuations in the prices that would destabilise the market (Fang et al., 2018). Since 2016 the carbon support price has been frozen at £18 t<sup>-1</sup> C; however, it is predicted to rise to £30 t<sup>-1</sup> C after 2023 and further to £70 t<sup>-1</sup> C by 2030 (Hirst and Keep, 2018, Treasury, 2021). In the UK emission reductions (catalysed by ETS and linked to divestment from coal power/transition to renewable energy sources) of 77 Mt yr<sup>-1</sup> from 1990 – 2018 have been reported (Edenhofer et al., 2017, DBEIS Department for Business, 2018).

*Carbon pricing (Table 2.1)*, is an alternative method of disincentivising emissions, achieved through the use of specialised taxes that charge for carbon emission (Zakeri et al., 2015). Carbon pricing gives a greater flexibility to which goods and services can be taxed directly; thus, offering a non-market coupled price for carbon (Haites, 2018). Carbon pricing methods are often not a one size fits all value, and prices are instead adjusted for each good or service, based on the total potential environmental and social costs of the emissions (Rausch et al., 2011, Stiglitz et al., 2017, Haites, 2018).

Sweden operates direct taxation of carbon emission (alongside EU ETS membership) (Shmelev and Speck, 2018, Tvinnereim and Mehling, 2018). Sweden's carbon tax is amongst the highest in the world, with a value of ~€130 t CO<sub>2e</sub> (Tvinnereim and Mehling, 2018); following yearly increases since adoption in 1995, when 1 t CO<sub>2e</sub> was valued at €23 (Jonsson et al., 2020). The scheme operates on the *polluter pays* principal; where the carbon tax is placed upon any activities that emit CO<sub>2</sub> (e.g. use of fossil fuels) (Gaines, 1991, Lin and Li, 2011), and includes government, industry, and private individuals (Shmelev and Speck, 2018). This carbon tax has facilitated substantial decreases in total emissions, especially within the

transportation sector where reductions of 11% were measured (equivalent to 2.5Mt CO<sub>2</sub>e (Andersson, 2019)) and has culminated in total emissions observed in 2010 to be equivalent to those of 1960, despite continued national growth (Shmelev and Speck, 2018).

Disincentive carbon payment methods often show positive results, such as within the UK, Sweden, and wider EU ETS schemes, where large reductions in GHGs have been achieved (Edenhofer et al., 2017, DBEIS Department for Business, 2018). However, these schemes are not without their issues. Disincentive payment options are often unpopular, with widespread criticism and political opposition prevalent (Pearse and Böhm, 2014) (**Box 2.1**). These options are often seen as another form of government levied tax, where the proceeds go to funding projects unrelated to climate change abatement (Hepburn, 2007), or through financial squeezing, seen to limit competitiveness and development in the global marketplace (Pearse and Böhm, 2014, Arlinghaus, 2015). Although trade of carbon and direct taxation of emissions facilitate emission reductions, there are often no explicit links to carbon sequestration from the atmosphere. Rather, carbon revenues support a diversity of activities that directly and/or indirectly aspire to deliver lower GHG emissions in the future. Thus, significant steps to reduce or resolve the effects of climate change will only transpire when emissions are reduced, *and* the anthropogenic atmospheric carbon load is re-sequestered concurrently.

Decarbonisation of the agricultural sector brings significant opportunity to reduce GHG emissions and re-carbonise soil (**Section 2.5**). Validated offsetting schemes that re-sequester carbon in soils may provide this required level of additionality (sequestration or offset activity that would not otherwise occur) to

contemporary carbon markets, over and above low emission investment. Such an approach holds enormous potential to not only rejuvenate soil carbon stocks, but to realise collateral benefits for soil ecosystems and the manifold ecosystem services they support (**Section 2.3**). To achieve this aspiration three key elements are needed: i) a soil carbon sequestration price (**Section 2.7**), ii) a soil carbon trading platform (**Section 2.8**), and iii) assurances on long-term soil carbon storage (**Section 2.9**).

## **2.7 Establishing a Soil Carbon Price**

Given their clear connection to climate change adaptation/mitigation, soil carbon stocks and carbon sequestration have tangible value (**Section 2.5**) (Pereira et al., 2018, Lal et al., 2018b, Baveye et al., 2020a). However, the re-carbonisation of soil will underpin manifold benefits for soil health and the delivery of soil ecosystem services (SES) (**Section 2.3**); it is arguably, the holistic value of these outcomes that should be used to establish the soil carbon price. Herein lies the challenge: to accurately value the provision of SES in a way that connects the regulating influence of soil carbon with the direct and wider value it represents to SES provision (including, but not limited to GHG emission mitigation).

The importance of ecosystem service valuation for sustainable growth and development has been recognised since the late 1960s, where the need to include natural resource stocks within decision-making was first identified (Costanza and Daly, 1992, Dominati et al., 2010, Braat and De Groot, 2012). Environmental economic approaches have since been applied to define the *relative* value of ecosystem services (Costanza et al., 1997, Braat and De Groot, 2012, Costanza et al.,

2017), and more recently, the monetisation of ecosystem services has emerged as a useful tool to support payments linked to ecosystem conservation (Ola et al., 2019, Balvanera et al., 2020). A well-established global exemplar of payments for ecosystem services is the REDD+ Scheme that, under an *incentive*-based mechanism, facilitates payments for afforestation and forestry management (Lederer, 2012, Strassburg et al., 2014, UNFCCC, 2016, Sheng, 2019).

Encouragingly, dialogue between economists and soil scientists, aligning the economic value of ecosystem services to land-use decision-making processes, has gone some way to promoting the ideas of SES valuation and payment (Dominati et al., 2010, Robinson et al., 2014, Pereira et al., 2018, Latawiec et al., 2020a). However, with the notable exception of Carbon Farming (**Box 2.1**) in Australia, the evaluation and inclusion of payment for soil carbon (or SES) in the mainstream of national/international policy has not yet transpired (Latawiec et al., 2020a, Willemen et al., 2020). This circumstance is juxtaposed with the economic costs associated with soil degradation (**Section 2.4**). It has been estimated that the total financial cost associated to soil degradation in the EU exceeds €38 bn yr<sup>-1</sup>, with associated crop loss costing more than €1.25bn yr<sup>-1</sup> (EEA, 2019), while in the US, soil degradation and reductions in soil carbon are estimated to cost at least US\$44 bn yr<sup>-1</sup> (Eswaran et al., 2001). Globally, the effects of soil degradation compound to a total estimated cost of approximately US \$300bn each year (Nkonya et al., 2016).

The challenge in setting a soil carbon price is not as straightforward as simply linking the soil carbon price to the monetary values associated with the costs of soil degradation. Rather, there is a real need for an expansive and more holistic valuation and assessment that embraces wider SES provision and a broad range

of worldviews. Thus, while we recognise that soil carbon markets are a powerful tool to demonstrate the value of soil to decision makers, we highlight that non-monetary value of soil should, in an ideal world, be promoted concurrently (a view reflected in the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), through its concept of nature's contributions to people (Pascual et al., 2017)). Through a more holistic framing, wider public support for, and adoption of, a soil carbon economy will be facilitated (i.e. from a shared perspective, that everyone will benefit, engagement will be stronger (Ascui and Lovell, 2011)).

Notwithstanding foregone ideals, the overriding proviso remains, soil carbon sequestration prices must square with the carbon market price currently used to facilitate existing carbon trading schemes. Reconciling prices to these already established carbon markets (**Table 2.1**), will be essential to mitigate issues pertaining to *offset undermining* of emissions reductions, arising from lower offset costs (Hu et al., 2015, Naegele, 2018), or low uptake on a soil carbon market due to an over-priced soil carbon unit. Furthermore, soil carbon prices must be substantial enough to encourage uptake by farmers and landowners. Stakeholders are only likely to adopt soil carbon sequestration practices where there are valid economic incentives to do so, especially where significant investment in time and resources are involved (Kragt et al., 2012, Burke et al., 2019)

## **2.8 A Soil Carbon Trading Platform**

Assuming a soil carbon price can be ascribed, a platform upon which to trade soil carbon units/credits will be needed to bring a soil carbon economy to fruition. A *voluntary* market (**Table 2.1**), trading in carbon offsets, could be the way forward to

incentivising payment for those that sequester carbon (Lee et al., 2016, Sapkota and White, 2020). In 2010, 131 Mt CO<sub>2</sub>e were traded through voluntary carbon markets; at a total value of US \$424 million (Benessaiah, 2012) (estimated increase to 141Mt CO<sub>2</sub>e traded in 2019). A significant proportion (29%) of the total revenue was associated with the REDD+ carbon market (Benessaiah, 2012). The REDD+ initiative (Lederer, 2012, Strassburg et al., 2014, UNFCCC, 2016) provides financial reward for developing countries that reduce GHG emissions through actions that redress deforestation and forest degradation (facilitated through CDM (Naegele, 2018)). Thus, REDD+ incentivises forest carbon stock improvements, while realising collateral benefits for sustainable management of forest conservation (Lederer, 2012, Strassburg et al., 2014). Voluntary offset markets offer ancillary benefits to farmers and landowners through increased opportunity to diversify production, reduce costs (if following carbon farming methods) and provide new revenue streams (De Pinto et al., 2010). Through monetisation of soil carbon sequestration/storage as a soil *good*, these markets can provide win-win opportunities for farmers/landowners, sustainable development, and the wider global community (Benessaiah, 2012, Lee et al., 2016).

Voluntary offset markets hold great potential for increasing the provision of soil (and wider) ecosystem services (**Section 2.3**) (Milder et al., 2010, Lee et al., 2016). It is highlighted that many voluntary offset buyers are also willing to pay higher *premium-offset* rates where wider ecosystem service, biodiversity and societal net-gains are delivered in parallel (Benessaiah, 2012, Lee et al., 2016). The voluntary carbon market might also facilitate the processing of payments linked to personal or private offsets (i.e. such as is seen from citizen payments to offset GHG emission

associated with air travel (Gössling et al., 2007, Mair, 2011)). However, if this course is to be followed, standardisation and verification measures must be established to ensure the validity of any subsequent sequestration and offsets that are created (**Section 2.9**). At present, there are several verifying bodies that set and monitor standards and methods within the voluntary offset market (Verra, SCS Global and GoldStandard). These organisations act as a point of registration and verification for projects and offsets credits sold on the voluntary or compliance (ETS) markets, ensuring their validity and additionality. The vast majority of these verified offsets are projects based in the global south and developing nations, focussing on REDD+ in nations such as Brazil, or energy efficiency projects in Kenya keeping costs low. However, for voluntary markets to truly gain traction and effect large-scale change, efforts must be made to better incorporate projects in more developed regions.

Although steps have been taken to increase the scope of offset projects, there is yet to be a mainstream provider or standardising body focussing on soil-based offsets. An issue we believe pertains to the complexity of soil carbon measurement/monitoring, and the assurity of long-term carbon storage (**Section 2.9**).

As an alternative method soil carbon payment mechanism could be integrated into the framework of an existing system: Agri-environmental schemes encourage environmentally friendly and sustainable agriculture/land management practice. These incentivised voluntary schemes, providing financial incentives to farmers/land managers to adopt best practice, have been active in the EU since the 1990s (Gatto et al., 2019, Kiryluk-Dryjska and Baer-Nawrocka, 2019, Simoncini et al., 2019). Aligning a soil carbon economy with such schemes makes sense, as these schemes fundamentally seek to evoke environmental net-gains, many of which link to

soil carbon sequestration. In addition, these schemes have pre-existing framework and infrastructure to facilitate relevant payments.

The UK is currently developing new national agriculture regulations (Agriculture Act, 2020) and a new agricultural payment system: Environmental Land Management scheme (ELMS). Central to developing the policy and payment scheme is the pledge to use public money to pay for public goods (2020a, 2020c). Thus, payments are anticipated to follow on farm interventions that deliver public goods, for example, biodiversity net-gains. There is also possibility that ELMS could support payments for soil re-carbonisation (**Box 2.2**).

A recent addition to the private sector carbon trading marketplace is the US based company IndigoAg. Following release of their Terraton Initiative in 2019, the company has vowed to initiate its own carbon off-set platform. The Initiative aspires to the goal of sequestering 1 Tt (1 Tt =  $10^{12}$  tonnes) of C globally (IndigoCarbon) via incentivised carbon farming (**Box 2.1**). To facilitate this aspiration IndigoAg propose to offer a minimum carbon price of USD \$15-20 per 1t CO<sub>2</sub>e sequestered, such a value corresponds to that proposed as the 'feasible minimum carbon price' for significant and effective carbon sequestration in farmland soils (Burke et al., 2019). At the time of writing, the UK Government had recently announced, in its 2021 Budget Statement (Treasury, 2021). Contained within which was an aspiration to grow a *green taxonomy*, with the UK at the centre of an expanded global voluntary carbon market.

While sequestration projects (such as REDD+) and Terraton, are recognised as effective carbon offset methods (facilitated through the CDM compliance markets, and the voluntary market, respectively) (UNFCCC, 2016, Vacchiano et al., 2018, Sapkota and White, 2020) (**Section 2.6**), there has been limited uptake and trade of

these offset permits within more formal carbon trading schemes (i.e EU ETS (**Section 2.6**)). There are three primary reasons for this reticence; firstly, the complexity of the environmental economics involved in sequestration accountancy makes auditing lengthy, bureaucratic, and difficult; secondly, lack of standardisation in carbon measurement, and thirdly; uncertainty regarding the permanence of carbon sequestration (Lovell, 2010, van der Gaast et al., 2018, Vacchiano et al., 2018, Sapkota and White, 2020).

### **Box 2.2 A Soil-Centric Approach – Case Study UK**

As the UK withdraws from the EU, it will need a UK specific successor to current EU agri-environmental schemes. The UK Government, through the Department for Environment, Food and Rural Affairs (DEFRA), is in the process of creating a UK-centric Environment Land Management Scheme (ELMS). The development of ELMS coincides with the adoption of the new Agriculture Act (2020), and Environment Bill (Klaar et al., 2020a). ELMS will provide a mechanism through which to meet Net Zero targets set by DEFRA and the National Farmers Union (NFU), by respectively, 2050 and 2040 (2018, NFU, 2019, Burke et al., 2019, 2020c). ELMS will be centred on the philosophy of using ‘public money to pay for public goods’ (2020a, Klaar et al., 2020a, Gosal et al., 2020). It is ELMS mission to deliver manifold environmental benefits by providing farmers, foresters and other land managers with opportunities, incentives, and financial reward for enhancing or maintaining the environment and essential ecosystem services while protecting UK natural capital (2020a, Gosal et al., 2020, Klaar et al., 2020a).

ELMS will provide a three-tiered management scheme. Payments will be made to farmers and landowners for the ecosystem services provided, rather than payments

based on total farm area, livestock herd size and general environmentally sensitive practices as seen previously (Klaar et al., 2020a). The public goods that will likely be paid for under ELMS include the provision of clean & plentiful water, clean air, protection from & mitigation of environmental hazards (e.g. flooding), mitigation of & adaptation to climate change, thriving plants & wildlife, habitat protection & expansion, beauty, heritage and engagement (2020a, 2020c).

The payment mechanisms under which ELMS will operate are currently under discussion but are likely to include instruments such as: Government based price setting (fixed prices), market coupled price setting (linking to the commodified value of carbon, allowing private sector investments and offset payments similar to ETS), direct payment mechanisms or payment by results, where a portion or all of the payment is delayed until adequate benefit has been attained (2020a).

ELMS is an evolving payment system, and while no formal pledge has been made to incorporate payment to farmers and landowners to sequester carbon in soils such an outcome could transpire (NFU, 2019, 2020a, 2020c, Klaar et al., 2020a). Payments to support soil carbon sequestration could be implemented as a component of ELMS or, perhaps more likely, could be developed in parallel to ELMS under a voluntary off-set scheme. ELMS is currently in the planning and trial stage (2021-2024) prior to full national adoption post 2024. It is likely, in the interim, that pressure will be placed on the government to instate '*carbon farming*' policies into ELMS before the full roll out of the scheme.

Linking together the Agricultural Act (2020) and the Environmental Bill to ELMS, alongside the NFU Net Zero 2040 aspiration, will ensure agricultural profitability and sustainability in the UK; enhancing the environment and working towards climate

change goals in an effective manner (Klaar et al., 2020a). Payment for re-carbonisation of soils, through ELMS, would help catalyse the transition of the UK agricultural sector to Net Zero, and provide opportunities for enhanced delivery of ecosystem services (De Groot et al., 2002a, MA, 2005, Power, 2010). Such a soil-centric approach would provide a fast-track to optimising the delivery of public goods and services, while cementing and improving profitability of the agricultural sector in the UK.

## 2.9 Assuring Long-term Soil Carbon Storage

It would be inappropriate to make payments for short-lived uplifts associated with labile/degradable carbon, on the auspices of GHG mitigation/offset. Payments for soil carbon sequestration should be linked to interventions that deliver *long-term* carbon storage. However, to assess changes through direct measurement of SOC every time an intervention is made, would be too costly and time consuming to be pragmatic (Smith et al., 2020b). Thus, numerical modelling is an essential tool to deliver confidence in soil-based carbon sequestration potential in a cost-effective way. SOC simulation models are varied but are generally based on empirical relationships or underlying processes, established using long-term field experiments as a primary data source (Campbell and Paustian, 2015, Paustian et al., 2019). SOC models predict SOC dynamics regionally and globally in response to climatic changes, land use and land management (Shirato and Yokozawa, 2005, Guo et al., 2007, WorldBank, 2012). SOC models have been successfully used to predict the impact of agricultural activities on SOC and CO<sub>2</sub> emissions, allowing farmers and regulators to predict SOC storage and stability in implementing and developing suitable land management options (e.g. soil

amendment application) (Taghizadeh-Toosi et al., 2014, Campbell and Paustian, 2015).

Here, several models have been developed and are well established for predicting SOC turnover in agricultural soils, for example, the *RothC Model* (Falloon et al., 1998, Coleman and Jenkinson, 2014a), *CENTURY* (Parton, 1996), and *ICBM* (Andrén and Kätterer, 1997). Each model considers the SOC held in different pools with varying decomposition rates; the temporal dynamics of carbon leaving/entering these pools then propagates through, for increasing timeframes. Significantly, the *RothC*, and other models, have been calibrated with measured data drawn from long term experiments (Shirato and Yokozawa, 2005, Skjemstad et al., 2004, Guo et al., 2007, Powlson et al., 2012a) and have been modified to predict the fate of exogenous organic inputs (e.g. compost, agri-industrial waste and digestate) (Yokozawa et al., 2010b, Peltre et al., 2012b, Mondini et al., 2017b). However, further validation is still required to ensure predicted carbon sequestration potential is corroborated/verified for soils and climates directly relevant to locations where soil re-carbonisation is delivered.

Verification and validation processes need to be transparent if incentives for land-based carbon sequestration are to be made credible (Lee et al., 2016). Placing focus on improving verification and validation processes will save time and expense, thus lowering the barrier for entry, increasing uptake, and developing further environmental economic potential for soil re-carbonisation. In our view, only with tailored assessment of carbon stability prognoses (that lock to specific bioclimatic regimes, land use and specific interventions) can payments be appropriately reconciled with soil re-carbonisation.

## **2.10 Outlook: Soil Carbon Payments and Multiple Net-gains**

Existing carbon trading mechanisms have highlighted the enormous potential for economic levers to deliver sizable reductions in GHG emissions (**Section 2.5**). Furthermore, it is expected that in the short to medium term commodification of carbon will continue to gain traction, through increasing adoption of carbon taxes, expansion of emissions trading schemes (**Table 2.1, Table SI2.1**), validated carbon pricing, payment for carbon offsets (**Section 2.7**) and the alignment of private sector markets (**Section 2.8**) (Stiglitz et al., 2017, Skovgaard et al., 2019). While increased delivery of disincentive carbon payments will facilitate greater efficiency and encourage divestment from high emission activities (Eggleston et al., 2006, Lundie et al., 2009, Rosenbloom et al., 2020), it will not redress the fundamental problem of elevated GHG loads already in the atmosphere. To grasp a Net Zero future, historic anthropogenic carbon emissions must also be removed. Thus, it is our view that incentivised carbon payment metrics (linked to sequestration of carbon from the atmosphere) are needed, as a mainstream compliment to these more dominant disincentive (emissions reduction) metrics.

Given the large historic transfer of carbon from soil to the atmosphere (**Section 2.4**), re-carbonisation of soil makes intuitive sense. Re-carbonising soils can provide efficient and cost-effective carbon sequestration potential (**Section 2.5**), without required development of new technology or techniques, and can be applied at scale with relative ease (Lal et al., 2018b, Soussana et al., 2019a, Smith et al., 2020b). Furthermore, through rejuvenation of soil carbon stocks, benefits for soil health and optimisation of ecosystem services can be achieved (**Section 2.4**): a win-win outcome.

Assigning a price for *soil* carbon (**Section 2.7**), within the prevailing carbon economy, offers enormous potential to not only to combat climate change via economic leverage (Alexander et al., 2015), but also achieve wider societal economic net-gains through uplifted delivery of ecosystem services (Benessaiah, 2012, Lee et al., 2016, IPBES, 2018b, Latawiec et al., 2020a, Bouma, 2020). Carbon sequestration payments may also provide opportunity to assist in the sustainable development of underperforming and industrialising regions, contributing to sustainable development goals through payment for beneficial land management practice (IPBES, 2018b, Rumpel et al., 2020, Bouma, 2020). However, setting a soil carbon price is not trivial. Proportionate payment for carbon sequestration must be established to reconcile economic difficulties faced by farmers/land managers in achieving sequestration aspirations.

With the projected increases in both the price of carbon and adoption of carbon pricing initiatives, financial incentives to sequester carbon will intensify (Rumpel et al., 2020). Herein, lies opportunity to formalise a soil carbon economy while the carbon market is in its formative stage (**Section 2.8**). A market-coupled approach, would see steady increase in the carbon price through successive yearly increases in the value of emissions permits, simultaneously providing a source of offsets that may be used in conjunction with emissions reductions (**Figure 2.1**). Setting minimum carbon payment levels and price floors within these adopted schemes (between \$15-20 t CO<sub>2</sub>e), would provide adequate economic incentive to sequester carbon in soil and rectify many of the economic difficulties faced by farmers (Burke et al., 2019). Such an approach of integrating sequestration payments into current carbon markets however, would need to address issues of *lowest cost purchasing*. Specifically, offsets

must not be valued at a substantially lower price than emissions credits, effectively encouraging emitters to buy cheap offsets rather than curb emissions. Assuming a price balance can be achieved, an approach that requires incentive-disincentive linkage could catalyse a ground-shift that would reduce *and* mitigate emissions, actively addressing climate change issues.

Interventions to which payments are linked will also require clear articulation (**Section 2.7**). These elements, while procedural, are arguably, the greatest challenge to address. With appropriate and proportional political momentum, it will be possible to encourage swift adoption and wide-scale participation in a soil carbon economy by multiple stakeholders (including, farmers and landowners, scientists, economists, and policy makers). Specifically, for global re-carbonisation of soil to be realised, communication defects and gaps, arising from the convergence of disparate fields (i.e. ecology, economics, agriculture, soil science and governmental policy), must be reconciled (Dominati et al., 2010, Bristow et al., 2010, Latawiec et al., 2020a). Monetary valuation of ecosystem services offers potential here. However, we continue to recognise the importance of non-monetary valuation of soil, and that this should be accommodated in soil carbon price setting.

Furthermore, consensus needs to be reached on how carbon stocks are defined, and stock changes verified through i) SOC measurement and ii) SOC durability. With regards to these aspects, international agreement is needed to define the depth to which carbon stocks should be assessed, and in what form the carbon (labile/recalcitrant) is considered “eligible” for remuneration. In considering the durability of a carbon stock, agreement will be required regarding the timeframe of the sequestration prognosis and standardisation of SOC fate-model variables (**Section**

**2.9).** Much of the uncertainty surrounding soil carbon markets can be mitigated by drawing upon contemporary literature, corroborating the environmental and economic value of soil carbon sequestration. It is our view, however, that this be tempered with a pro-active approach, where evidence may be gathered and synthesised through ongoing action/intervention – actively engaging with soil carbon payments.

In conclusion, the re-carbonisation of soils has the capacity to deliver a significant portion of the required intervention to offset current emissions and remove historic emissions. Furthermore, re-carbonisation of soil will deliver climate change mitigation with co-benefits for soil quality, health, the delivery of SES and wider societal benefits. Capturing a *soil-based* carbon economy is a grand challenge, but with urgent and assertive political action, one that is attainable in the decade ahead.

## 2.11 Supporting Information

**Table SI2.1:** Operational and proposed emissions trading schemes (ETS) globally (2020)

REGION	SCHEME	CURRENTLY OPERATING	PLANNED SCHEMES	PROPOSED SCHEMES	EMISSIONS CONTROLLED	
<b>Europe</b>	EU ETS	EU27, UK, Lichtenstein, Norway, Iceland			Industry, power generation and intra-member states aviation	
	National /EU ETS	Switzerland			Linked to EU ETS	
	National		Ukraine		Planning phase	
	National		Montenegro		Industry and power	
	National		Germany		Heating and transport fuel (in conjunction with EU ETS)	
<b>Asia</b>	National			Turkey	Proposed for energy generation sector	
	National	Kazakhstan			Power generation and industry	
	National			Pakistan	Planning phase	
	City /Provincial	<b>China:</b> Beijing Tianjin Shanghai Fujian Shenzhen Guangdong Chongqing Hubei				Inclusion of aviation, power generation, transport, industry, and non-industrial sources that emit >10,000 t CO <sub>2</sub> e yr <sup>-1</sup>
	National		China			Continuation and expansion of regional initiatives with national threshold of >26,000 t CO <sub>2</sub> e yr <sup>-1</sup>
	National	Republic of Korea				Power generation, industry, waste, building, transportation public sector and domestic aviation
	City	<b>Japan:</b> Tokyo Saitama				Fuel use, and public/private entities that require energy equivalent to 1500kL yr <sup>-1</sup> of crude oil
	National				Japan	Planning phase
	National				Taiwan	Planning phase
	National				Philippines	Industrial and commercial sectors
	National				Indonesia	Emissions and waste
	National				Vietnam	Industry and waste
	National				Thailand	Planning phase
<b>Oceania</b>	National	New Zealand			Forestry, energy generation, industry, waste, and agriculture (2025)	
	National			Chile	Planning phase	

<b>South America</b>	National		Brazil	Planning phase
	National		Columbia	Planning phase
	National	Mexico		Energy generation and industry
<b>North America</b>	Regional (Western Climate Initiative (WCI))	California		Industry, energy generation, combustion of fossil fuels, imports (linked to Quebec ETS)
	Regional greenhouse gas initiative (RGGI)	<b>USA:</b> Connecticut Delaware Maine Maryland Massachusetts New Hampshire New Jersey New York Rhode Island Vermont		Power generation from fossil fuel installations That produce >25MW yr <sup>-1</sup>
	State	Massachusetts		Energy Generation (linked to RGGI)
	Regional carbon pricing		New England Region	Transport
	State		Virginia	To link with RGGI
	City		New York City	Buildings sector
	State		North Carolina	Energy generation
	State		New Mexico	Planning phase
	State		Oregon	Energy generation and industry
	State		Washington	Industry and emissions of >100,000 t CO <sub>2</sub> e yr <sup>-1</sup>
	Province (Western Climate Initiative (WCI))	Quebec		Energy generation, industry, fuel consumption, transport, construction, (emissions >25,000 t CO <sub>2</sub> e yr <sup>-1</sup> ) and voluntary participants (>10,000t CO <sub>2</sub> e yr <sup>-1</sup> )
	Province	Nova Scotia		Industry, energy generation and fuel suppliers

ETS schemes currently in operation, planning or proposition phases around the world and the definition for inclusion 2020. Adapted from <https://icapcarbonaction.com/en/ets-map> (2020b)



## **Chapter 3:**

# **Recycling Paper to Recarbonise Soil**

### 3.1 Abstract

Soil organic carbon can be increased through sympathetic land management and/or directly by incorporating carbon rich amendments. Herein, a field experiment amended paper crumble (PC) to soil at a normal deployment rate of 50 t ha<sup>-1</sup>, and at higher rates up to 200 t ha<sup>-1</sup>. The nominal 50 t ha<sup>-1</sup> PC amendment resulted a mean increase in soil carbon of 12.5 g kg<sup>-1</sup>. Using a modified ROTH-C carbon fate model, the long-term (50 years) carbon storage potential of a 50 t ha<sup>-1</sup> PC amendment was determined to be 0.36 t<sub>C</sub> ha<sup>-1</sup>. Modelling a rotational (4 yearly) 50 t ha<sup>-1</sup> PC amendment indicated 6.65 t<sub>C</sub> ha<sup>-1</sup> uplift would accrue after 50 years. Contextualised for the average farm in the East of England (~120 ha, with 79% as arable), PC derived increases in SOC would be equivalent to 2310 t CO<sub>2</sub>e. These results support the use of PC to deliver significant levels of soil recarbonisation. Beyond carbon, PC was observed to influence other soil properties. Benefits observed included, decreased bulk density, increased water holding capacity, and increased cation exchange capacity. While PC amendment did not significantly increase wheat (*Triticum aestivum*) crop yield, manifold benefits in terms of increased SOC, long-term carbon storage potential, and improved soil quality sustains PC as a beneficial soil conditioner.

### 3.2 Introduction

More than 200 million hectares of agricultural land worldwide have been acknowledged as dangerously degraded, where soil carbon stocks are reduced by ≥ 50% (Lal, 2001). Due to the shaping influence on soil physical, chemical, hydrological, and biological properties, soil carbon, or more broadly soil organic matter (SOM), constitutes one of the most important factors underpinning soil

health, and by extension the maintenance/delivery of soil ecosystem services (Abiven et al., 2009b, Bhogal et al., 2009b, Power, 2010, Powlson et al., 2012a, Keenor et al., 2021). Society relies upon these essential ecosystem services for the provision of goods (food and resources), environmental regulation (water filtration and flood mitigation), and to support environmental functions (carbon cycling and sequestration) (Dominati et al., 2010, Latawiec et al., 2020b, Keenor et al., 2021). Soils rich in organic matter are often regarded as having greater resilience to the environmental pressures, for example, drought and erosion (Bhogal et al., 2009b, Powlson et al., 2012a).

Soil organic carbon (SOC) degradation linked to agriculture, forestry and land use change has underpinned a considerable emission of carbon to the atmosphere, contributing significantly to climate change (Smith, 2008). An estimated 135 Pg C have been lost over the past 150 years with a further 36 Pg C of projected loss by 2050 (IPBES, 2018a, Lal, 2018a). Furthermore, soil derived emissions from agriculture and land use change account for approximately 24% of the global annual GHG emission (Smith et al., 2014c). SOC reductions have been linked to decreased crop yields (Follett, 2001b, Kimetu et al., 2008b, Gomiero, 2016, Ivits et al., 2018), loss of soil biodiversity (Lal, 2001, Tsiafouli et al., 2015), altered soil hydrology and nutrient provision (Lal, 2006, Kimetu et al., 2008b), soil erosion (Olson et al., 2016, Lal, 2019), and impairment of soil ecosystem services (Power, 2010). Consequently, SOC loss and soil degradation have significant implications for present and future food security, resource sustainability and essential ecosystem service provision (Follett, 2001b, Lal, 2006, Power, 2010, Gomiero, 2016, Lal, 2016).

To combat these issues, interventions are urgently needed to restore SOC, and mitigate the negative effects of this legacy loss. By adopting soil-centric land management practices, the effects of soil degradation may be arrested; thus, better protecting existing soil carbon stocks and stimulating additional carbon sequestration (Latawiec et al., 2020b). Interventions that enable soils to sequester rather than emit C, such as, less aggressive tillage, reduced agrochemical input, cover-crop rotations, and ‘feeding’ soil with C-rich amendments, provide opportunity to rejuvenate soils and capture carbon (Lal, 2004b, Powlson et al., 2012a, Soussana et al., 2019b, Keenor et al., 2021).

Direct intervention methods, such as augmenting soil with C-rich amendments (manures (Lal, 2004b), composts and paper waste (Chantigny et al., 1999, Powlson et al., 2012a) and biochar (Lehmann et al., 2006b, Smith, 2016), afford a means of increasing SOC stocks over a short timeframe and without the need for a radical shift in land management practice.

Paper crumble (PC), the focus of this research, is a co-product of the paper and cardboard recycling process. Comprised of wood pulp fibre, PC contains high levels of carbon (up to ~37% dry weight dependant on feedstock), 20-30% of which may be considered recalcitrant (Zibilske et al., 2000, Powlson et al., 2012a).

To date, several papers have evaluated the benefits of PC as a soil amendment in a variety of agricultural and environmental contexts (Chantigny et al., 1999, Zibilske et al., 2000, Foley and Cooperband, 2002a, Chow et al., 2003, EA, 2005, Abiven et al., 2009b, Powlson et al., 2011, Gallardo et al., 2012, Powlson et al., 2012a, Rasa et al., 2021b). Previous publications have reported beneficial effects on soil physical, chemical, and hydrological properties. PC has been observed to minimise surface

water runoff and associated soil erosion (Zibilske et al., 2000, Foley and Cooperband, 2002a, Powlson et al., 2012a, Rasa et al., 2021b), and substantially increase SOM/SOC content. However, this evidence is fragmented with respect to different soils and contrasting PC materials. In addition, the permanence of SOC uplift following PC amendment has, to date, not been reported.

To consolidate evidence regarding the potential for PC to increase soil carbon stocks and its wider influence upon soil properties (physical, chemical, hydrological) and crop yield, a field experiment was undertaken using applications of PC between 50 and 200 t ha<sup>-1</sup>. Soils were assessed to establish soil organic matter (SOM) / total carbon (TC) contents. Subsequently thermogravimetric analysis (TGA) was used to profile PC-carbon stability. This data was then used to inform a modified ROTH-C carbon fate model to evaluate the long-term carbon storage potential of PC amended soil. In complement, the influence of PC on soil strength, bulk density, water holding capacity, pH, cation exchange capacity (CEC) and major/trace element concentrations were assessed. Finally, a laboratory batch-equilibration study was undertaken to explore potential interactions between PC and N-fertiliser. Given the size of the PC resource (e.g., in the UK, ~1Mt produced p.a) (CPI, 2014), this research sought to evidence the opportunity for this resource to re-carbonise soil and to improve soil quality.

### **3.3 Materials and Methods**

#### **3.3.1 Field Experiment**

The field experiment was established at Estuary Farm (King's Lynn, Norfolk, UK; 52° 46'46.0"N 0° 24'08.8"E). Soil was of the Wallasea Series; a paleo-alluvial gley soil,

with stoneless A-horizon of silt clay texture (Hodge et al., 1984). Field measurements and samples were collected in 2019: soil physical data (January/May), hydrological data (January), chemical data (January) and crop data (August).

PC was provided by Palm Paper Ltd (King's Lynn, Norfolk, UK). PC was applied to fields using a Bunnings Spreader and then incorporated to a depth of c. 5cm by culti-pressing and flat-lifting the soil (September 2018). PC was applied at rates of 50, 100, 150 and 200 t ha<sup>-1</sup> to 36 x 400 m strips of the same field. Soils were drilled with winter wheat (*Triticum aestivum*) (September 2018). The field margin was used to benchmark outcomes in PC amended soil. The properties of the PC are provided in **Table 3.1**.

**Table 3.1.** PC properties including: OM, TC, TN, C:N, Water Holding Capacity, Bulk Density, Cation Exchange Capacity, pH, Essential and Non-essential Elements (n = 4; mean ± std dev).

Parameter	Unit	Value
OM <sup>a</sup>	% dry mass	29.9 ± 0.3
Total C	% dry mass	24.4 ± 1.6
Total N	% dry mass	0.55 ± 0.06
C:N	dimensionless	45:1
WHC <sup>b</sup>	%	131 ± 10.81
Bulk density	g cm <sup>-3</sup>	0.39 ± 0.01
CEC <sup>c</sup>	me/100g	89.4 ± 2.7
pH	dimensionless	6.94 ± 0.04
<b>Essential major elements</b>		
K	mg kg <sup>-1</sup> dry mass	67.4 ± 3.8
Mg	mg kg <sup>-1</sup> dry mass	142 ± 3.9
Na	mg kg <sup>-1</sup> dry mass	781 ± 18
P	mg kg <sup>-1</sup> dry mass	5.61 ± 0.84
<b>Essential trace elements</b>		
B	mg kg <sup>-1</sup> dry mass	0.37 ± 0.01
Zn	µg kg <sup>-1</sup> dry mass	BDL
Cu	µg kg <sup>-1</sup> dry mass	0.26 ± 0.01
Ni	µg kg <sup>-1</sup> dry mass	0.11 ± 0.01
Mo	µg kg <sup>-1</sup> dry mass	0.14 ± 0.01
<b>Non-essential elements</b>		
Cr	µg kg <sup>-1</sup> dry mass	0.01 ± 0.002
Cd	µg kg <sup>-1</sup> dry mass	BDL
Hg	µg kg <sup>-1</sup> dry mass	BDL
Pb	µg kg <sup>-1</sup> dry mass	BDL

Note. In several instances available concentrations of elements were below the detection limit for the method; where this is the case values have been annotated "BDL".

<sup>a</sup> OM: organic matter.

<sup>b</sup> WHC: water holding capacity.

<sup>c</sup> CEC: cation exchange capacity.

### **3.3.2 Soil Sampling**

Soil samples (0-20 cm; n=4) were obtained in January using a Dutch Auger, sieved *in-situ* (1 cm) and further sieved in the laboratory (2 mm). Soil samples were sealed and retained in cold storage ( $\leq 4$  °C) prior to laboratory analysis.

### **3.3.3 Soil Organic Matter, C & N Content and Thermal Analysis**

SOM content was measured by loss on ignition (ISO, 1995a). Briefly, soil (10 g; n = 4) was dried (74 °C for 16 h) and then combusted (470 °C for 36 h). For Total Carbon (TC) and Total Nitrogen (TN), milled dry soil samples (5 mg; n = 4) were packed in tin capsules (8 × 5 mm). TC and TN were measured using an elemental analyser (Exeter CHNS analyser).

The thermal stability of PC was assessed using a Thermo-gravimetric analyser (Mettler Toledo TGA/DSC 1). Samples (n=3) were heated in a nitrogen atmosphere, at a rate of between 10 to 20 °C min<sup>-1</sup> from 25 to 1000 °C. To benchmark the PC samples, cellulose and lignin (obtained from Sigma-Aldrich) were assessed using the same method.

### **3.3.4 Carbon Fate Modelling**

The Rothamsted Carbon (RothC) Model (Coleman and Jenkinson, 1996) is a widely used model for assessing the turnover of soil organic carbon (SOC). The recent versions of the model include four active carbon pools and one inert carbon pool (inert organic matter; IOM). The model divides incoming organic inputs into decomposable plant material (DPM) and resistant plant material (RPM), both

decomposing to form microbial biomass (BIO), humified organic matter (HUM) and CO<sub>2</sub>. The standard model considers plant residues and farmyard manure as organic carbon (OC) inputs and uses a pre-defined ration of DPM/RPM. In order to be suitable for PC amendment, the RothC model was modified and propagated using TGA/DTG assigned organic carbon fractions (**Section 3.4.2**).

Soil carbon modelling was performed in RStudio. Within the model initial soil carbon level was set to zero (thus, only PC carbon was considered). The model runs considered PC applied to soil under the following two scenarios: i) a single application of 50 t ha<sup>-1</sup> in year 0; and ii) a 50 t ha<sup>-1</sup> application every 4 years from year 0 to year 49. The input parameters used to inform the model are summarised in the supporting information (**Table SI 3.1**).

### **3.3.5 Soil Physical Attributes**

Penetration resistance (Eijkelkamp Hand-Penetrometer; 13mm diameter 30° cone, 10mm diameter rod (n = 16)) and shear resistance (Pilcon 19mm soil shear vane (n = 24)), were measured *in situ* (January/May). Soil core samples (n = 4) were obtained using a Dent soil corer (core sleeves 7.5 cm height and 8.8 cm diameter) (January). Cores were oven dried (74 °C) and soil bulk density calculated (n = 4).

### **3.3.6 Soil Hydrological Attributes**

Water holding capacity (WHC; n = 4) was assessed by placing soil (~ 20 g) in a filter funnel (Whatman No.1 filter paper) and saturating the soil with distilled

water. Samples were allowed to drain until gravity release of water had stopped. Moisture content of the soil was determined (drying at 74 °C for 16 h).

### **3.3.7 Soil Chemical Attributes**

Soil pH (n = 4) was measured (ISO, 1994) in 1:10 soil/water suspension using a pH electrode (Mettler Toledo Pro pH) and pH meter (Mettler Toledo 5 Easy).

Cation Exchange Capacity (CEC) (n = 4) was assessed by sodium acetate exchange method (USEPA, 1986). In brief, soil (5 g) was mixed with 1M sodium acetate (30 ml), shaken (16 h), centrifuged and the supernatant discarded (repeated two further times, with 1 h shake times). Thereafter, acetone (30 ml) was used to rinse the soil pellet (1 h shake, centrifuged and supernatant discarded; repeated three times). Finally, 1M ammonium acetate (30 ml) was added to the sample, followed by agitation (16 h) and centrifugation. The supernatant was decanted into a volumetric flask (100 ml) through a No.1 filter paper. This procedure was repeated two further times and the samples were made up to volume with ammonium acetate (1M). Sodium (Na) content was measured by ICP-AES (Varian Vista Pro CCD Simultaneous).

Major and trace elements concentrations (n = 4) were measured following soil/PC extraction with 0.01 M CaCl<sub>2</sub> solution (Quevauviller, 1998). Samples (50g) were mixed with 0.01 M CaCl<sub>2</sub> solution (500 ml), shaken for 3h and allowed to settle (30 mins). Aliquots (50ml) of the extract were then centrifuged and filtered (0.45 µm). Major and trace element concentrations were measured using ICP-AES (see above).

### **3.3.8 Nitrogen Species Interaction with Soil and PC**

A fertiliser solution was prepared by dissolving 0.357g of ammonium nitrate in 1 L Milli-Q water (18.2 MΩ.cm). Fertiliser solution (40ml) was added to Wallasea soil or soil/PC mixtures (equivalent to a PC application of 50 t ha<sup>-1</sup>) (4 g, n =4). The fertiliser addition represented 200 kg N ha<sup>-1</sup> (i.e. assumes a mixing depth of 1.5 cm and soil bulk density of 1.03 g cm<sup>-3</sup>). The samples were shaken (18h), centrifuged and filtered (0.45 µm). In parallel, Milli-Q water (40 ml) was added to soil or soil/PC mixtures (4g, n = 4) and the same process was repeated. Ammonium and nitrate concentrations in the resultant solutions were measured using a Skalar San++ Flow Analyser.

### **3.3.9 Crop Sampling**

Seed heads (quadrat, 0.25m<sup>2</sup>; n = 4) were collected prior to harvest in August. Samples were dried (74 °C for 24 h) and 100 undamaged seed heads separated and threshed. Total yield was then calculated using threshed sample mass and an average number of plants per m<sup>2</sup> of 460 per m<sup>2</sup> (Wheat Growth Guide, 2018)); and scaled to t ha<sup>-1</sup>.

### **3.3.10 Statistical Analysis**

On-way analysis of variance (One-way ANOVA) was used to test the variable addition of PC on soil carbon, physical, hydrological and chemical attributes, nitrogen species interaction and crop yields in field margin and PC amended soils. Significance level was set to 95% ( $P \leq 0.05$ ) and determined by a *post hoc* test with Tukey's HSD comparison. This procedure was completed using IBM SPSS 25.

Statistical analysis results are displayed in bar charts along with mean values and standard deviation (SD).

### 3.4 Results and Discussion

#### 3.4.1 SOM, TC, TN, and C:N Ratio

SOM measured as loss on ignition (LOI) increased significantly ( $P \leq 0.05$ ) in all PC treatments  $\geq 100 \text{ t ha}^{-1}$ , relative to the field margin soil (**Figure 3.1A**). SOM in the field margin soil was 7.5%, while in the 50, 100, 150 and 200  $\text{t ha}^{-1}$  treatments, SOM contents were 10.5%, 12.8%, 15.4%, and 14.7%, respectively (**Figure 3.1A**). The maximum increase in SOM, observed in the 150  $\text{t ha}^{-1}$  treatment, was 2.1-fold higher than the field margin benchmark; with 150 and 200  $\text{t ha}^{-1}$  treatments showing no significant difference ( $P \geq 0.05$ ) to each other (**Figure 3.1A**).

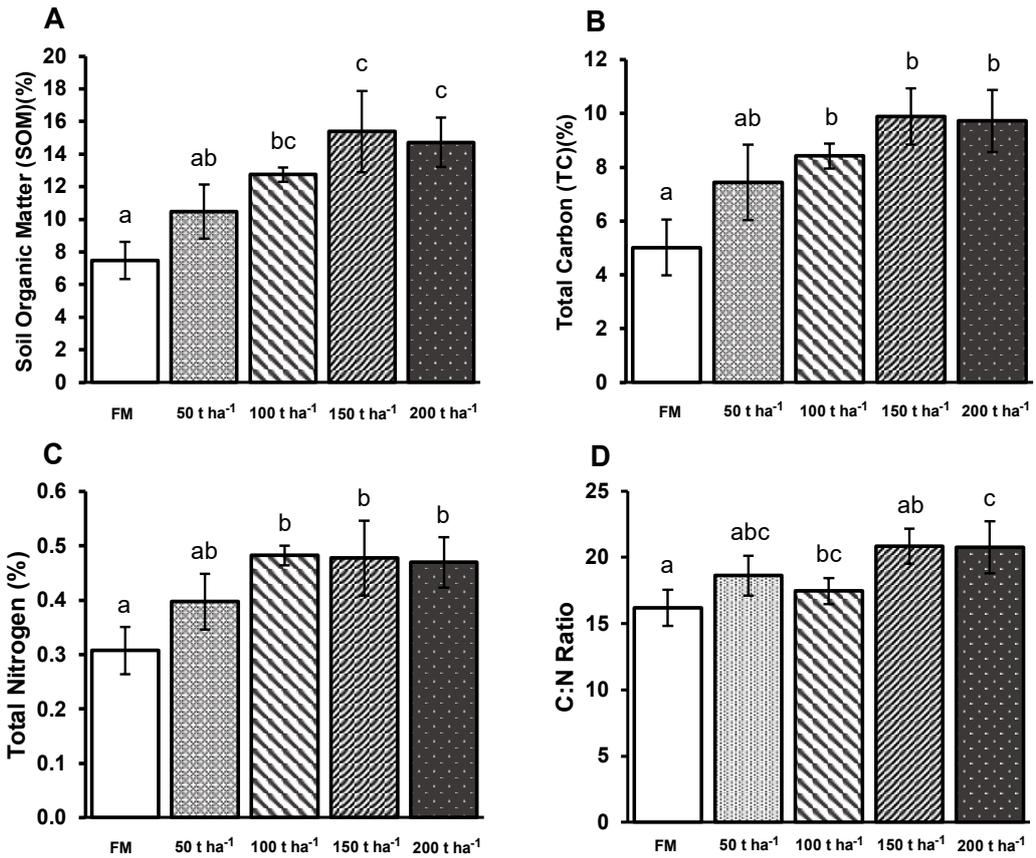
Considering TC (measured by elemental analysis), a similar trend was observed to that of the SOM (**Figure 3.1B**). All PC treatments increased TC content, with these increases being significant ( $P \leq 0.05$ ) in treatment  $\geq 100 \text{ t ha}^{-1}$  (**Figure 3.1B**). TC in the field margin soil was 5.0%, while in the 50, 100, 150 and 200  $\text{t ha}^{-1}$  treatments, TC contents were 7.4%, 8.4%, 9.9%, and 9.7%, respectively (**Figure 3.1B**). As was the case with SOM, TC increases plateaued (with no further significant increase ( $P \geq 0.05$ )) at PC amendment levels of 150 and 200  $\text{t ha}^{-1}$  (**Figure 3.1 A; B**). Increases in TC for each 50  $\text{t ha}^{-1}$  increment up to this plateau were 1.25 % (i.e.  $12.5 \text{ g}_C \text{ kg}^{-1}_{\text{soil}}$ ). Given that the PC used in this investigation had a carbon content of  $244 \text{ g kg}^{-1}$  (**Table 3.1**) an amendment of 50  $\text{t ha}^{-1}$  could theoretically deliver  $1.22 \times 10^7 \text{ g}_C \text{ ha}^{-1}$ . Assuming incorporation of PC to a depth of 5 cm and a soil bulk density of  $0.98 \text{ g cm}^{-3}$  (calculated for the Wallasea series soil using empirical-pedogenic method, the predicted uplift in

TC per 50 t PC applied to 1 ha would be 14.9 g<sub>C</sub> kg<sup>-1</sup>. Thus, the observed TC uplift was in keeping with the expected outcome. Small discrepancies (noted for applications up to 150 t ha<sup>-1</sup>) may be attributed to some decomposition of the PC over the intervening 5-month period between incorporation and sampling. In the high amount (200 t ha<sup>-1</sup>) treatment, the observed plateau in SOM/TC (and divergence for the expected uplift), suggests incomplete incorporation of the amendment with subsequent loss by wind action.

TC contents were converted to C stocks per unit area (assuming an incorporation depth of 5 cm and soil bulk density of 0.98 g cm<sup>-3</sup>). C uplifts, (above the C stock in field margin (24.5 t C ha<sup>-1</sup>)), were: +11.8 t C ha<sup>-1</sup> (50 t ha<sup>-1</sup> PC treatment), +16.7 t C ha<sup>-1</sup> (100 t ha<sup>-1</sup> PC treatment), +24.0 t C ha<sup>-1</sup> (150 t ha<sup>-1</sup> PC treatment) and +23.0 t C ha<sup>-1</sup> (200 t ha<sup>-1</sup> PC treatment).

Total nitrogen (TN) content followed a similar trend to TC (**Figure 3.1C**). Like TC, TN was observed to increase in all PC amendment treatments, with 100, 150 and 200 t ha<sup>-1</sup> treatments reaching a plateau where no significant difference ( $P \geq 0.05$ ) between these treatments was observed (**Figure 3.1C**). TN in the field margin was 0.31%, increasing to 0.40% (in the 50 t ha<sup>-1</sup> PC treatment) and to a maximum of 0.47-0.48% (in the 100 to 200 t ha<sup>-1</sup> PC treatments) (**Figure 3.1C**).

The C:N ratio was higher in all PC treated soils when compared to the field margin (**Figure 3.1D**). However, increases were only significant ( $P \leq 0.05$ ) in treatments  $\geq 150$  t ha<sup>-1</sup> (**Figure 3.1D**). C:N in the field margin was 16:1, increasing to a maximum value of 21:1 in the 150 t ha<sup>-1</sup> treatment. C:N in other PC products previously studied have been reported to range between  $\geq 70:1$  to  $\leq 20:1$  (Foley and Cooperband, 2002a), the C:N (45:1) of PC was central within this range.

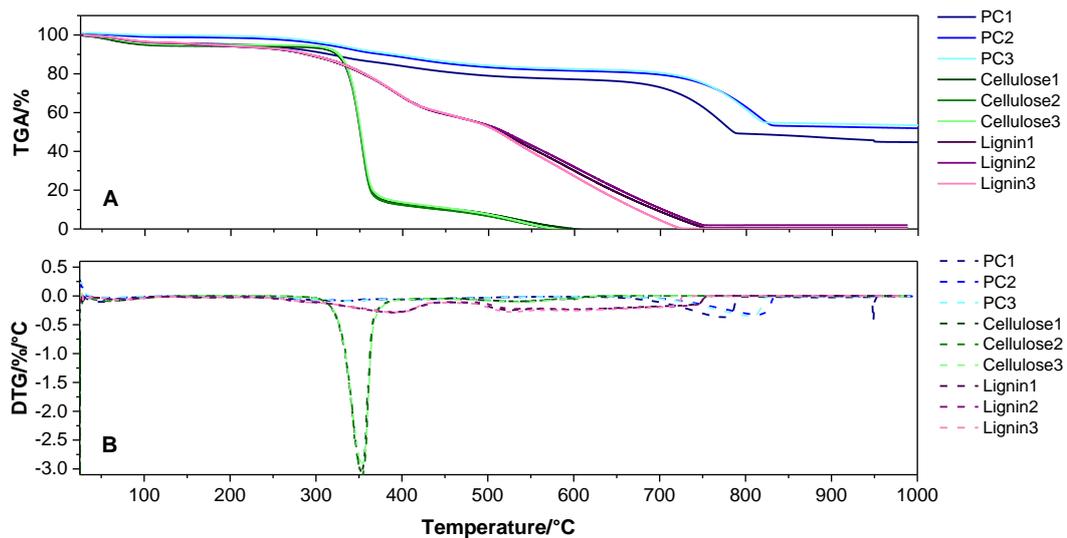


**Figure 3.1** Soil organic matter (A), total carbon (B), total nitrogen (C), C:N ratio (D) in field margin soil (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

### 3.4.2 TGA Carbon Stability Profiling and Long-term Carbon Storage

Cellulose and lignin were used to benchmark the TGA profiles. These components of biomass represent relatively degradable and recalcitrant carbon, respectively (McKendry, 2002). Cellulose is an unbranched glucose polymer, while lignin is highly branched phenolic polymer; as such these different chemical structures influence their relative stability (Lu et al., 2017, Yang et al., 2007). Thermal stability of materials has been related to the biodegradation of different organic matter or organic carbon pools to determine labile carbon (such as cellulose-C) that quickly degrades and stable carbon (such as lignin-C) that decomposes slowly (Plante et al., 2005a, Capel et al., 2006).

By benchmarking temperature zones for attrition of cellulose and lignin, carbon fractions in PC were distinguished in terms of this relative stability. Following initial moisture loss from 25 to 125 °C (Raveendran et al., 1996, Yang et al., 2007), the cellulose sample remained stable until a temperature of 210 °C was reached. Thereafter rapid attrition of cellulose was observed between 210 – 400 °C (**Figure 3.2**). C attrition in this temperature range was ~81.8%. Above a temperature of 400 °C but below 600 °C, a smaller amount of cellulose residue (~13%) was pyrolyzed. All cellulose was pyrolyzed by a temperature of 600 °C. Attrition of lignin was protracted over a wider temperature range (220 – 750 °C) (**Figure 3.2**). As observed with the cellulose sample, initial moisture loss from the lignin occurred between 25 and 125 °C, thereafter the sample remained stable until a temperature of 220 °C was reached. Between 220 - 450 °C steady attrition of lignin was observed, with the maximum mass loss rate (0.29 %/°C) observed at ~390 °C. Thereafter mass loss accelerated slightly between 450 and 750 °C, with the maximum mass loss rate (0.25 %/°C) observed at ~530 °C (**Figure 3.2**). All lignin was pyrolyzed by a temperature of 750 °C. These results conform with previous studies (Rao and Sharma, 1998, Yang et al., 2007, Yaras et al., 2021) and confirm greater thermal stability for lignin over cellulose.



**Figure 3.2.** Thermogravimetric analysis (TGA) curves (A) and derivative thermogravimetric (DTG) curves (B) of paper crumble, cellulose, and lignin (n =3).

TGA profiling of PC, revealed three phases (**Figure 3.2**). The first phase occurred between 25 – 125 °C. This phase was assigned to moisture evaporation (Mendez et al., 2009, Yaras et al., 2021). The second phase occurred between 125 – 700 °C. This second phase was assigned to pyrolysis of organic matter (Mendez et al., 2011). The final phase between 700 – 820 °C was assigned to attrition of inorganic carbonates (Marouani et al., 2019, Mendez et al., 2009). Benchmarking the PC TGA profile against profiles for cellulose and lignin, the organic matter attrition between 150 – 375 °C was assigned to less stable (*labile*) components, while organic matter attrition between 375 – 700 °C was assigned to components of greater recalcitrance (*resistant*). Resulting TGA profiles from this investigation conform with the previous findings of TGA profiles for paper mill wastes and de-inking paper sludge (Mendez et al., 2009, Mendez et al., 2011).

PC had a moisture content of 40% and TC content of 24.4% (**Table 3.1**). Of this TC, 42.5% was associated with the OC fraction (125-700 °C) and 57.5% with the inorganic carbon fraction (700-1000 °C) (**Figure 3.2**). The amounts of labile and resistant carbon

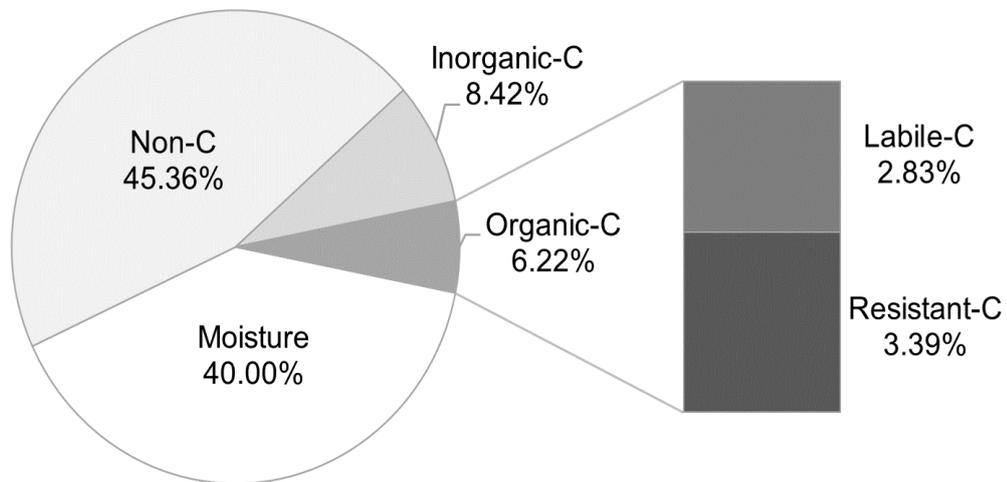
were evaluated to be 28.3 kg ton<sup>-1</sup> and 33.9 kg ton<sup>-1</sup> (on a bulk weight basis) (**Figure 3.3**).

Using these quotients of relatively degradable and relatively recalcitrant carbon, the ROTH-C carbon fate model (Coleman and Jenkinson, 1996) was used to predict long term carbon storage for PC amended to the Wallasea soil series of the field trial (input parameters are provided in **Table SI 3.1**).

The RothC model was modified to predict the carbon storage potential of high carbon soil amendments in previous studies. These modifications introduce additional exogenous organic matter pools, thus allowing the model to accept a range of exogenous organic inputs (e.g. compost, agri-industrial waste and digestate) (Mondini et al., 2017a, Peltre et al., 2012a, Yokozawa et al., 2010a) . Laboratory experiments were conducted to define the size and decomposition rates of addition entry pools in the additional exogenous organic matter pools (Mondini et al., 2017a). In this research, the RothC model was refined to take entry OC pools ascribed by the TGA/DSC profiling as either labile or resistant fractions. The RothC model was subsequently run to provide a prognosis on the longevity of carbon storage under PC amendment scenarios.

Two scenarios were modelled. Scenario 1 considered a single application of 50 t ha<sup>-1</sup> PC in year 0 and the fate of its carbon over the following 50 years. This scenario established short- medium- and long-term organic carbon uplifts (at 10, 25 and 50 years) to be 0.82, 0.48 and 0.36 t ha<sup>-1</sup>, respectively (**Table 3.2**). The residual proportion of PC carbon at 50 years was 12% of the OC mass initially amended. Scenario 2 assumed PC deployment to soil at an application rate of 50 t ha<sup>-1</sup> on a rotational basis (i.e. every 4 years). Using this scenario, the model established the short- medium- and

long-term OC uplifts (at 10, 25 and 50 years) to be 2.85, 5.08 and 6.65 t ha<sup>-1</sup>, respectively (**Table 3.2**).



**Figure 3.3.** Paper crumble composition in terms of moisture, non-carbon, inorganic-carbon, organic-carbon; and labile-carbon/resistant-carbon. Annotated values are % of C in undried “bulk” PC amendment.

**Table 3.2.** Rothamsted carbon (RothC) modelling outputs: organic carbon uplift at 10, 25 and 50 years under two modelling scenarios (single application and 4 yearly application of 50 t ha<sup>-1</sup> PC).

Scenario	Application rate t ha <sup>-1</sup>	OC per 50 t ha <sup>-1</sup> amendment t ha <sup>-1</sup>	Application scheme	OC uplift		
				10 year- t ha <sup>-1</sup>	25 year- t ha <sup>-1</sup>	50 year- t ha <sup>-1</sup>
Scenario 1	50	3.11	Single application	0.82	0.48	0.36
Scenario 2	50	3.11	Quadrennia l application	2.85	5.08	6.65

### 3.4.3 Soil Strength, Penetration Resistance, and Shear Resistance

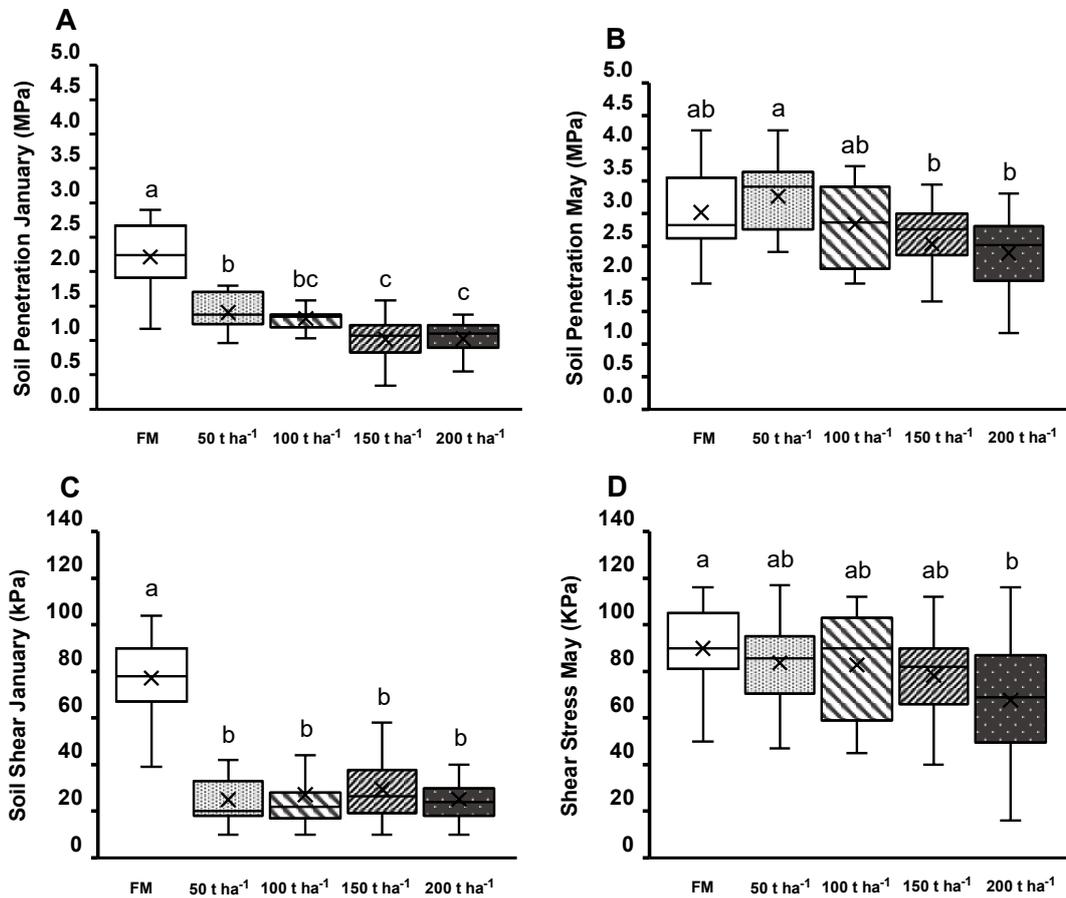
Soil penetration and soil shear resistances were measured *in situ* in both January and May. Management practices that influence soil structure and aggregation facilitate root growth and penetration and air and water storage in soil pores this supporting crop success (Pagliai et al., 2004). Soils with high penetration/shear resistance (and soil bulk density (**Section 3.4.4**)) may limit water infiltration, reduce water availability inhibit the growth of plants due to compaction (Taylor and Brar, 1991, Nawaz et al., 2013).

In January, penetration resistance in the field margin was 2.2 MPa (**Figure 3.4A**). At this time penetration resistance in all of the PC amended soil treatments was significantly lower ( $P \leq 0.05$ ); with values ranging from 1.0 to 1.4 MPa (**Figure 3.4A**). Similarly, soil shear resistance, in January, was much higher in the field margin (79.0 kPa) and significantly different ( $P \leq 0.05$ ) to the values observed in all of the PC amended treatments; wherein soil shear resistance varied from 24.9 to 29.4 kPa (**Figure 3.4C**). No significant differences ( $P \geq 0.05$ ) were observed between the different PC treatments (**Figure 3.4C**). It is likely that the substantial differences observed between the PC treatments and the FM at this stage in the season reflect the effects of soil tillage.

In May, ground conditions were much drier, and both penetration and shear resistance were higher (compared to January) (**Figure 3.4A; B vs. 3.4C; D**). A small (non-significant ( $P \geq 0.05$ )) increase in penetration resistance was observed in the 50 t ha<sup>-1</sup> treatment (3.3 MPa) relative to the FM soil (3.0 MPa) (**Figure 3.4B**). Reductions (not significant ( $P \geq 0.05$ )) in penetration resistance were observed in treatments of  $\geq 100$  t ha<sup>-1</sup> relative to the FM soil (**Figure 3.4B**). A stepwise decrease in soil shear resistance was observed with increased quantities of PC (**Figure 3.4D**). Significant decrease ( $P \leq 0.05$ ) in shear resistance was observed between the FM soil (89.9 kPa) and the 200 t ha<sup>-1</sup> PC treatment (67.6 kPa). No significant differences ( $P \geq 0.05$ ) were observed between the PC treatments (**Figure 3.4D**).

Of the penetration resistances measured in January (**Figure 3.4A**), no soil was observed to exceed the soil compaction threshold for heavy texture soils (2.1-2.5 MPa), suggesting no impediment to propagation of plant roots (Stirzaker et al., 1996, McKenzie et al., 2002). In contrast, all soils measured in May were found to meet or

exceed this limit. It is suggested that this increased soil strength was linked to PC-aggregate binding along with seasonal soil settlement, consolidation, and aggregation (Rajaram and Erbach, 1999, Chantigny et al., 1999).



**Figure 3.4.** Soil penetration, January (A), May (B) and soil shear January (C), May (D) in field margin soil (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). The top and bottom of the box indicates the upper and lower quartiles, the horizontal line indicates the median, the symbol (x) indicates the mean (N = 16 for penetration & N = 24 for shear-vane). Error bars represent SD of the mean. Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

### 3.4.4 Soil Structure, Bulk Density, and Hydrology

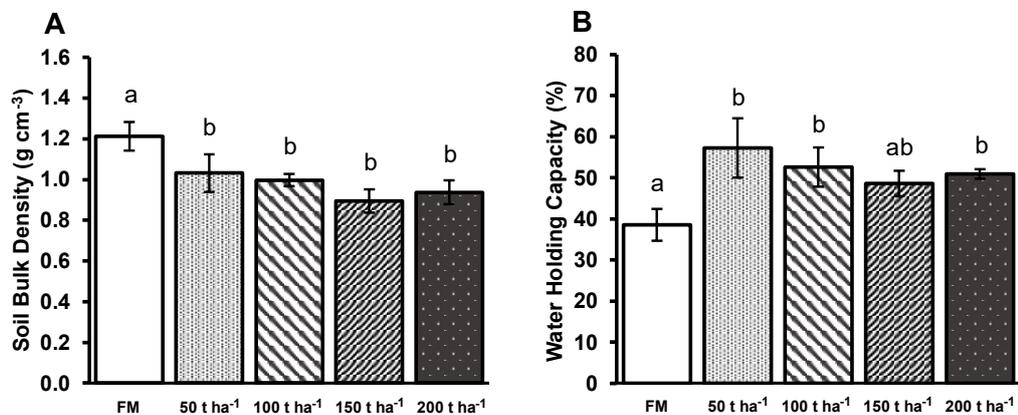
Soil structure, porosity and hydrological properties are underpinned by a variety of interconnecting factors; soil texture, structure, organic matter content, biological activity, moisture content and land management practice (Zibilske et al., 2000, Bormann and Klaassen, 2008). Changes in any of these soil attributes thus exhibit commensurate changes in the physical and hydrological properties of soil. Enhancing

and altering soil physical properties through direct SOM inputs, often leads to soil bulk density (SBD) reductions, in turn providing benefits to water infiltration and WHC (Franzluebbers, 2002). Increasing SOC/SOM contents is an effective method of enhancing soil hydrological properties, where WHC can be increased by 1 – 10 g per 1g SOM (Lal, 2006).

SBD was observed to decrease significantly ( $P \leq 0.05$ ) in all PC treated soils relative to the field margin (**Figure 3.5A**). SBD in the field margin was  $1.21 \text{ g cm}^{-3}$  and decreased to range between  $1.03 \text{ g cm}^{-3}$  ( $50 \text{ t ha}^{-1}$ ) and  $0.89 \text{ g cm}^{-3}$  ( $150 \text{ t ha}^{-1}$ ) (**Figure 3.5A**). No significant differences ( $P \geq 0.05$ ) were observed between different PC treatments (**Figure 3.5A**). The reductions in SBD in the PC treatments are likely due to the direct influence of the low-density of PC ( $0.39 \text{ g cm}^{-3}$ ) “diluting” the denser soil. Aligning SBD observations with soil strength observations, supports proposed interaction mechanism, wherein lower amounts of PC amendment assisted soil particle cohesion (acting as a “glue”) this promoting soil aggregation, enhancing soil porosity and decreasing SBD. In PC treatments exceeding  $100 \text{ t ha}^{-1}$  there were discrete PC zones (visually evident in soil samples), the presence of such zones (varying in diameter  $\sim 0.1 \text{ mm}$  to  $\sim 10 \text{ mm}$ ) may have resulted in a trade-off between PC-facilitated soil-aggregate cohesion and pockets of low-density PC offsetting gains in soil strength but offering zones for enhanced water holding capacity (*see below*).

PC amendment to soil was observed to significantly increase ( $P \leq 0.05$ ) soil WHC in all treatments relative to the field margin benchmark. WHC in the field margin was 38.6 % while WHC in the PC amended soils ranged between 48.6% and 57.3% (**Figure 3.5B**). No significant difference ( $P \geq 0.05$ ) was observed between the different PC treatments (**Figure 3.5B**). Changes in soil WHC linked closely to SBD changes

associated with PC addition, following the same overall stepwise decrease (from 50 to 150 t ha<sup>-1</sup>) and plateau (at 150 and 200 t ha<sup>-1</sup>) (**Figure 3.5**). Such changes may have arisen due to a combination of increased soil aggregation (*in low PC treatments*) and discrete PC zones (PC WHC of 131.4%; **Table 3.1**), these providing pockets where water could be adsorbed within the PC-soil matrix. These results are consistent with previous publications linking enhanced soil aggregate stability and increased water holding capacity (Chantigny et al., 1999, Zibilske et al., 2000, Foley and Cooperband, 2002a, Chow et al., 2003, Gallardo et al., 2012, Powlson et al., 2012a).



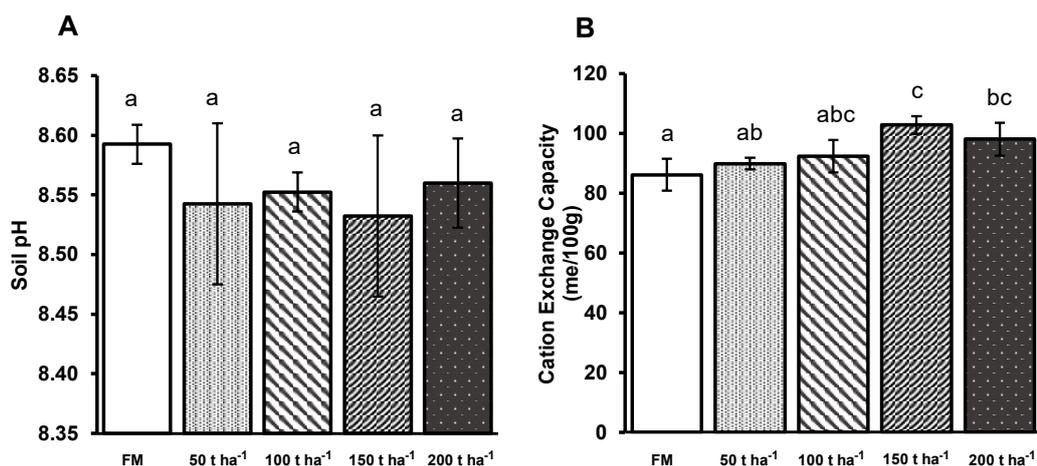
**Figure 3.5.** Soil bulk density (A) and water holding capacity (B) in field margin soil (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

### 3.4.5 pH and Cation Exchange Capacity

Soil pH and CEC are key to regulating chemical functions and thus have a direct effect upon soil fertility, biological activity, and productivity (some essential and trace elements are more or less available in different pH ranges) (Kemmitt et al., 2006, Alam et al., 1999, Hazelton and Murphy, 2007). Soil CEC provides a direct measure of the soils ability to absorb, hold and exchange cations within the soil matrix; these ions support crop growth, and may also assist in buffering soil pH (McCauley et al., 2009, Méndez et al., 2015).

PC had a circumneutral pH (6.94) (**Table 3.1**). Following PC amendment, soil pH generally decreased comparing to the field margin (8.63) but no significant changes ( $P \geq 0.05$ ) were observed, with all soils remaining alkaline in a range between 8.53 – 8.56 (**Figure 3.6A**). Previous studies have reported contrasting changes to soil pH following PC amendment; these contrasting outcomes, in part, are likely related to different pH values for the PC materials (dependant on the process and initial feedstock). Alkaline PC amendments (pH ~8.0) have been reported to increased soil pH (Chantigny et al., 1999, EA, 2005, Rasa et al., 2021b), while circumneutral PC amendments (~7.0) (Gallardo et al., 2012), and acidic PC amendments (~6.7), have been reported to slightly decrease soil pH (Foley and Cooperband, 2002a, Méndez et al., 2015). The results reported here (for circumneutral PC), are consistent with these previous reports for similar pH value PC products. Thus, circumneutral PC has the potential to beneficially reduce the soil pH in alkaline soil; however, long term experiments would be needed to support.

PC addition increased the CEC of all PC amended soils relative to the field margin (86 me/100g); with increase in CEC being significant ( $P \leq 0.05$ ) in the 150 and 200 t ha<sup>-1</sup> treatments (**Figure 3.6B**). CEC in PC amended soil ranged from 90 me/100g (50t ha<sup>-1</sup> treatment) to 103 me/100g (150 t ha<sup>-1</sup> treatment). Significant differences ( $P \leq 0.05$ ) in CEC were observed in the 50 and 150 t ha<sup>-1</sup> treatments while no significant differences ( $P \geq 0.05$ ) were observed between other PC treatments (**Figure 3.6B**). Relative to the field margin increases in CEC ranged between 1.0- and 1.2-fold. PC enhanced CEC was consistent with other studies that have reported increases in the CEC in PC amended soils (Fierro et al., 1999).



**Figure 3.6.** Soil pH (A) and cation exchange capacity (B) in field margin soil (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

### 3.4.6 Elemental Analysis

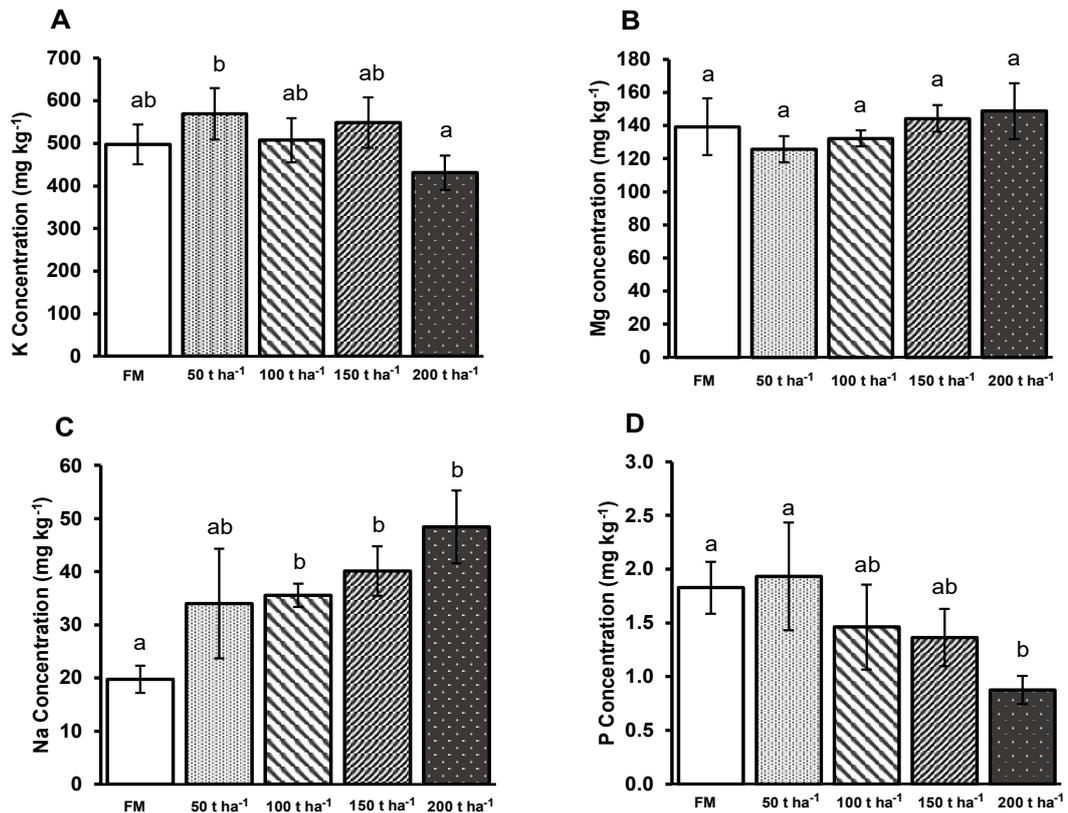
Soils should contain a variety of essential elements in sufficient concentrations to ensure effective uptake and use by plants in a variety of processes (Grusak, 2001). For an element to be considered essential it must be required for completion of the plant life cycle (i.e., underpinning a key metabolic process or function) and may not be entirely replaceable by another element (Grusak, 2001, Kirkby, 2012). 17 essential elements are required for plant growth, with 14 derived from the soil, including the major elements K, Mg P, and trace elements, B, Cu, Mo, Ni, Zn (those measured in this investigation) (Jones and Jacobsen, 2005, Mahler, 2004). Na is essential for some plants and being chemically similar to K it is beneficial when K is limited (Pilon-Smits et al., 2009). The major elements (macronutrients) are often observed in plants in concentrations ≥ 0.1% dry tissue weight, while trace element (micronutrients) concentrations are generally ≤ 0.025% (Grusak, 2001, Jones and Jacobsen, 2005). Alongside the measured major and trace elements, several non-essential and potentially toxic elements (Cr, Cd, Hg, Pb) were measured.

Of the essential major elements only Na and P were significantly influenced ( $P \leq 0.05$ ) by PC amendment.

Stepwise increases in available Na were observed with successive PC amendment, ranging from 1.7 - 2.5-fold (**Figure 3.7C**). Available Na concentrations in the field margin was  $19.7 \text{ mg kg}^{-1}$ , while in the  $50 \text{ t ha}^{-1}$  and  $200 \text{ t ha}^{-1}$  treatments it was  $34.0 \text{ mg kg}^{-1}$  and  $48.4 \text{ mg kg}^{-1}$ , respectively. Due to the high concentration of Na in the PC ( $781 \text{ mg kg}^{-1}$ ) it is likely that the increases observed in the soils are a function of amendment incorporation. Low concentrations of Na in soil can improve the yield of cereal crops, however at high concentrations Na may exhibit plant toxicity (Kronzucker et al., 2013, Rawlins et al., 2012). Soil structure decline and soil permeability decrease occur when the Na concentration exceeds the critical level 5% (Clancy, 2009, Horneck et al., 2007). The highest Na level observed in PC amended soils was 0.005% ( $48.4 \text{ mg kg}^{-1}$ ; **Figure 3.7C**) and is unlikely to cause plant Na stress.

PC had an available P content of  $5.61 \text{ mg kg}^{-1}$  (**Table 3.1**), while the available P in PC treated soils ranged from  $1.93 - 0.87 \text{ mg kg}^{-1}$  (**Figure 3.7D**). A small (non-significant ( $P \geq 0.05$ )) increase in available P was observed in the  $50 \text{ t ha}^{-1}$  PC treatment ( $1.93 \text{ mg kg}^{-1}$ ; field margin  $1.83 \text{ mg kg}^{-1}$ ). For all PC supplied available P this was not translated into increased available P in the treated soils (**Figure 3.7D**). Available P decreased in PC treatments  $\geq 100 \text{ t ha}^{-1}$ . This decrease was significant ( $P \leq 0.05$ ) in the  $200 \text{ t ha}^{-1}$  treatment ( $0.88 \text{ mg kg}^{-1}$ ) (**Figure 3.7D**). Maximum P-availability occurs between pH of 5.5-7.5 (Fernández and Hoef, 2009). Thus, available P delivered in the PC amendment may have been repartitioned due to soil pH being alkaline (8.5 – 8.7 in amended soils); this leading to the P-complexation by calcium ions (Fernández and Hoef, 2009, Siddique and Robinson, 2003). P is essential to plant growth and plays an important

role in energy transfer (Grusak, 2001, Jones and Jacobsen, 2005). While deficiency in P can lead to slow and stunted growth with yield losses (Shenoy and Kalagudi, 2005) in the present research no significant differences were observed in the crop yields (Section 3.4.8).



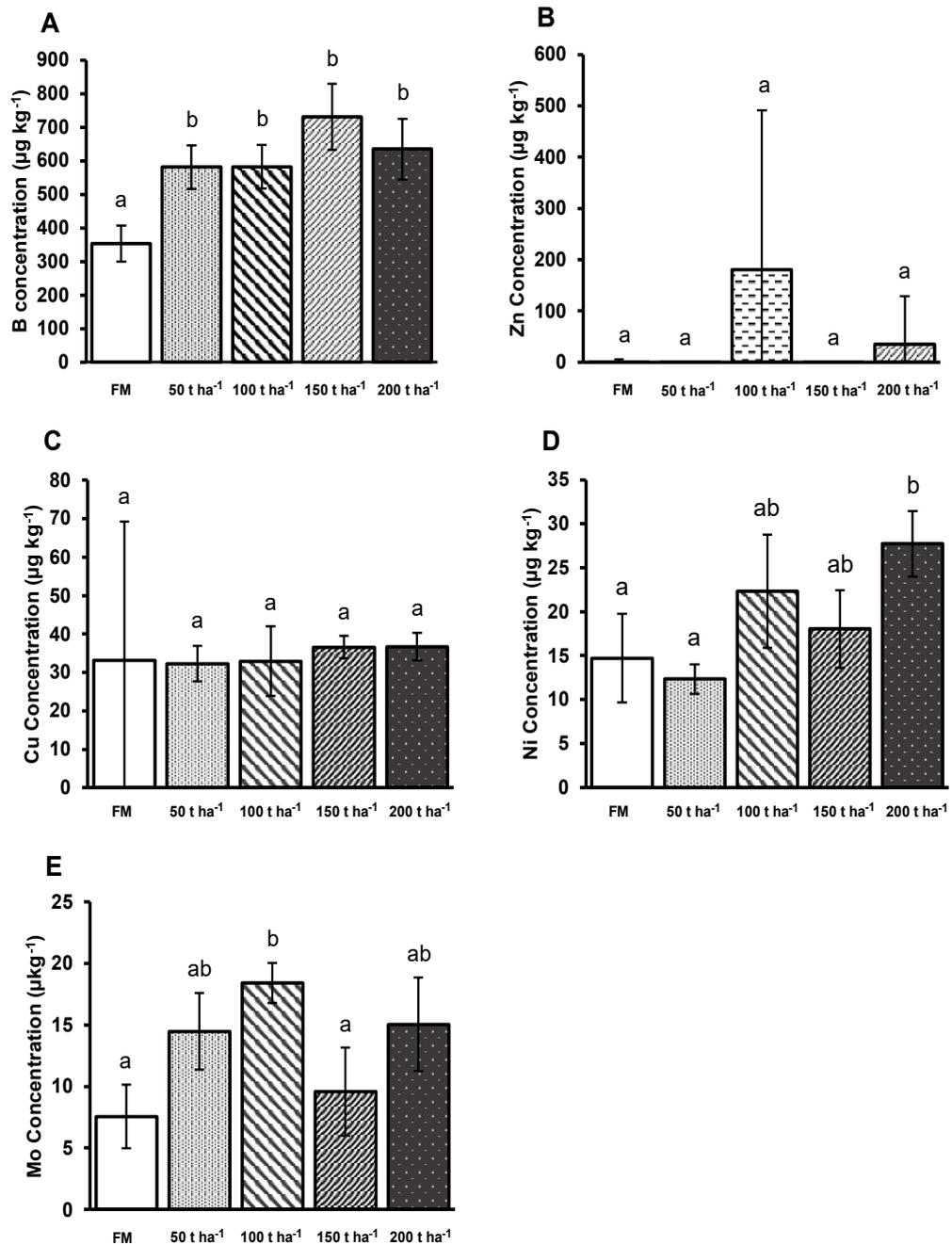
**Figure 3.7.** Essential major elements K (A), Mg (B), Na (C) and P (D) in field margin soil (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

Of the essential trace elements only B and Mo were significantly influenced (P ≤ 0.05) by PC amendment (Figure 3.8A; E).

Available B concentrations significantly increased (P ≤ 0.05) in all PC treated soils relative to the field margin soil (353 μg kg<sup>-1</sup>) and ranged between 582 – 731 μg kg<sup>-1</sup>, a 1.6- to -2.1-fold increase (Figure 3.8A). Uplift in available B concentration was broadly equivalent across all treatments, with the exception of the 150 t ha<sup>-1</sup> amendment rate where the largest available B concentration (731 μg kg<sup>-1</sup>) was observed (Figure 3.8A).

No significant differences ( $P \geq 0.05$ ) were observed between PC treatments (**Figure 3.8A**). B is an essential micronutrient and plays a vital role in creation and maintenance of plant cell walls (Koshiha et al., 2009, Rerkasem and Jamjod, 2004). Deficiency of B is the most wide-spread and frequent micronutrient deficiency (Gupta, 1980, Koshiha et al., 2009). There is a risk of deficiency in B when the concentrations are lower than  $150 - 500 \mu\text{g kg}^{-1}$  (Ahmad et al., 2012). Available B in PC treated soils were noted to be above this threshold (**Figure 3.8A**). Available Mo concentrations increased in all PC treatments relative to the field margin benchmark soil, however a significant increase ( $P \leq 0.05$ ) was only observed in the  $100 \text{ t ha}^{-1}$  treatment (**Figure 3.8E**).

Non-essential elements were below the limit of detection and thus were not significantly influenced ( $P \geq 0.05$ ) by PC amendment, suggesting the PC amendment does not represent a risk with respect to introducing potentially toxic elements to land.



**Figure 3.8.** Essential trace elements B (A), Zn (B), Cu (C), Ni (D) and Mo (E) in field margin soil (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

### 3.4.7 PC Interactions with Fertiliser N-Species

Organic fertiliser can release nitrogen from the time it was applied to as much as several years after application but to be useful to plants nitrogen (N) must be present as either ammonium ion or nitrate ions (Hue and Silva, 2000). Available concentrations

of ammonium in unfertilised soils and PC amended soils were below the limit of detection (**Figure SI 3.1A**). Thus, no changes in ammonium availability were observed in the presence of PC. Low concentrations of nitrate were observed in the unfertilised control soils (22 mg kg<sup>-1</sup>) and unfertilised PC amended soils (20 mg kg<sup>-1</sup>) (**Figure SI 3.1B**). No significant difference ( $P \geq 0.05$ ) was observed where soil only and soil with PC were compared in unfertilised tests. Where soils were equilibrated with fertiliser solution, no significant difference ( $P \geq 0.05$ ) in ammonium availability was observed between the control soils (409 mg kg<sup>-1</sup>) and 50 t ha<sup>-1</sup> PC treatments (444 mg kg<sup>-1</sup>) (**Figure SI 3.1A**). The application of PC (50 t ha<sup>-1</sup>) significantly increased ( $P \leq 0.05$ ) available nitrate (1.1-fold) in the fertilised treatments.

Previous reports have suggested PC to be a nitrogen deficient soil amendment with the potential to “lock-up” nitrogen (Foley and Cooperband, 2002a, Powlson et al., 2012a, Fierro et al., 1999). In contrast to these publications, the present results suggest, for this PC and the Wallasea soil, that PC amendment (50 t h<sup>-1</sup>) was of no detriment to ammonium nor nitrate species added to soil as N-fertilisers.

#### **3.4.8 Crop Yields**

PC addition was found to have no significant ( $P \geq 0.05$ ) effect upon the yield of wheat (**Figure SI 3.2**). Total grain yields varied from 5.69 t ha<sup>-1</sup> (in the 150 t ha<sup>-1</sup> treatment) to 6.28 t ha<sup>-1</sup> (in the 100 t ha<sup>-1</sup> treatment) (**Figure SI 3.2**). This outcome was likely underpinned by the use of agrochemicals throughout the crop cycle to optimise nutrients and suppress pests. These yields are notably low when contextualised, with average UK wheat yield 2000-2020 (DEFRA, 2020a), that range from 6.7 to 9.0 t ha<sup>-1</sup>. It has been reported that optimal wheat grows occur where soil pH is between 6.0 and

7.0 (Vitosh, 1994). Thus, the lower yields observed may in part be attributable to the alkaline pH of the soil used in this experiment.

### **3.4.9 Soil Carbon Uplift, Policy and Carbon Off-setting**

Restoring soil C stocks within agricultural soils, has moved to the forefront of the climate policy agenda in recent years. Championed by programmes such as the '4p1000' and UNFAO RECSOIL initiatives efforts to re-sequester C in soil are being integrated in national policy (Soussana et al., 2019b, Smith et al., 2020b, UNFAO, 2020). For example, in the UK, the 2020 Agriculture Act (DEFRA, 2020a) that seeks to lever increases in soil carbon stocks through a new environmental land management scheme (ELMs). This scheme proposes the use of public money to pay for public goods, including carbon storage, biodiversity net-gains, flood mitigation and climate change adaptation (DEFRA, 2020b, DEFRA, 2020c, Klaar et al., 2020b). Simultaneously, supporting soils to deliver greater C storage will assist in meeting societal obligations under the Paris Agreement, sustainable development goals and Net Zero aspirations (Soussana et al., 2019b, NCC, 2020, Latawiec et al., 2020b).

At a global scale soil recarbonisation offers technical potential for re-sequestration of up to ~5Gt C yearly (Soussana et al., 2019b, Smith et al., 2020b, UNFAO, 2020). It is salient to attaining short term goals that, management interventions such as no/minimum tillage, cover cropping, use of ground cover, land use change avoidance and increased use/application of organic amendments to soil are fully adopted to deliver recarbonisation at low cost and in short timeframes (Lal, 2004b, Powlson et al., 2012a, Soussana et al., 2019b, Smith et al., 2020b, UNFAO, 2020, Keenor et al., 2021).

The research presented herein highlights the significant potential for soil amendments, such as PC, to align with soil recarbonisation aspirations. Herein, modelling the fate of PC carbon, based on its stability profile on a rotational basis (re-application ever 4 years) returned an accrue a total carbon uplift of  $6.65 \text{ t}_{\text{OC}} \text{ ha}^{-1}$  over a 50-year period (**Table 3.2**). In the context of '4p1000' initiative, where an annual uplift of  $4 \text{ g}_{\text{OC}} \text{ kg}^{-1}$  (0.4%) is aspired to, PC accrued carbon over 50 years would be  $\sim 200 \text{ g}_{\text{OC}} \text{ kg}^{-1}$ ; using the same assumptions as those presented in **Section 3.4.2**, this uplift would equate to  $0.9 \text{ t}_{\text{C}} \text{ ha}^{-1}$ . Comparison  $6.65$  vs  $0.9 \text{ t}_{\text{C}} \text{ ha}^{-1}$ , highlights the significant recarbonisation potential of PC amendment.

It is highlighted that the carbon stability profiling coupled to the modelling approach defined long-term stable C (stable to 50 years) associated with a  $50 \text{ t ha}^{-1}$  PC amendment to be  $0.36 \text{ t}_{\text{C}} \text{ ha}^{-1}$  (**Table 3.2**). This is a small portion (11.5%) of the total carbon ( $3.11 \text{ t}_{\text{C}}$ ) entrained in a  $50 \text{ t ha}^{-1}$  PC amendment at time of application. It is highlighted that while this amount of carbon is small, the approach used to define it ensured the quantification of carbon stored with "permanence" (*here 50 years*). The evaluation of C storage "permanence" is fundamentally important to appraising soil recarbonisation strategies. There is little merit in claiming  $\text{CO}_2$  removal from the atmosphere to soil if the prospect of long-term carbon storage is wanting.

Results presented indicate that an average farm, in the East of England of 120 ha (with 79 % as arable) (Defra, 2021), would achieve an OC uplift (associated with a 4-year rotational application of PC;  $50 \text{ t ha}^{-1}$ ) equate to  $630 \text{ t C}$  (equivalent to  $2310 \text{ t CO}_2\text{e}$ ). At time of writing the unit price of soil C has not been equilibrated in the formal carbon market, however, other carbon off-sets have been tested under market forces

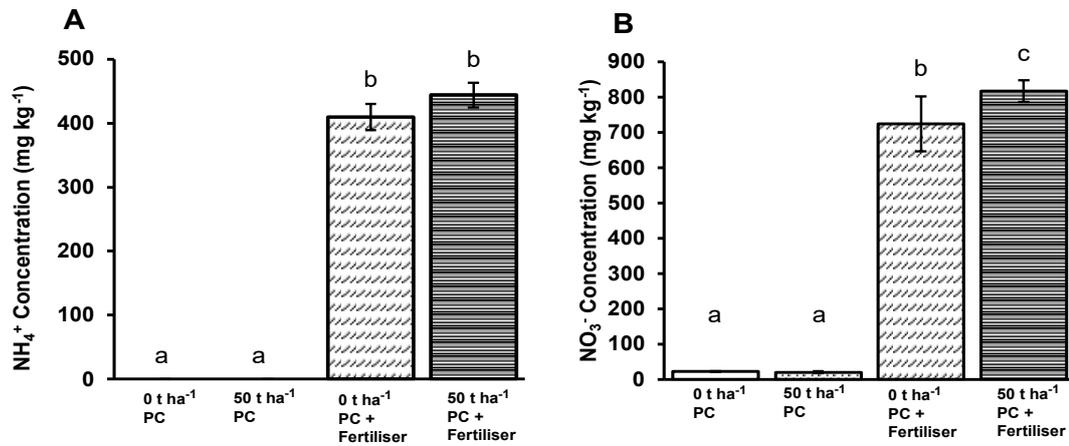
for many years (i.e., the EU emissions trading scheme (EU ETS) (European Commission, 2015)). At the time of writing the EU ETS market price for 1 t CO<sub>2</sub>e was EUR €95 (February 2022; having increased from EUR €40 per 1 t CO<sub>2</sub>e the same time of the previous year) (EU Carbon Permits, 2022). Applying this carbon price of €95 per 1 t CO<sub>2</sub>e to the calculated uplift of 2310 t CO<sub>2</sub>e, the value of carbon sequestration could be €219,450 (equivalent to €46 ha<sup>-1</sup> y<sup>-1</sup>). Conflating the estimated PC resource in the UK ~1Mt (CPI, 2014) with its long-term stable carbon quotient (0.0072 t<sub>C</sub> t<sub>PC</sub><sup>-1</sup>), yields 7200 t<sub>C</sub>; equivalent to 26,400 t CO<sub>2</sub>e of permanent storage. At EUR €95 t CO<sub>2</sub>e this long-term carbon storage could potentially leverage an off-set value of €2.5m p.a.

### 3.5 Conclusion

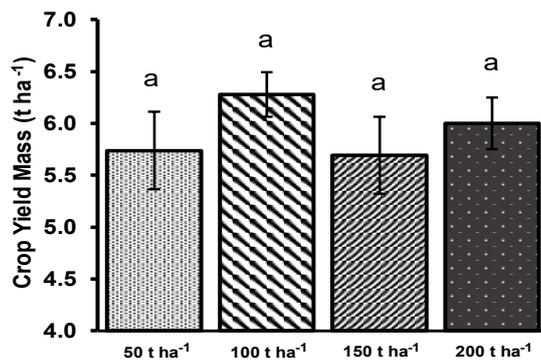
Protection and recovery of soil carbon stocks is of paramount importance to sustaining productive agriculture, improving food security, and more broadly to improve the delivery of ecosystem services (Power, 2010, Latawiec et al., 2020b, Doran, 2002, Adhikari and Hartemink, 2016b). PC, as detailed herein, has the potential to make a significant contribution to soil recarbonisation in terms of both uplift per unit area and, given the amount of PC resource available (e.g. in the UK ~1Mt (CPI, 2014)), at a meaningful scale. While PC was not observed to increase crop yield in this research (*likely due to high levels of agricultural intervention*) it may afford benefits to crops where soil fertility is lower and agricultural inputs more restricted. Results reported herein support such a premise, in so much as they indicate significant benefits to soil nutrient concentrations, CEC, soil bulk density and WHC. Further research to explore the influence of PC on soil fertility in low management intensity systems, regenerative agriculture systems and

across a range of soil types and geographies is recommended to broaden understanding of PC influence on soils.

### 3.6 Supporting Information



**Figure SI 3.1.** Ammonium (NH<sub>4</sub><sup>+</sup>; A) and nitrate (NO<sub>3</sub><sup>-</sup>; B) concentrations in unamended soils and PC amended (50 t ha<sup>-1</sup>) soils both in the absence (white) and presence (grey) of augmented ammonium nitrate fertiliser. Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).



**Figure SI 3.2.** Crop yield mass in field margin (FM) and PC treated soil (50, 100, 150 and 200 t ha<sup>-1</sup>). Error bars represent SD of the mean (n = 4). Bars that share a lower-case letter are not significantly different (P ≥ 0.05).

**Table SI 3.1.** Input parameters for carbon modelling.

<b>Soil conditions</b>												
Modelling site	Estuary Farm, King's Lynn, UK (52°46'46.0"N 0°24'08.8"E)											
Soil clay content (%)	48											
Soil sample depth (cm)	5											
<b>Land management data</b>												
C input	Determined by the two scenarios											
Soil cover	Soil is covered with vegetation every month											
<b>Weather data</b>												
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Monthly air temperature (°C)*	4.7	4.9	6.5	9	12.2	15.1	17.4	17.1	14.9	11.7	7.7	5.3
Monthly rainfall (mm)*	60	49	51	52	57	66	64	68	64	69	67	61
Monthly open pan evaporation (mm)**	14.7	18.7	37.3	60.0	90.7	125.3	148.0	134.7	101.3	62.7	30.7	20.0

\* Monthly average air temperature (°C) and average rainfall (mm) were obtained for King's Lynn (1999 – 2019) from Climate-Data.org (2021).

\*\* Open-pan evaporation (mm) data was not available. Therefore, monthly potential evapotranspiration (PET) was obtained from the Müller's collection (Müller, 2012) using Cromer (52°56'N 1°17'E) as the most similar site to King's Lynn. PET values were converted to open-pan evaporation by dividing by 0.75 (Coleman and Jenkinson, 2014b).



**Chapter 4:**  
**Influence of Paper Crumble on Soil Hydrology  
and Soil Carbon Stocks**

#### 4.1 Abstract

This research, a field experiment undertaken for a drought prone sandy soil, evaluated the influence of paper crumble (PC; a carbon rich soil amendment (ca. 235 g C kg<sup>-1</sup>)), on soil bulk density (BD), water holding capacity (WHC), infiltration rate, and soil organic carbon content (SOC). PC was amended to soil at doses of 50, 100 and 200 t ha<sup>-1</sup>. PC treatment significantly ( $p \leq 0.05$ ) increased WHC under all treatments 1 year post amendment (17.3% in the 200 t ha<sup>-1</sup> treatment); and significantly increased ( $p \leq 0.05$ ) in the 200 t ha<sup>-1</sup> treatment in year 3 (18.1%). Furthermore, significant ( $p \leq 0.05$ ) improvement in infiltration rates (more than double under all PC treatments), and significant ( $p \leq 0.05$ ) increases in SOC content under treatments of 100 and 200 t ha<sup>-1</sup> both 1 year and 3 years post amendment were observed. Given a soil bulk density of 1.60 g cm<sup>-3</sup> and the total increase in SOC stock after 3 years, the 200 t ha<sup>-1</sup> application delivered 64.8 t CO<sub>2</sub>e ha<sup>-1</sup> of carbon sequestration uplift and increase water storage capacity by more than 5100 L ha<sup>-1</sup>. These results highlight the opportunity for PC to deliver substantial hydrological improvements to sandy soils while concurrently increasing soil carbon stocks, offering synergistic wins for climate change mitigation and improved food security.

#### 4.2 Introduction

From food to fuels and fibres, climate and water regulation, to carbon sequestration, the contribution of soils to global ecosystem services and to sustaining the world around us is enormous (de Groot et al., 2002b, Dominati et al., 2010, Power, 2010, Lal, 2013, Adhikari and Hartemink, 2016b, Latawiec et al., 2020b). Fundamental to the provision of these services are soil carbon stocks and soil hydrological function

(Vereecken et al., 2022). Well managed hydrologically effective soils exhibit resilience to extreme weather conditions; buffering the environment and providing resistance to erosive pressures and surface water run off by effectively absorbing, storing, conserving, and releasing water (Garbowski et al., 2023); while soil carbon helps provide structure and underpins the provision of myriad soil functions and services (Adhikari and Hartemink, 2016b, Keenor et al., 2021). Together, these properties of soil improve resilience and support the provision of a positive water balance between periods of rainfall and under drought conditions, continuing to provide water to vegetation and crops and minimising yield losses (Lal, 2006).

However, as a consequence of rapid changes in the climate and intensification of agricultural practices, soils have been subjected to, and remain at significant risk of damage and degradation, greatly impairing soil productivity and ecosystem service provision as a result of reduced hydrological function and soil carbon loss (Lal, 2001, Pimentel, 2006, Smith, 2008, Gregory et al., 2015, Pereira et al., 2018, Bateman and Muñoz-Rojas, 2019).

Damaged soils presently represent more than 20% of the total global soil resource, with further degradation estimated in the order of 5-10 million hectares per year (Stavi and Lal, 2015, Bateman and Muñoz-Rojas, 2019). Furthermore, this is estimated to translate to direct emission of more than 176 Gt C to the atmosphere as a result of soil carbon destabilisation and decomposition, with a further 36 Gt C by 2050 (IPBES, 2018b, Lal, 2018b). Such declines perpetuate and reinforce damage within the soil environment (Lal, 2015), weakening structural and aggregate stabilities (Lal, 2004c, Stavi and Lal, 2015), and aggravating the impairment of soil hydrological functions and susceptibility to erosive pressures (Pimentel, 2006, Lal, 2015). Furthermore, soils

depleted in SOM/SOC require increased water and nutrient input to maintain crop yields raising barriers to food security in a variety of geographical and climatic contexts (Lal et al., 2004, Lal, 2009).

In contrast, soils rich in organic matter, sustain greater structural stability, aggregation and cohesion (Abiven et al., 2009b); thus imparting substantial benefit to the infiltration and water holding capacities of soils (Franzluebbers, 2002, Lal, 2020b). Even marginal increases (e.g. 1%) in soil organic matter content have been reported to greatly assist in improving water storage capacities (Libohova et al., 2018, Lal, 2020b). As such, improving soil hydrological properties and soil carbon stocks are of vital importance to environmental resilience, enhancing agricultural productivity, soil health and fertility and mitigating the effects of climate change (Lal, 2009).

Adequate and resilient soil water regulation is especially important in the present and near future; given the context of: i) continued population growth and socio-economic improvements, with much of this growth projected in water/food insecure regions (Lal, 2006, Sposito, 2013); ii) increased frequency of extreme weather due to climate change: where severe drought, and flooding events may become more frequent (Porporato et al., 2004, Trenberth, 2011, Reidmiller et al., 2017); and/or iii) where environmental/pedological contexts mean soil moisture potential is a limiting factor in crop production (e.g. drought/flood prone soils) (Lal, 2004c, Lal, 2009). With the propensity for both too much and too little water to cause issues, it is imperative that soils be managed in such a way that improvements in soil structure, hydrological function and carbon stocks are realised.

Adopting land management strategies that seek to enhance soil carbon content and hydrological function, will simultaneously aid in protecting soils from further

degradation, enhance the provision of ecosystem services and soil sustainability, and improve food security (Lal, 2006, Smith et al., 2016). Where this can be achieved alongside enhancements in other soil properties (e.g. soil carbon uplift), opportunity arises to reverse soil degradation and mitigate climate change (through recarbonisation of soils) (Latawiec et al., 2020b, Keenor et al., 2021). Furthermore, such efforts may help to meet United Nations Sustainable Development Goals (SDGs) such as zero-net land degradation and the United Nations Convention to Combat Desertification (Stavi and Lal, 2015, Bateman and Muñoz-Rojas, 2019).

One approach to improve soil hydrological properties and rejuvenate soil carbon stocks is the application of organic matter rich soil amendments. Such amendments hold potential to: facilitate changes to soil bulk density, improve of water holding capacity and infiltration rates, sequester and store carbon, and improve essential nutrient availability in soils (Lal, 2014, Mao et al., 2022, Leuthold et al., 2023). These benefits contribute to enhancing soil function and improving overall soil health, quality and fertility (Zebarth et al., 1999, Tejada et al., 2009, Lal, 2014, Mao et al., 2022, Garbowski et al., 2023, Leuthold et al., 2023).

A variety of different organic matter soil amendments have been investigated with respect to their potential for improving soil carbon content and enhancing soil hydrological properties, including but not limited to: compost, biochar, digestate, paper waste and manures (Chantigny et al., 1999, Lal, 2004c, Lima et al., 2009, Diacono and Montemurro, 2011, Powlson et al., 2011, Powlson et al., 2012b, Omondi et al., 2016, Mao et al., 2022, Rivier et al., 2022, Garbowski et al., 2023). With the incorporation and use of amendments, such as compost, being observed to significantly improve soil water infiltration and water holding

capacities, retaining up to 80% of rainfall and reducing run off by 60%, simultaneously improving long term water storage and reducing soil erosion (Faucette et al., 2007, Garbowski et al., 2023).

Furthermore, once conventional wastes, which would be diverted to landfill or incinerated, may be repurposed as agricultural amendments, helping to minimise the loss of nutrients from the agricultural/environmental system and close nutrient cycles while also reducing greenhouse gas emissions in the process (Diacono and Montemurro, 2011, Amundson et al., 2015, Eden et al., 2017, Zhu et al., 2017).

Paper crumble (PC), the focus of this investigation, has been previously evaluated for its ability to enhance soil physical and hydrological properties, through the improvement of soil bulk density and creation of soil aggregate structures (Chantigny et al., 1999, Zibilske et al., 2000, Abiven et al., 2009b), and significantly enhance soil carbon stocks in both the short- and long-term, improving soil function and fertility, and sequestering carbon (Powlson et al., 2012b, Mao et al., 2022). Furthermore, organic matter rich amendments, including PC, have been observed to minimise surface water run-off and associated erosion, while increasing soil water holding capabilities (Zibilske et al., 2000, Foley and Cooperband, 2002b, Powlson et al., 2012b, Rasa et al., 2021a); with such benefits being of particular importance in drought prone soils, or those at risk of degradation.

To evaluate the efficacy of PC, for enhancing soil hydrological function, and improving soil organic carbon (SOC) stocks, a field experiment was conducted using variable rates of PC addition (0 to 200 t ha<sup>-1</sup>). Soils were evaluated with respect to, soil bulk density (BD), soil water holding capacity (WHC), water infiltration rates and soil

carbon content (SOM/SOC). Subsequently this data was used to estimate potential increases in soil water storage capacities and soil carbon stocks and sequestration.

### 4.3 Materials and Methods

#### 4.3.1 Field Experiment

The field experiment was established on a 10 ha field at Lexham Estate (Lexham, Norfolk, UK; 52° 43' 29" N, 0° 44' 37" E). The soil type was Newport 4 Series (loamy sand to sandy loam texture, moderately stoney, and well drained) (UKSO, 2024). The PC amendment discussed within this report provided by Palm Paper Ltd (King's Lynn, Norfolk, UK), derived from recycled newsprint, and is considered a type 1 paper crumble (*non-virgin, de-inked*) (**Table 1**). This product undergoes treatment with bio-digestate and ink sludge reintroduction post processing, enriching the product in nitrogen and incorporating clay (*kaolinite*) into the final product. Further evaluation of the PC product with respect to elemental analysis can be found in Mao et al. (2022) (**Chapter 3**). The trial was established with 12 (36 x 36m) grids in 2 columns on the east and west sides of the field, leaving a 36m untreated buffer between these strips; additionally, an 18m untreated buffer was established between the north-south axis of the grids (Figure SI 4.1). PC was applied to fields (August 2020) using a Bunnings' Spreader and then incorporated to a depth of c. 5 cm by culti-pressing and flat-lifting the soil (Figure SI 4.2;4.3).

**Table 4.1:** PC Properties: Bulk Density, Moisture Content, WHC, SOM, SOC, Total Nitrogen, C:N, and pH, (n = 10; Mean ± SD).

Parameter	Unit	Value
<b>Bulk Density</b>	g cm <sup>-3</sup>	0.39 ± 0.01
<b>Moisture Content</b>	%	36.8 ± 2.1
<b>WHC</b>	%	131 ± 10.8
<b>SOM</b>	% dry mass	29.9 ± 0.3
<b>SOC</b>	% dry mass	23.5 ± 1.3
<b>Total N</b>	% dry mass	0.5 ± 0.1
<b>C:N</b>	dimensionless	45 : 1
<b>pH</b>	dimensionless	7.8 ± 0.1

Field measurements and samples were collected prior to PC addition (August 2020), then subsequently 1 year (August 2021) and 3 years (August 2023) post amendment; assessments were made post-harvest and prior to soil cultivation. Crop rotations were rye (2020/21), vining peas (2021/22), potatoes (2022), and winter wheat (2022/23). Soil core samples (from which soil bulk density, soil moisture, water holding capacity, SOM/SOC were measured), were collected at consistent sample points (located using GPS) pre amendment (2020) and post amendment in years 1 and 3. Soil infiltration data were collected only in year 1.

#### 4.3.2 Soil Sampling and Soil Bulk Density

In all three sampling years soil core samples (7.5 cm depth, 8.8 cm Diameter; n = 18), were obtained using a soil Dent Corer, and subsequently sieved (2mm) in the laboratory to remove stones and gravel (accounted for in soil bulk density calculations). Core samples were subsequently dried (74°C for 24h) and soil bulk density calculated. Dry soil samples were then sealed and retained in cold storage (≤ 4°C) prior to further analysis.

### **4.3.3 Soil Organic Matter**

SOM content was measured by *loss on ignition* (ISO 10694: (1995b)); where dried soil (10 g; n = 18) was placed in a crucible, and then transferred to a furnace (470 °C for 24 h), removed and subsequently re-weighed. SOC content was measured by dry combustion in an elemental analyser (Exeter CHNS analyser), from approximately 15 mg of sample (n = 18).

### **4.3.4 Soil Hydrological Attributes**

Soil field moisture (n = 18) was determined from drying cores obtained from the field (sieved 2 mm) (dried 74 °C for 24h). WHC (n = 18) was assessed by placing sieved (2 mm) soil (20 g) in a Whatman No.1 filter paper in filter funnel and saturating the soil with distilled water. Samples were allowed to drain under gravity until release of water had stopped. Moisture content of the soil was determined (dried 74 °C for 16 h). Infiltration measurements were obtained using a Decagon Mini-Disk Infiltrometer following manufacturers guidelines (METERGroup, 2021a) (n = 24). Measurements were obtained 4 days after a rain event (*ca.* 25 mm (August 2021)). The soil surface was cleared of litter and the top 1 cm of soil removed to provide uniform surface for the infiltrometer to interface with. A measurement interval of 30 seconds and a total elimination of 25 ml was used to ascertain infiltration rate, (suction set to 4 cm), to minimise excess loss of water to large soil pores or voids arising from soil arrangement, texture and bioturbation/biopores (METERGroup, 2021a). Calculations were subsequently carried out using the software and guidance provided by Decagon Devices (METERGroup, 2021b).

### 4.3.5 Statistical Analysis

Significant differences (ANOVA; IBM SPSS 25) was determined using a *post hoc* test with Tukey's HSD, data significance set to 95% ( $P \leq 0.05$ ). ANOVA was used to determine the significance of both intra-year data, comparing the effect of the different PC amendment rates at a given time; and inter-year data, comparing the effects of PC amendment rates over time relative to the pre-amendment control soil. Extreme outliers were representing 3x the interquartile range  $\pm$  the upper/lower quartile were removed (Barbato et al., 2011).

## 4.4 Results and Discussion

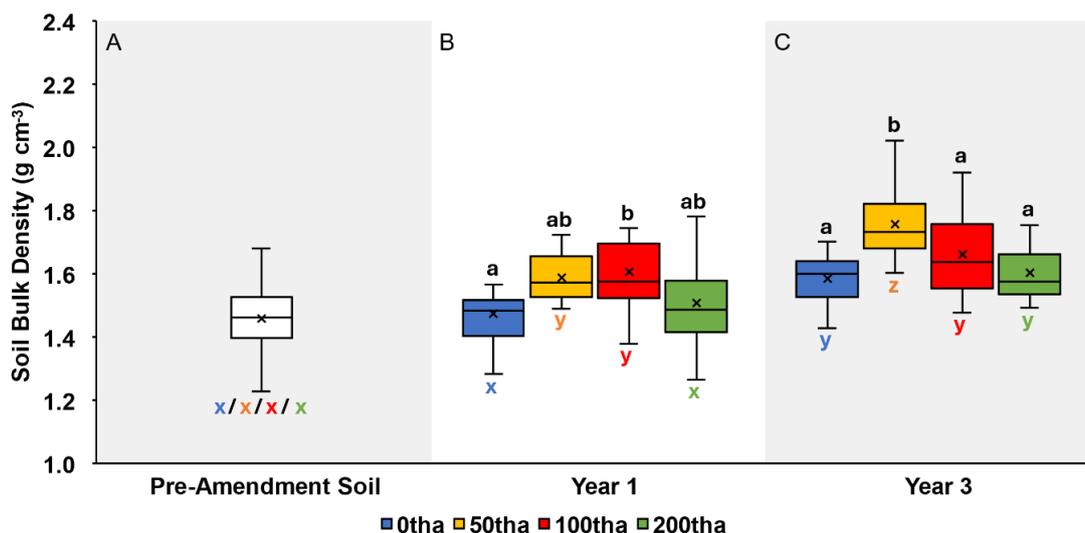
### 4.4.1 Soil Bulk Density

One year following PC amendment, soil bulk density (SBD) was observed to increase under all PC doses relative to the control; however, this increase was only significant ( $p \leq 0.05$ ) in the 100 t ha<sup>-1</sup> treatment (increasing from 1.47 g cm<sup>-3</sup> to 1.61 g cm<sup>-3</sup>) (**Figure 4.1B**). No significant differences ( $p \geq 0.05$ ) were observed between the PC treatments in the year 1 data (**Figure 4.1B**). Additionally, 3 years post amendment, SBD was also observed to increase under all PC treatments relative to the control; however, this increase was only significant ( $p \leq 0.05$ ) in the 50 t ha<sup>-1</sup> treatment (increasing from 1.58 g cm<sup>-3</sup> to 1.76 g cm<sup>-3</sup>) (**Figure 4.1C**). Furthermore, the 50 t ha<sup>-1</sup> treatment was observed to have a significantly higher ( $p \leq 0.05$ ) SBD than all other PC treatments (**Figure 4.1C**). The observed increases in SBD in both years 1 and 3 suggest the incorporation of PC influenced soil aggregation and structural cohesion, in line with previous observations (Chantigny et

al., 1999, Zibilske et al., 2000, Abiven et al., 2009b, Mao et al., 2022); potentially enhancing resistance to erosion and improving the flow and storage of water as a result of soil structural changes (Franzluebbers, 2002, Basso et al., 2013). However, commensurate reductions in SBD following amendment with organic matter were not observed.

When compared year to year for a given treatment regime, some significant differences ( $p \leq 0.05$ ) were observed (**Figure 4.1 A-C**). Between the control soils and the unamended soil SBD increased significantly ( $p \leq 0.05$ ) with time in the year 3 soil, relative to both the pre-amendment and year 1 values (rising from  $1.46 \text{ g cm}^{-3}$  in the pre amendment soil, to  $1.47 \text{ g cm}^{-3}$  in year 1 soil, and to  $1.58 \text{ g cm}^{-3}$  in year 3 soil) (**Figure 4.1 A-C**). At applications of  $50 \text{ t ha}^{-1}$  PC, SBD increased stepwise and significantly ( $p \leq 0.05$ ) in each subsequent year (rising from  $1.46 \text{ g cm}^{-3}$  in the pre-amendment soil, to  $1.59 \text{ g cm}^{-3}$  in year 1, and to  $1.76 \text{ g cm}^{-3}$  in year 3) (**Figure 4.1 A-C**). At application of  $100 \text{ t ha}^{-1}$  PC, SBD increased significantly ( $p \leq 0.05$ ) in both year 1 and year 3 relative to the pre-amendment soil (from  $1.46 \text{ g cm}^{-3}$  in the pre-amendment soil, to  $1.61 \text{ g cm}^{-3}$ , to  $1.66 \text{ g cm}^{-3}$  respectively) (**Figure 4.1 A-C**). However, there was no significant difference ( $p \geq 0.05$ ) in SBD between the year 1 and year 3 soils. At application rates of  $200 \text{ t ha}^{-1}$  small increases in SBD were observed in both year 1 and year 3 relative to the pre-amendment soil. However, this increase was only significant ( $p \leq 0.05$ ) of the year 3 soil (rising from  $1.46 \text{ g cm}^{-3}$  in the pre-amendment soil to  $1.60 \text{ g cm}^{-3}$  in the year 3 soil) (**Figure 4.1 A-C**). Despite increases in SBD, this remained below limiting levels to both crop root expansion and water ingress in all soils (below  $1.8 \text{ g cm}^{-3}$  in sandy soil) (Shaheb et al., 2021, Kaufmann et al., 2010, Chaudhari et al., 2013). The observed increases in

SBD likely related to PC mediated soil cementation and binding, as a consequence of PC-aggregate surface coating and the in-filling of soil pores with fine PC particles, as previously observed by Chantigny et al. (1999) and Mao et al. (2022). This observed binding effect was especially significant ( $p \leq 0.05$ ) at PC doses of 50 – 100 t ha<sup>-1</sup>, while at doses of 200 t ha<sup>-1</sup> greater opportunity for discrete PC only zones to form within may have subsequently exerted the opposite effect, weakening soil structural and aggregate strength due to reduced adhesion and reducing the overall size of this effect (Mao et al., 2022). Additionally, further cultivation and the resultant spreading of the PC over the proceeding years may have provided a more conducive environment for PC-aggregate binding and soil pore infill, (potentially mediated by the presence of both high organic matter content, and the kaolinite clay in the PC amendment), ultimately reducing discrete PC only zones, leading to the uplifts in SBD measured in year 3 relative to the year 1 soil (**Figure 4.1 B; C**). However, it is highlighted that the changes observed in year 3 SBD were not limited to solely the PC amended soil, and thus may be (*or also*) related to soil settlement and restructuring following the complete soil cultivation required by the potato crop (Kimble et al., 2000, Grandy et al., 2002).



**Figure 4.1:** Soil bulk density ( $\text{g cm}^{-3}$ ) (2020  $n=72$ ; 2021/2023  $n=18$ ) of soil; pre amendment (A), 1 year post amendment (B), and 3 years post amendment (C), with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values ( $3\times$  interquartile range  $\pm$  upper quartile/lower quartile, respectively). Black letter values shown above the bars indicate the outcomes of significance testing (ANOVA) for data in each frame (A/B/C). Coloured letter values shown below the bars indicate the outcomes of significance testing (ANOVA) for the data between frames based on the same PC treatment dose (A Vs. B Vs. C). Bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

#### 4.4.2 Soil Hydrology

PC applications were observed to enhance WHC and infiltration rates of treated soils, as well as increasing the *in-situ* field soil moisture content. Uplifts in soil hydrological capacities were especially strong in the short term (1 year post amendment), and at higher dose rates in the longer term (3 years post amendment).

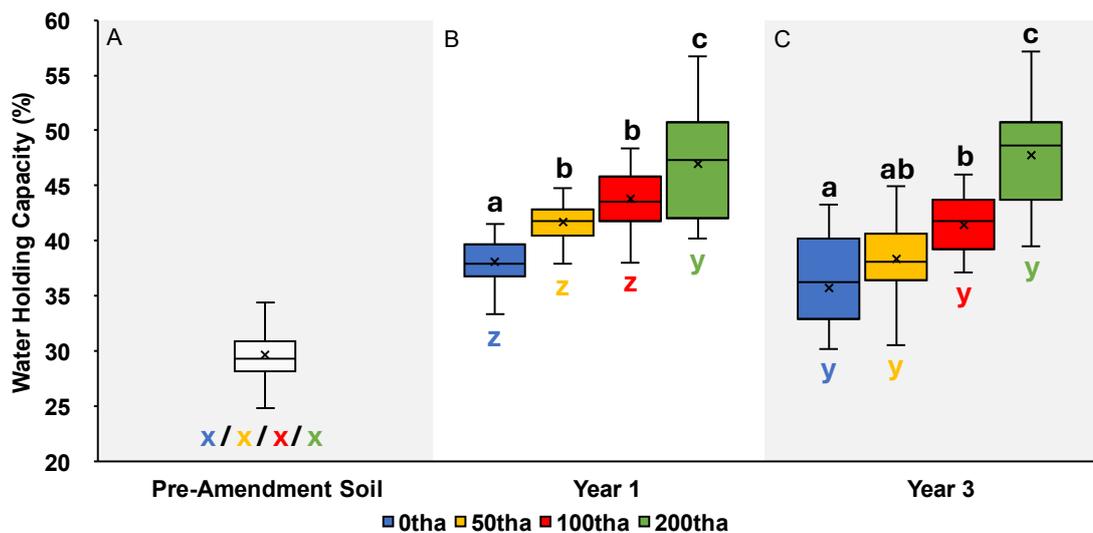
One year post amendment, WHC was observed to increase stepwise with increasing PC dose, with significant increases ( $p \leq 0.05$ ) in all PC treatments relative to the control soil (increasing from 38.1% in the control, to 41.7%, 43.8%, and 46.9% in the 50  $\text{t ha}^{-1}$ , 100  $\text{t ha}^{-1}$ , and 200  $\text{t ha}^{-1}$  PC treated soils, respectively) (**Figure 4.2 B**). Furthermore, treatments with 200  $\text{t ha}^{-1}$  PC dose had significantly greater ( $p \leq 0.05$ ) WHC than the other PC application doses (**Figure 4.2 B**).

Similarly, 3 years post amendment, WHC was observed to increase stepwise with increasing PC dose, with significant increases ( $p \leq 0.05$ ) observed in both  $100 \text{ t ha}^{-1}$  and  $200 \text{ t ha}^{-1}$  treatments relative to the control (increasing from 35.7% to 41.4% and 47.7% respectively) (**Figure 4.2 C**). Additionally, the  $200 \text{ t ha}^{-1}$  PC treatments had measured significantly greater ( $p \leq 0.05$ ) WHC than the other PC application levels (**Figure 4.2 C**).

When compared year to year for a given treatment regime, WHC was seen to increase significantly ( $p \leq 0.05$ ) in all PC treatments relative to the pre-amendment soil (**Figure 4.2 A-C**). Additionally, significant differences ( $p \leq 0.05$ ) were observed between the year 1 and year 3 WHC (**Figure 4.2 B; C**): with significantly greater ( $p \leq 0.05$ ) WHC was observed in treatments of 50 and  $100 \text{ t ha}^{-1}$  in the year 1 samples relative to the year 3 samples. At applications of  $200 \text{ t ha}^{-1}$  WHC was found to be broadly congruent with no significant differences ( $p \geq 0.05$ ) between the year 1 and year 3 samples (**Figure 4.2 B; C**). WHC of the control soils increased significantly ( $p \leq 0.05$ ) relative to the pre-amendment soil (rising from 29.6% in the pre-amendment soil, to 38.1% and 35.7% in years 1 and 3 respectively (**Figure 4.2 A-C**)). Given the lack of PC input to the control soils, it is likely that the observed changes in WHC over time related to both the crop rotation (with rye straw incorporation post-harvest in year 1), and the subsequent soil cultivation and structural changes.

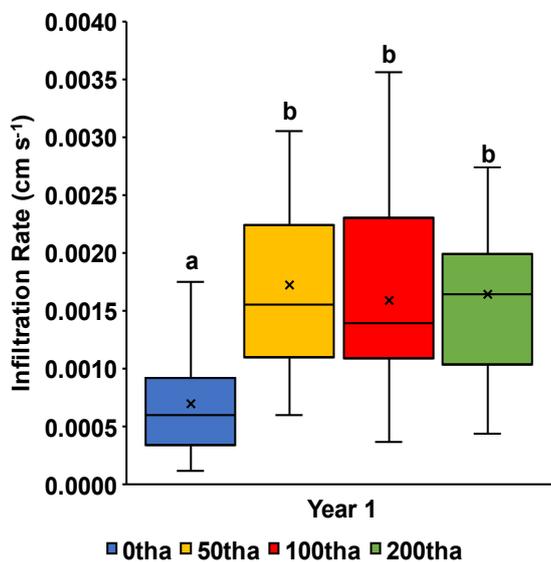
Soil field moisture content offered an appreciation of the in-field water holding capacity under field conditions. Soil moisture content initially followed the same trend as WHC: with increase in moisture content under all PC doses relative to the control soil in year 1 but was only significant ( $p \leq 0.05$ ) at a dose of  $200\text{-t ha}^{-1}$

(rising from 6.7% to 11.0% respectively)) (**Figure SI 4.4 A**). In year 3, soil moisture measured initial reductions (not significant ( $p \geq 0.05$ ) in the 50 and 100 t ha<sup>-1</sup> doses relative to the control, before significant ( $p \leq 0.05$ ) increase in the 200 t ha<sup>-1</sup> treatment (from 11.7% to 14% respectively) (**Figure SI 4.4 B**). No inter-year comparisons were made for soil moisture content due to prevailing weather and climate differences between sample collection dates rendering such comparisons arbitrary. The similarity in data trends between WHC (**Figure 4.2**) and soil moisture (**Figure SI 4.4**), highlight that the uplifts in WHC translate to *in-situ* outcomes, supporting the use of PC as an effective method of increasing soil water storage (**Section 4.4.3**). Such improvements increased soil moisture content by 4.3% and 2.3% (after 1 year and 3 years respectively) (**Figure SI 4.4 A; B**).



**Figure 4.2:** Soil water holding capacity (%) (2020 n= 72; 2021/2023 n=18) of soil; pre amendment (A), 1 year post amendment (B), and 3 years post amendment (C), with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values ( $3 \times$  interquartile range  $\pm$  upper quartile/lower quartile, respectively). Black letter values shown above the bars indicate the outcomes of significance testing (ANOVA) for data in each frame (A/B/C). Coloured letter values shown below the bars indicate the outcomes of significance testing (ANOVA) for the data between frames based on the same PC treatment dose (A Vs. B Vs. C). Bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

Water infiltration rates were observed to increase significantly ( $p \leq 0.05$ ) under all PC treatment doses, substantially enhancing the rate at which water ingress occurred, and improving the ability of the soil to collect and absorb water before surface run off occurs (Vereecken et al., 2022). Infiltration rates more than doubled under all PC treatments relative to the control: increasing from  $0.0007 \text{ cm s}^{-1}$  in the control, to  $0.0017 \text{ cm s}^{-1}$  in the  $50 \text{ t ha}^{-1}$  treatment, and  $0.0016 \text{ cm s}^{-1}$  in the  $100 \text{ t ha}^{-1}$  and  $200 \text{ t ha}^{-1}$  treatments, respectively (**Figure 4.3**). No significant differences ( $p \geq 0.05$ ) in infiltration rate were observed between the different PC treatments



**Figure 4.32:** Infiltration Rate ( $\text{cm s}^{-1}$ ) ( $n= 24$ ) of 2021 soil samples, with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values ( $3 \times$  interquartile range  $\pm$  upper quartile/lower quartile, respectively). Black letter values shown above the bars indicate the outcomes of significance testing (ANOVA) between the data, bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

(**Figure 4.3**), suggesting that increases in the PC dose rate, while enhancing the overall water holding capacities, offers no further benefit to water infiltration. As such, lower dose PC treatments may be optimal as a cost effective method of increasing infiltration in soils subject to high precipitation loads but that may not be at risk of drought – potentially offering benefits to soil erosion mitigation

(Vereecken et al., 2022).

### 4.4.3 Soil Water Storage Potential

Improvements observed to the soil hydrological properties support the findings of prior study and evaluation of PC amendments, and furthermore offer potential to improve soil resilience and resistance to adverse conditions (by reducing surface water runoff and associated erosion as a consequence of amendment addition) (Chantigny et al., 1999, Zibilske et al., 2000, Foley and Cooperband, 2002b, Powelson et al., 2012b, Rasa et al., 2021b, Mao et al., 2022).

Enhancement of the soil water holding capacity and infiltration rate can significantly improve soils' ability to collect and retain water during heavy precipitation events and increase the water storage potential in times of drought (Williams et al., 2016) (both predicted to increase in many temperate regions as a result of climate change (Porporato et al., 2004, Trenberth, 2011)). Even marginal increases in water storage offer potentially significant improvements: increasing water availability to crops by 5-10 days as a result of SOM increase (Lal, 2006). Indeed, potentially available water content in the soil of the 200-t ha<sup>-1</sup> PC treatment 3 years post amendment, was calculated to increase by approximately 5100 L ha<sup>-1</sup> (at a depth of 30 cm) relative to the control soil (increasing from 17,800 L ha<sup>-1</sup> to 22,900 L ha<sup>-1</sup>) following a 2.18% increase in organic matter content (**Section 4.4.4**). Although, it must also be considered that the introduction of kaolinite clay through amendment with PC may have conferred some level of hydrological benefit. However, given the positive correlations between SOC and WHC were evident in both year 1 and year 3 ((year 1) gradient = 0.42 ; r<sup>2</sup> = 0.73); ((year 3) gradient = 0.55 ; r<sup>2</sup> = 0.44) (**Figure SI 4.5; Section 4.4.2 and 4.4.4**); thus, it is likely overall enhancement of soil hydrological properties was primarily

underpinned by commensurate increases in soil carbon (**Section 4.4.4**) (Libohova et al., 2018, Lal, 2020b).

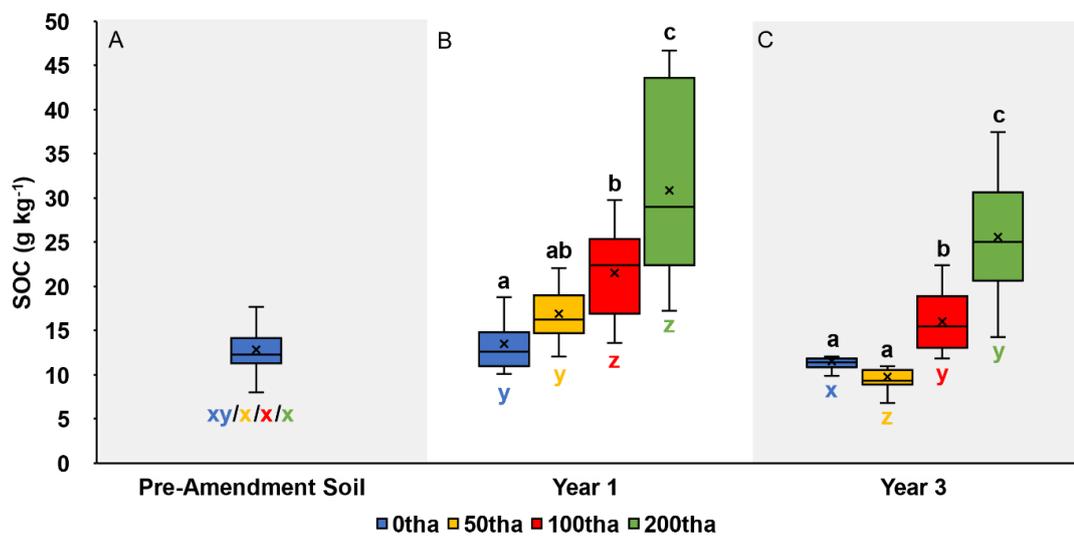
#### **4.4.4 Carbon Sequestration**

SOM/SOC increased commensurately with increasing PC dose, with greater influence in the short term (1 year post amendment) and under higher PC treatment doses (200 t ha<sup>-1</sup>).

One year post amendment SOC was observed to increase stepwise with increasing PC dose; rising (not significantly ( $p \geq 0.05$ )) from 13.4 g kg<sup>-1</sup> in the control to 16.9 g kg<sup>-1</sup> in the 50 t ha<sup>-1</sup> PC treatment, then significantly ( $p \leq 0.05$ ) to 21.5 g kg<sup>-1</sup> in the 100 t ha<sup>-1</sup> PC treatment, and significantly again ( $p \leq 0.05$ ) to 30.9 g kg<sup>-1</sup> in the 200 t ha<sup>-1</sup> PC treatment (**Figure 4.4 B**). 3 years post amendment, SOC was observed to initially decrease (not significantly ( $p \geq 0.05$ )) from 11.4 g kg<sup>-1</sup> in the control to 9.7 g kg<sup>-1</sup> in the 50 t ha<sup>-1</sup> PC treatment, before then increasing stepwise (significantly ( $p \leq 0.05$ )) with each additional PC dose to a total of 25.5 g kg<sup>-1</sup> in the 200 t ha<sup>-1</sup> PC treatment (**Figure 4.4 C**).

When compared year to year for each given PC treatment regime SOC was observed to decrease significantly ( $p \leq 0.05$ ) with time under all PC treatment doses (**Figure 4.4 A-C**). Between the pre-amendment soil and control soils, SOC increased (not significantly ( $p \geq 0.05$ )) in year 1, from 12.8 g kg<sup>-1</sup> C to 13.4g kg<sup>-1</sup> C and decreased to 11.4 g kg<sup>-1</sup> C by year 3, significantly ( $p \leq 0.05$ ) below that of the year 1 soil, but not significantly different ( $p \geq 0.05$ ) to the pre-amendment soil (**Figure 4.4 A-C**). Initial increases observed to the untreated control soil in year 1 (and also potentially affecting all PC treated soils) may have been due to the influence of the

crop rotation, where a crop of rye had been grown over the preceding year, and once harvested the stubble and waste straw had been re-incorporated into the soil during cultivation, likely replenishing soil carbon within the soil in the short term. Under all treatments of PC, SOC was observed to decrease significantly ( $p \leq 0.05$ ) between year 1 and year 3: from 16.9 g kg<sup>-1</sup> C to 9.7 g kg<sup>-1</sup> C in the 50 t ha<sup>-1</sup> treatment; from 21.5 g kg<sup>-1</sup> C to 16.1 g kg<sup>-1</sup> C in the 100 t ha<sup>-1</sup> treatment; and from 30.9 g kg<sup>-1</sup> C to 25.5 g kg<sup>-1</sup> C in the 200 t ha<sup>-1</sup> treatment (**Figure 4.4 A-C**).



**Figure 4.4:** SOC (g kg<sup>-1</sup>) (2020 n= 72; 2021/2023 n=18) of soil; pre amendment (A), 1 year post amendment (B), and 3 years post amendment (C), with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values (3x interquartile range  $\pm$  upper quartile/lower quartile, respectively). Black letter values shown above the bars indicate the outcomes of significance testing (ANOVA) for data in each frame (A/B/C). Coloured letter values shown below the bars indicate the outcomes of significance testing (ANOVA) for the data between frames based on the same PC treatment dose (A Vs. B Vs. C). Bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

SOM was observed to follow a similar trend to SOC, increasing stepwise with PC dose, with significant ( $p \leq 0.05$ ) increases observed in the 200 t ha<sup>-1</sup> treatment relative to the control one year post amendment, and significant increases ( $p \leq 0.05$ ) measured in both treatments of 100 and 200 t ha<sup>-1</sup> PC 3 years post amendment (**Figure SI 4.6 B; C**). When compared year-to-year for a given treatment regime, SOM was observed to decrease with time (Significantly ( $p \leq 0.05$ )) in the control, and (not significantly ( $p \geq$

0.05)) in the 50 t ha<sup>-1</sup> treatment; and increase (not significantly ( $p \geq 0.05$ )) under the higher PC doses of 100 t ha<sup>-1</sup> and 200 t ha<sup>-1</sup> (**Figure SI 4.6 A-C**). Furthermore, the increases in SOM/SOC likely contributed to the changes observed with respect to the improvement and function of soil hydrological properties (**Section 4.4.2**), where increases in SOC were observed to correlate with increases in WHC (**Section 4.4.3; Figure SI 4.5**).

When converting SOC to a t ha<sup>-1</sup> basis, acknowledging the influence of soil BD and the volume of soil (0 – 7.5 cm in depth) per hectare, the resultant carbon stocks reflected the same trend of that for the data presented as g kg<sup>-1</sup> (**Figure 4.5 A-C**). One year post amendment SOC increased stepwise with increasing PC dose, these increases were significant ( $p \leq 0.05$ ) in both the 100 t ha<sup>-1</sup> and 200 t ha<sup>-1</sup> treatments relative to the control, increasing from 16.02 t C ha<sup>-1</sup> to 27.43 t C ha<sup>-1</sup> and 36.47 t C ha<sup>-1</sup> respectively (**Figure 4.5 B**). 3 years post amendment, PC was observed to increase SOC significantly ( $p \leq 0.05$ ) under treatments of 100 t ha<sup>-1</sup> and 200 t ha<sup>-1</sup> relative to the control, from 14.51 t C ha<sup>-1</sup> to 21.61 t C ha<sup>-1</sup> and 32.61 t C ha<sup>-1</sup> respectively. However, a decrease (not significant ( $p \geq 0.05$ )) was observed under a treatment of 50 t ha<sup>-1</sup> PC, reducing to 13.15 t C ha<sup>-1</sup> (**Figure 4.5 C**).

When compared year to year, SOC was measured to decrease under all PC treatments from year 1 to year 3, with significant ( $p \leq 0.05$ ) decreases in the 50 t ha<sup>-1</sup> and 100 t ha<sup>-1</sup> applications and a non-significant decrease ( $p \geq 0.05$ ) in the 200 t ha<sup>-1</sup> treatment (**Figure 4.5 B; C**). Additionally, a total decrease in SOC (not significant ( $p \geq 0.05$ )) was also observed between the pre-amendment soil and the 50 t ha<sup>-1</sup> PC treated soil of year 3. However, SOC was subsequently observed to increase

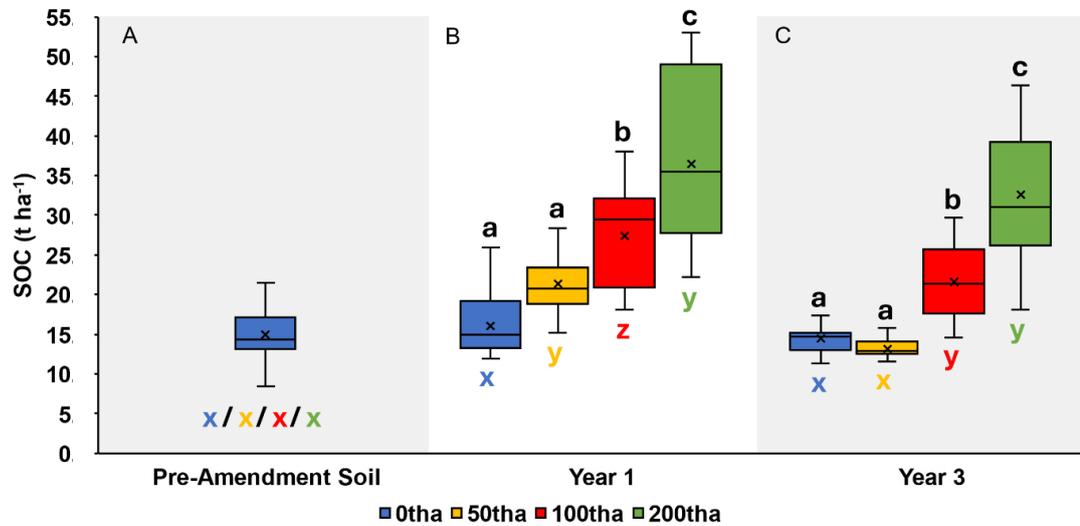
(significantly ( $p \leq 0.05$ )) in the 100 t ha<sup>-1</sup> and 200 t ha<sup>-1</sup> PC treated soils relative to the pre-amendment control (**Figure 4.5 A; C**).

PC applications introduced additional carbon to the soil in amounts of 7.71, 15.42 and 30.84 t C ha<sup>-1</sup> in the 50, 100 and 200 t ha<sup>-1</sup> PC treatments respectively, leading to total carbon contents (immediately post amendment) of 14.91, 22.62, 30.33, and 45.75 t C ha<sup>-1</sup> in the control, 50, 100, and 200 t ha<sup>-1</sup> PC treatments (**Table 4.2**).

Over the 3-year period SOC was observed to decrease (not significantly ( $p \geq 0.05$ )) in the control soil by 0.4 t C ha<sup>-1</sup>, or a reduction of 2.68% relative to the pre-amendment soil carbon content (**Table 4.2; Figure 4.5 A; C**). In the soils treated with PC, SOC was observed to decrease (not significantly ( $p \geq 0.05$ )) in the 50 t ha<sup>-1</sup> PC dose (by 1.76 t C ha<sup>-1</sup>, or a reduction of 11.8%); and increase significantly ( $p \leq 0.05$ ) in treatment doses of 100 t ha<sup>-1</sup> PC (by 6.70 t C ha<sup>-1</sup>, or increase of 44.9%), and 200 t ha<sup>-1</sup> PC (by 17.7 t C ha<sup>-1</sup>, or increase by 119%) when compared with the pre-amendment soil (**Table 4.2; Figure 4.5 A; C**).

Thus, as a consequence of PC addition 3 years post amendment, carbon stocks were doubled in the 200 t ha<sup>-1</sup> treatment (increasing from 14.9 t C ha<sup>-1</sup> to 32.6 t C ha<sup>-1</sup>) (**Table 4.2; Figure 4.5 A; C**) and equating to a total carbon sequestration value of 64.8 t CO<sub>2</sub>e ha<sup>-1</sup>. This result highlights the significant potential for PC to deliver considerable carbon sequestration outcomes in the short term. When benchmarked against other carbon stock increases reported for alternative soil amendments PC was observed to perform well. With PC delivering 17.7 t C ha<sup>-1</sup> uplift, compared to farmyard manure (7.6 t C ha<sup>-1</sup>), biosolids (22.7 t C ha<sup>-1</sup>), sewage sludge (16.4 t C ha<sup>-1</sup>) and green waste compost (7.6 t C ha<sup>-1</sup>) (Powlson et al., 2012b)

(assuming 200 t ha<sup>-1</sup> amendment application and moisture content equivalent to that of PC).



**Figure 4.5:** SOC stock (t ha<sup>-1</sup>) (2020 n= 72; 2021/2023 n=18) of soil; pre amendment (A), 1 year post amendment (B), and 3 years post amendment (C), with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values (3x interquartile range ± upper quartile/lower quartile, respectively). Black letter values shown above the bars indicate the outcomes of significance testing (ANOVA) for data in each frame (A/B/C). Coloured letter values shown below the bars indicate the outcomes of significance testing (ANOVA) for the data between frames based on the same PC treatment dose (A Vs. B Vs. C). Bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

**Table 4.2:** PC carbon amendment and resultant soil carbon stocks in PC amended soil.

	0 t ha	50 t ha	100 t ha	200 t ha
<b>Pre-amendment Soil Carbon Stock (t C ha<sup>-1</sup>)</b>	14.9	14.9	14.9	14.9
<b>Total PC Addition (Dry Weight) (t ha<sup>-1</sup>)</b>	0.00	31.6	63.2	126
<b>Total PC Carbon Addition (t C ha<sup>-1</sup>)</b>	0.00	7.71	15.4	30.8
<b>Post Amendment Soil Carbon Stock (t C ha<sup>-1</sup>)</b>	14.9	22.6	30.3	45.8
<b>3-year Soil Carbon Stock (t C ha<sup>-1</sup>)</b>	14.5	13.2	21.6	32.6
<b>Total 3-year Carbon Uplift (%)</b>	-2.68	-11.8	44.9	119

## 4.5 Conclusion

The addition of PC to sandy soil was observed to significantly improve soil hydrological function and increase soil carbon stocks. Overall, PC applications were observed to enhance the soil moisture, WHC and infiltration rates of treated soils and significantly increase soil carbon stocks. This was particularly true 1 year post amendment, where all PC treatments were observed to enhance water holding capacity (significantly ( $p \leq 0.05$ )), soil moisture content (significant ( $p \leq 0.05$ ) with treatments of  $200 \text{ t ha}^{-1}$ ); infiltration rates (significantly ( $p \leq 0.05$ )), and soil carbon content (significant ( $p \leq 0.05$ ) with treatments of  $100 \text{ t ha}^{-1}$  and  $200 \text{ t ha}^{-1}$ ); and at treatment rates of  $200 \text{ t ha}^{-1}$ , maintaining significantly ( $p \leq 0.05$ ) improved hydrological capacities and carbon uplifts up to 3 years post amendment. Indeed, 3 years post amendment, treatment with  $200 \text{ t ha}^{-1}$  PC afforded a carbon sequestration potential of  $64.8 \text{ t CO}_2\text{e ha}^{-1}$ , and improved potential water storage by  $5100 \text{ L ha}^{-1}$ .

Furthermore, the use of soil amendments such as PC aligns with broader regenerative or holistic agricultural practices, aimed at reducing chemical inputs, enhancing soil organic matter, and promoting a shift toward sustainable agriculture principals. As such, PC represents a valuable tool in the pursuit of sustainable land management and food security in a changing climate.

However, it must be appreciated that there are a range of different PC products, derived from variable types of paper feedstocks, and are treated with variable manufacture and recycling processes. Much like the range of different compost amendments available, paper crumble is not one homogenous amendment – as such, consideration must be given to these additional factors (i.e. clay fraction content) with regards to the influence this might exert on soil textural and hydrological properties

alongside the influence of PC organic matter fractions. Therefore, in addition to the required further study regarding the influence of PC amendments upon the soil health, soil process and long-term carbon storage; comparison of these different types of PC, with respect to their intrinsic properties, must also be considered. With research in both areas needed to further elucidate and evaluate the holistic influence of PC soil amendments for improving soil ecosystem services and the environment more broadly.

## 4.6 Supporting Information



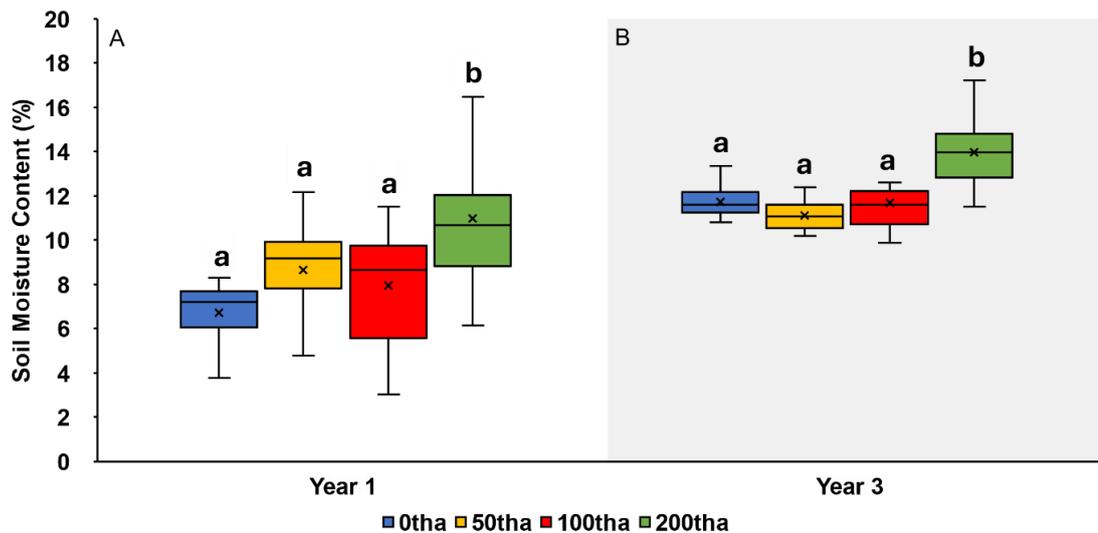
**Figure SI 4.1:** Lexham Estate farm trial field site ((Lexham, Norfolk, UK; 52° 43' 29" N, 0° 44' 37" E).), annotated with defined paper crumble (PC) spreading zones, in quantities of 0, 50, 100, and 200 t ha<sup>-1</sup> PC amendment, and buffer strips across the sample site.



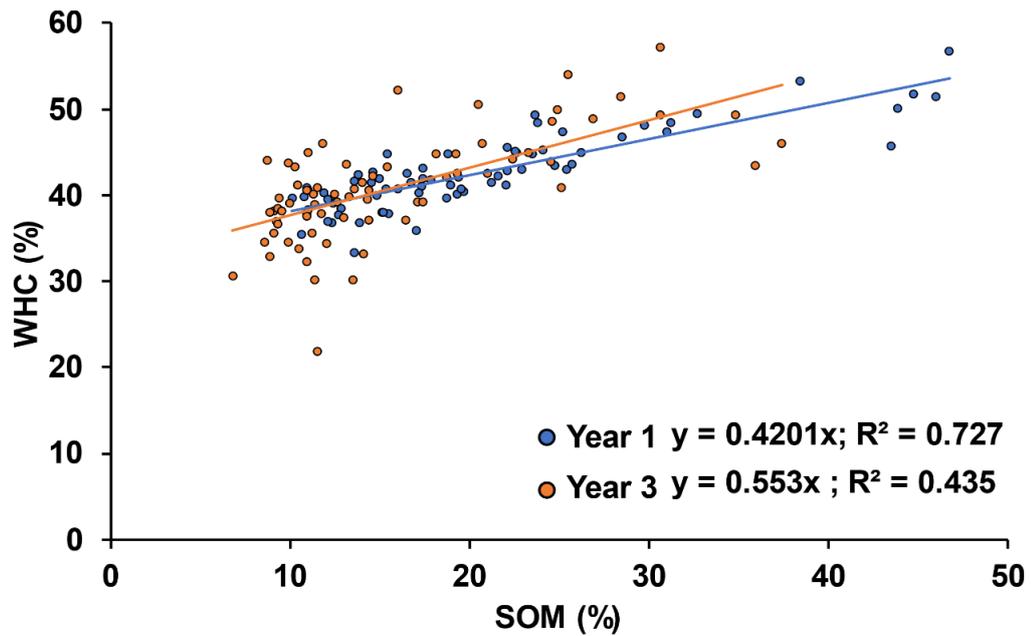
**Figure SI 4.2:** Paper crumble (PC) soil amendment being deployed to the field site ready for spreading (August 2020)



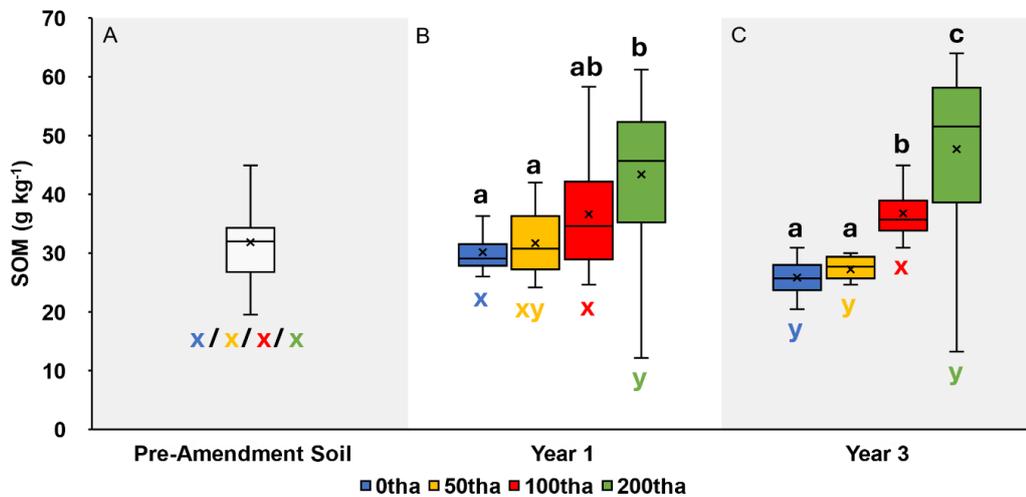
**Figure SI 4.3:** Paper crumble (PC) soil amendment spread at a rate of 200 t ha<sup>-1</sup> to the trial field (August 2020).



**Figure SI 4.4:** Soil Moisture Content (%) (2020 n = 24; 2021/2023 n=18) of soil; 1 year post amendment (A), and 3 years post amendment (B), with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values (3x interquartile range ± upper quartile/lower quartile, respectively). Letter values shown above the bars indicate the significance test (ANOVA) for data in each frame (A/B), Bars that share a lower-case letter indicate no significant differences (P ≥ 0.05).



**Figure SI 4.5:** Linear regression analysis of soil organic matter content vs. water holding capacity (2021 n = 72; 2023 n = 72) in soil 1 year post amendment (blue) and 3 years post amendment (orange).



**Figure SI 4.6:** SOM (%) (2020 n = 72; 2021/2023 n = 18) of soil; pre amendment (A), 1 year post amendment (B), and 3 years post amendment (C), with increasing doses of paper crumble (PC). The box indicates the upper and lower quartiles, with the midline representing the median value, the symbol (x) represents the mean, whiskers represent the highest and lowest values ( $3 \times$  interquartile range  $\pm$  upper quartile/lower quartile, respectively). Black letter values shown above the bars indicate the outcomes of significance testing (ANOVA) for data in each frame (A/B/C). Coloured letter values shown below the bars indicate the outcomes of significance testing (ANOVA) for the data between frames based on the same PC treatment dose (A Vs. B Vs. C). Bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).



**Chapter 5:**  
**Physical Protection of Soil Carbon Stocks**  
**Under Regenerative Agriculture**

## 5.1 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, soils from blackcurrant fields under regenerative management (0 to 7 years) were analysed with respect to: soil bulk density (SBD), aggregate fractionation (water stable aggregates vs. non-water stable aggregates (WSA and NWSA respectively)), soil carbon content, and carbon stability (recalcitrant vs. labile carbon). From this, long term carbon sequestration potential was calculated from both recalcitrant and physically occluded carbon stocks (stabilised carbon). Results indicated favourable shifts in the proportion of NWSA:WSA with time. This ratio increasing from 27.6%:5.8% (control soil) to 12.6%:16.0% (alley soil), and 16.1%:14.4% (bush soil) after 7 years, along with their relative enrichment in carbon. While no significant ( $p \geq 0.05$ ) changes in recalcitrant carbon stocks were observed after 7 years, labile carbon stocks increased significantly ( $p \leq 0.05$ ) from  $10.44 \text{ t C ha}^{-1}$  to  $13.87 \text{ t C ha}^{-1}$ . As a result, total sequesterable carbon (stabilised carbon) increased by  $1.7 \text{ t C ha}^{-1}$  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 mechanisms of soil carbon stabilisation under regenerative agriculture practices and highlights the importance of soil aggregates in physically protecting carbon net-gains.

## 5.2 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, 2018b). 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, 2004c, Smith et al., 2020a). 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., 2014a, Newton et al., 2020). To date it is estimated that more than 176 Gt of soil carbon has been lost to the atmosphere (IPBES, 2018b), 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., 2018a, Smith et al., 2020a). 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 degradation of soil, is the loss and destruction of stable soil aggregates and loss of SOC (Smith, 2008, Baveye et al., 2020b). Soil aggregate formation, as facilitated by SOC, assists the stabilisation and storage of carbon and imparts resilience to soils against erosion and climate change while providing hydrological benefits and enhancing soil fertility (Lal, 1997, Abiven et al., 2009b, Chaplot and Cooper, 2015, Veenstra et al., 2021).

In addition to mitigating the negative effects of soil degradation, the formation and persistence of stable soil aggregates is instrumental in soil carbon sequestration (Lal, 1997, Six et al., 1998, Abiven et al., 2009b); in particular due to physical protection of labile carbon within the soil aggregates; thus minimising biogenic and oxidative decay of SOC (Brodowski et al., 2006, Smith, 2008, Schmidt et al., 2011, Berhe and Kleber, 2013).

However, 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 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., 2002b, Dominati et al., 2010, Power, 2010, Baveye et al., 2016b, Keenor et al., 2021)).

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., 2009b, Bhogal et al., 2009b, Baveye et al., 2020b, 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., 2004b, 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, 2004c). 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<sub>2</sub>e have been achieved globally since the 1970s (Al-Kaisi and Lal, 2020).

To evaluate the influence of transitioning a soft fruit production enterprise from a regime of conventional cropping and tillage to a regenerative approach, 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, and 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). 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 conventional arable farming to regenerative soft fruit production would increase total soil carbon stock with time and that this carbon stock would become increasingly stabilised, either associated with WSA (i.e. physically protected) and/or of greater recalcitrance.

## **5.3 Materials and Methods**

### **5.3.1 Field Experiment**

This research was undertaken at Gorgate Farm, Norfolk, UK (52°41'58"N 0°54'01"E). The farm is part of the wider Wendling Beck Environment Project (WBNRP, 2024) a regenerative farming and landscape management program set in C. 750 ha. The field experiment comprised 5 blackcurrant fields established in 2019, 2017, 2015, and 2013 (1, 3, 5, and 7 years since soil disturbance) and a conventionally managed arable field as a datum (0 years since soil disturbance; field history in the arable regime (2014-2021) is shown in **Figure SI 5.1**.

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. Currants 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, and are treated with sprays of compost tea and organic fertiliser. Comparatively the control comprised a

conventionally managed arable field adjacent to the blackcurrant fields, cultivated yearly and drilled with winter wheat, with stubble re-incorporation. Samples were collected in late June, immediately prior to the harvest of both crops.

### **5.3.2 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 field using a soil Dent 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 ( $\leq 4$  °C) prior to laboratory analysis. Soil cores were subsequently oven dried (40 °C for 24hrs) and soil bulk density calculated for each field (n = 5).

### **5.3.3 Soil Fractionation**

Soil fractionations, namely, Water Stable Aggregates (WSA), Non-Water Stable Aggregates (NWSA) and sand, were established using a capillary-wetting wet sieving method, adapted from Seybold and Herrick (2001): Briefly, the previously dried bulk density samples (n = 5) were dry sieved (2 mm) to remove all debris. Subsequently, 2mm sieved bulk soil (100 g) was placed on 63  $\mu\text{m}$  sieves. 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  $\mu\text{m}$  sieve was collected and dried (40 °C for 24 hours) and then weighed, this fraction was defined as NWSA (n = 5). The soil retained on the 63  $\mu\text{m}$  sieve was further processed using in sodium hexametaphosphate solution (0.02 M), to disaggregate the WSA aggregates and

separate from the sand fraction. The material remaining on the 63  $\mu\text{m}$  sieve was then dried (40  $^{\circ}\text{C}$  for 24 hours); and designated as the sand fraction ( $n = 5$ ). The WSA fraction (That which passed through the 63  $\mu\text{m}$  sieve) was subsequently established by calculation ( $n = 5$ ) (Eq. 4.1):

$$\text{Eq.4.1} \quad \% \text{ WSA} = \left( \frac{\text{Bulk Soil Mass}_{\text{dry}} - (\text{Sand Mass}_{\text{dry}} + \text{NWSA Mass}_{\text{dry}})}{\text{Bulk Soil Mass}_{\text{dry}}} \right) \times 100$$

#### 5.3.4 Total C, and N Content by Elemental Analysis

Dry bulk soil, and soil fractions, were milled to produce a fine powder and samples (20 mg;  $n = 5$ ) packed in  $8 \times 5$  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  $\mu\text{g}$ ), a blank sample (empty capsule) and an organic blank sample (benzoic acid 1700  $\mu\text{g}$ ) prior to sample analysis, and standard reference materials (acetanilide 1500  $\mu\text{g}$ ) were run alongside samples (every 6<sup>th</sup> run) for QA/QC (a precision threshold of  $\pm 1\text{SD}$  of the mean from the standard reference material) (Hemming, N.D.).

#### 5.3.5 Thermogravimetric Assessment of SOC Stability

Thermal stability of the SOC in bulk soil, NWSA and sand fractions were assessed using a Thermo-gravimetric analyser (Mettler Toledo TGA/DSC 1). Samples ( $n = 5$ ) were contained in 70  $\mu\text{l}$  platinum crucibles. Samples were heated, in an inert atmosphere, at a rate of 10  $^{\circ}\text{C min}^{-1}$  from 25  $^{\circ}\text{C}$  to 1000  $^{\circ}\text{C}$ . TGA data was subsequently used to ascribe stable/not-stable carbon and inorganic carbon content of the bulk soil and soil fractions. Data was split into 4 distinct phases by temperature range according to organic matter attrition windows as stated in Mao *et al.* (2022): i) 25  $^{\circ}\text{C}$  – 125  $^{\circ}\text{C}$

(moisture evaporation), ii) 125 °C – 375 °C (labile components), iii) 375 °C – 700 °C (recalcitrant components), iv) 700 °C – 1000 °C (inorganic carbon).

### 5.3.6 Carbon Assessment

Soil carbon was assessed as total SOC, soil fraction C, total labile/recalcitrant C and physically protected/unstabilised C. In addition, C was further assessed on a total field carbon stock basis (in t ha<sup>-1</sup>). To calculate the total field carbon stock in t ha<sup>-1</sup> (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. 4.2):

$$\text{Eq.4.2} \quad C \text{ } t h a^{-1} = \left( 0.6(C_{\text{Alley}} \times SBD_{\text{Alley}} \times \text{Depth}) \right) + \left( 0.4(C_{\text{Bush}} \times SBD_{\text{Bush}} \times \text{Depth}) \right)$$

### 5.3.7 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% ( $p \leq 0.05$ ) (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).

## 5.4 Results and Discussion

### 5.4.1 Soil Bulk Density

Soil bulk density (SBD) provides insights into soil structures, arrangement of soil particles, and the extent of soil aggregation arising from the influence of 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 can act as a key soil condition indicator (Chaudhari et al., 2013, Allen et al., 2011). 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  $1.4 \text{ g cm}^{-3}$  (clay rich soils), and  $1.8 \text{ g cm}^{-3}$  (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 (**Figure**

**5.1).** The highest overall SBD was measured in the control soil ( $1.75 \text{ g cm}^{-3}$ ) and the lowest SBD in the year 3 bush soil ( $1.07 \text{ g cm}^{-3}$ ) (**Figure 5.1**).

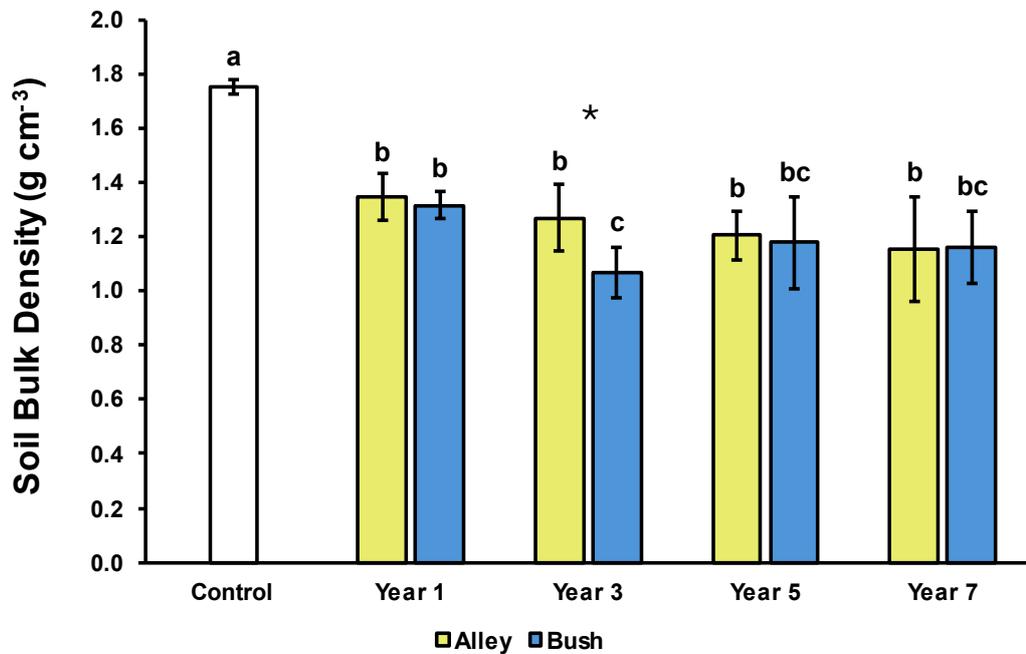
In the alley soils SBD was observed to decrease significantly ( $p \leq 0.05$ ) in all of the regeneratively managed soils compared to the conventional control (**Figure 5.1**). Between the regeneratively managed soils SBD was observed to decrease (not significantly ( $p \geq 0.05$ )) successively with each additional year under regenerative management; from  $1.35 \text{ g cm}^{-3}$  in 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 conventional control soil) (**Figure 5.1**).

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 (**Figure 5.1**). 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  $1.32 \text{ g cm}^{-3}$  in to  $1.07 \text{ g cm}^{-3}$ , before increasing (not significantly ( $p \geq 0.05$ )) in years 5 and 7 (to  $1.18 \text{ g cm}^{-3}$  and  $1.16 \text{ g cm}^{-3}$  respectively)(**Figure 5.1**).

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  $1.27 \text{ g cm}^{-3}$  and  $1.07 \text{ g cm}^{-3}$  respectively (**Figure 5.1**).

None of the soils measured in this investigation were observed to exceed the root limiting soil density factor of  $1.8 \text{ g cm}^{-3}$  suggesting no significant detriment to the growth of plants from soil compaction. Furthermore, the overall trend of soil bulk density reduction seen over the course of the 7-year period (**Figure 5.1**) is likely a consequence of both increased aggregate stability and quantity of stable aggregates

(Section 5.4.2) alongside increases in soil carbon stocks (Section 5.4.3), 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).



**Figure 5.1:** 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.

#### 5.4.2 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., 2009b).

Proportions of WSA and NWSA were seen to change significantly ( $p \leq 0.05$ ) in both the alley and bush soils (**Figure 5.2**). While the sand fraction also observed significant changes ( $p \leq 0.05$ ) between some of the alley and bush soils (**Figure 5.2**), the overall change in sand fraction has been discounted from further discussion as this fraction cannot be created or altered relative to the 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 ( $p \leq 0.05$ ) over the 7 years of establishment, while the WSA fractional proportion increased significantly over time ( $p \leq 0.05$ ) (**Figure 5.2; Table SI 5.1**). Such changes were likely due to the effects 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 commensurate with soil carbon increases (**Section 5.4.3**) and SBD decreases (**Section 5.4.1**), collectively these changes would enhance soil aggregate stability and cohesion (Abiven et al., 2009b, 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; significant reductions ( $p \leq 0.05$ )

were measured between the control soil and all regeneratively managed soils, additionally, NWSA in the year 7 soil was measured to be significantly lower ( $p \leq 0.05$ ) than all other regeneratively managed soils (**Figure 5.2; Table SI 5.1**).

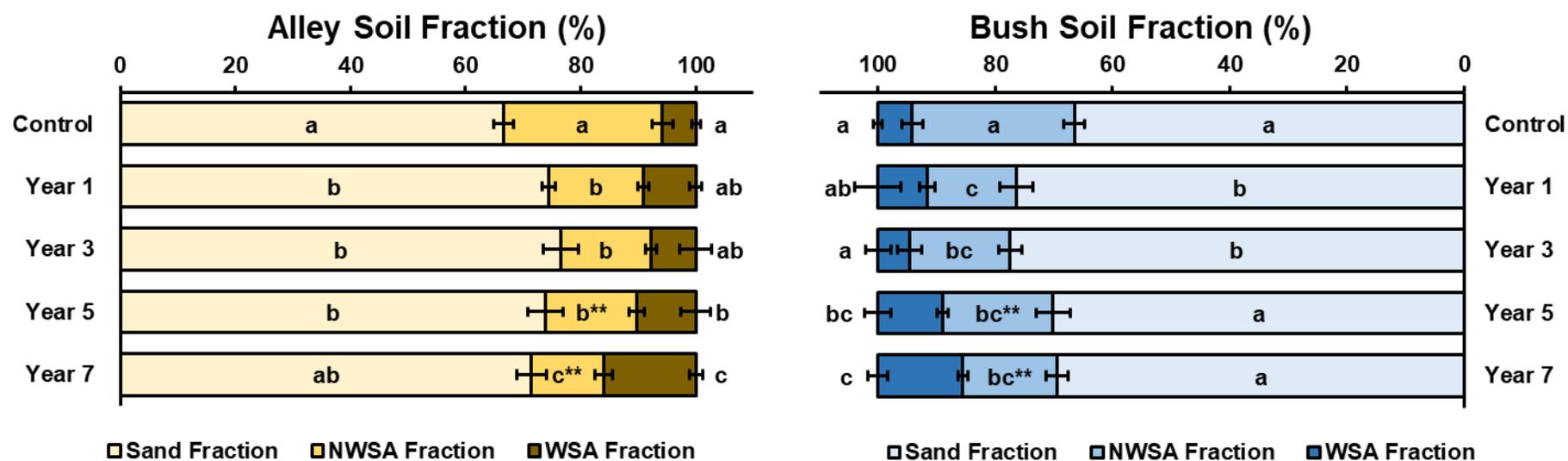
In the bush soil, NWSA fractions were also observed to decrease significantly ( $p \leq 0.05$ ) in all regeneratively managed soils relative to the control, ranging between 27.6% in the control to 15.2% in the year 1 soil (**Figure 5.2; Table SI 5.1**). However, this decrease was not successive, as the greatest reduction was measured in the year 1 soil and increased (not significantly ( $p \geq 0.05$ )) to then broadly plateau in subsequent years (**Figure 5.2; Table SI 5.1**). 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 (**Figure 5.2; Table SI 5.1**). 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) (**Figure 5.2; Table SI 5.1**).

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), (**Figure 5.2; Table SI 5.1**). Additionally, the WSA fraction in year 7 was observed to be significantly greater ( $p < 0.05$ ) than in all other regeneratively managed soils (**Figure 5.2; Table SI 5.1**).

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)

(**Figure 5.2; Table SI 5.1**). Between 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 (**Figure 5.2; Table SI 5.1**). 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 (**Figure 5.2; Table SI 5.1**).



**Figure 5.2:** 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.

### 5.4.3 Soil Carbon and Thermal Stability

Soil organic carbon (SOC) underpins a wide range of ecosystem processes and functions (Power, 2010, de Groot et al., 2002b, Adhikari and Hartemink, 2016b, Baveye et al., 2016b, 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).

SOC was observed to increase in both the alley and bush soils over time (**Figure SI 5.2**), with significant increases ( $p \leq 0.05$ ) in the year 5 bush soil ( $22.3 \text{ g kg}^{-1} \text{ C}$ ) and 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 the control soil ( $16.6 \text{ g kg}^{-1} \text{ C}$ ) (**Figure SI 5.2**). 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 (**Figure SI 5.2**)

Thermal techniques such as thermogravimetric analysis can provide effective means of characterising organic matter pools in the soil, defining the profile of SOC stability (Plante et al., 2005b, Dell'Abate et al., 2000, Dell'Abate et al., 2003, Plante et al., 2011, Mao et al., 2022). Furthermore, thermal stability can provide a proxy for biogenic decay and degradation of soil organic matter and carbon stocks (Plante et al., 2005b, 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 (**Figure 5.3**). 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 (**Figure 5.3**).

Labile soil carbon measured in the alley soils increased broadly stepwise with age of establishment, with all regenerative managed soils increasing in labile carbon relative to the control soil, these increases were significant ( $p \leq 0.05$ ) in both the year 5 and year 7 soils relative to the control (increasing from  $7.9 \text{ g kg}^{-1} C_{\text{labile}}$  (control) to  $13.6 \text{ g kg}^{-1} C_{\text{labile}}$ ,  $17.6 \text{ g kg}^{-1} C_{\text{labile}}$  respectively), i.e., an increase of  $9.7 \text{ g kg}^{-1} C_{\text{labile}}$  (**Figure 5.3**). 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 (**Figure 5.3**).

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, significantly greater ( $p \leq 0.05$ ) carbon stocks were measured in the year 5 and year 7 soils relative to the control (increasing from  $7.9 \text{ g kg}^{-1} C_{\text{labile}}$  to  $12.4 \text{ g kg}^{-1} C_{\text{labile}}$  and  $13.9 \text{ g kg}^{-1} C_{\text{labile}}$ , respectively) i.e., an increase of  $4.0 \text{ g kg}^{-1} C_{\text{labile}}$  (**Figure 5.3**). 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) (**Figure 5.3**).

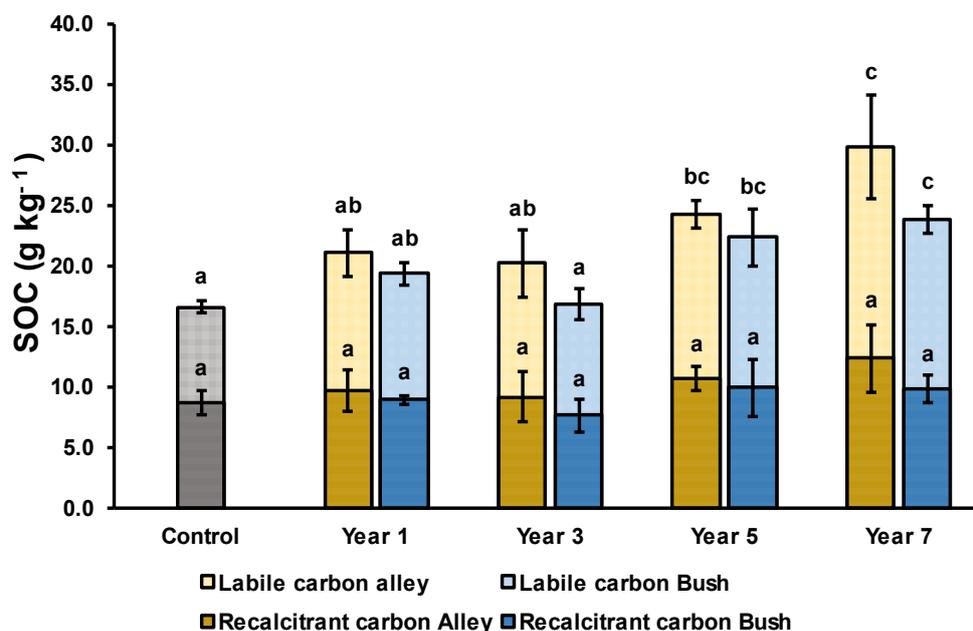
When compared pairwise, labile carbon in the alley soil increased by a total of  $9.7 \text{ g kg}^{-1} C_{\text{labile}}$ , vs. Increase of  $4.0 \text{ g kg}^{-1} C_{\text{labile}}$  in the bush soil after 7 years of regenerative

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 in any given year) (**Figure 5.3**).

Recalcitrant carbon measured in the alley soils increased broadly stepwise with increasing age of establishment, with all regeneratively managed soils increasing relative to the conventional control, however none of these increases were significant ( $p \geq 0.05$ ) (**Figure 5.3**). Over the 7 year period recalcitrant carbon in the alley soils increased (not significantly ( $p \geq 0.05$ )) 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 7 soils) (**Figure 5.3**).

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 (**Figure 5.3**). Recalcitrant carbon in the bush soil increased (not significantly ( $p \geq 0.05$ )) 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  $1.2 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$  (**Figure 5.3**).

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 (**Figure 5.3**). By year 7, the alley soil was observed to contain a total carbon content of  $29.9 \text{ g kg}^{-1} \text{ C}$  (split 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 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 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  $8.7 \text{ g kg}^{-1} \text{ C}_{\text{recalcitrant}}$ ) (**Figure 5.3**).



**Figure 5.3:** SOC split by recalcitrant (hashed) and labile (plain) carbon pools ( $n = 5$ ) in the alleyway (yellow) and bush (blue) regimes. Error bars represent  $\pm 1SD$ . 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.

#### 5.4.4 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 (**Figure 5.4**). It is highlighted that despite their smaller fractional share (**Section 5.4.2**), 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 (**Figure 5.4A**).

When analysed by fraction, the labile carbon pool in the NWSA fraction was observed to significantly decrease ( $p \leq 0.05$ ) with increasing years of establishment, 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 (**Figure 5.4A**).

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 (**Figure 5.4A**). 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 (**Figure 5.4A**).

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, 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 (**Figure 5.4B**).

Within the NWSA fraction no significant differences ( $p \geq 0.05$ ) were observed between the control and any regeneratively managed soil (**Figure 5.4B**). Labile carbon was initially measured to decrease 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%) (**Figure 5.4B**).

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 (**Figure 5.4B**). 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 (**Figure 5.4B**).

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 (**Figure 5.4 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 (**Figure 5.4C**).

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% ) (**Figure 5.4C**). 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 (**Figure 5.4C**).

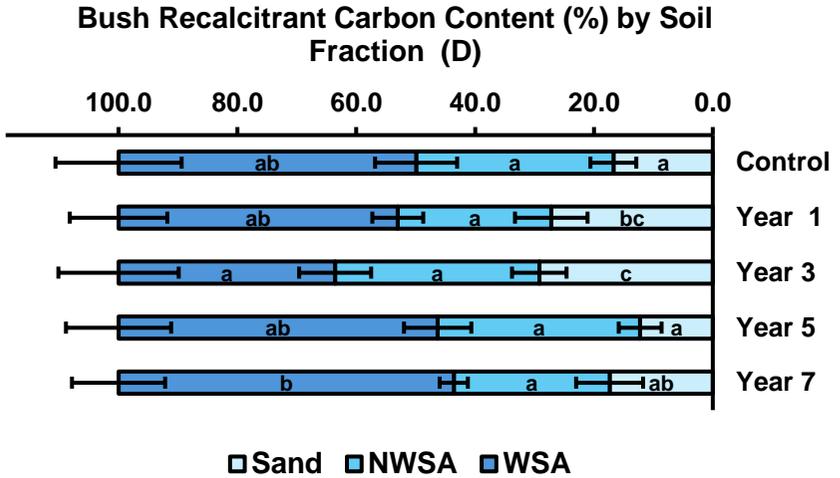
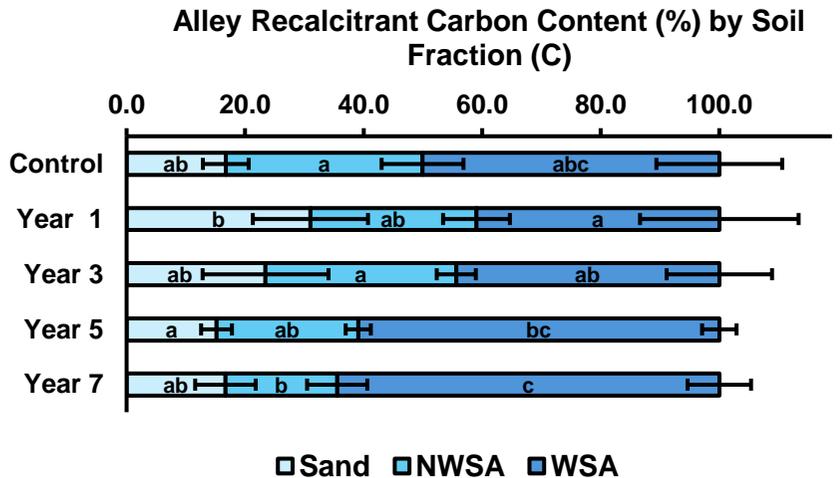
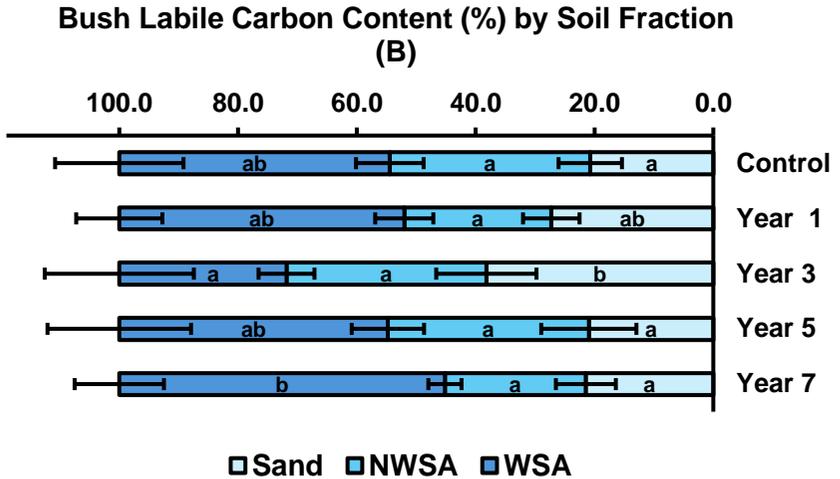
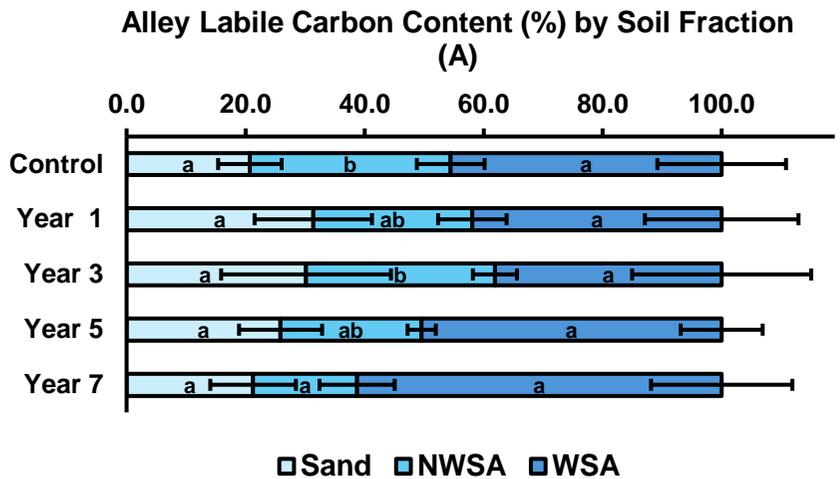
In the WSA fraction the recalcitrant carbon pool was observed to increase (not significantly ( $p \geq 0.05$ )) with time, increasing from 50.1% in the control to 64.5% in the year 7 soil (**Figure 5.4C**). Initial decreases in recalcitrant carbon were observed in the year 1 soil relative to the control (decreasing (not significantly ( $p \geq 0.05$ )) to 41.0 %); thereafter subsequent stepwise increases in all other regeneratively managed soils were observed (**Figure 5.4C**).

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 (**Figure 5.4D**).

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 (**Figure 5.4D**).

Within the WSA fraction, recalcitrant carbon was observed to increase overall from the control to year 7, with initial reductions measured in year 1 and 3 relative to the control soil, decreasing (not significantly ( $p \geq 0.05$ )) from 50.1% in the control to 36.4% in the year 3 soil, before subsequently increasing stepwise to a total of 56.4% in year 7 (not significantly different ( $p \geq 0.05$ ) to the control) (**Figure 5.4D**).

When compared pairwise significant differences ( $p \leq 0.05$ ) were observed between 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 (**Figure 5.4 C/D**).



**Figure 5.4:** 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  $\pm 1SD$ . 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.

#### 5.4.5 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).

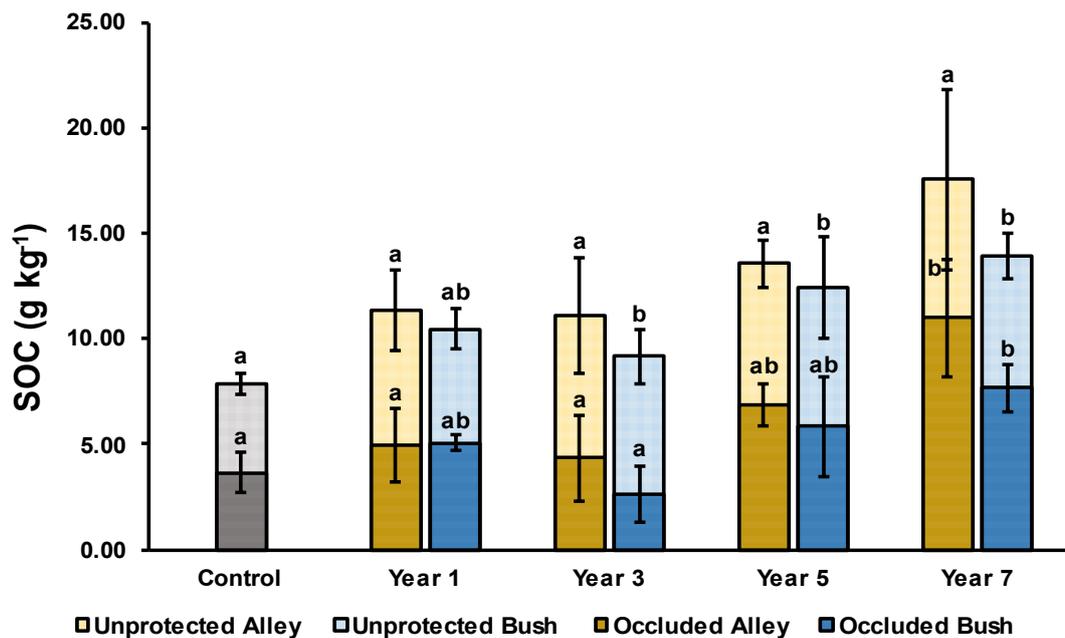
Stable soil aggregates can also confer potentially long-term storage to soil carbon, through stabilisation and occlusion, physically separating the carbon from its potential vectors of degradation (Schrumpf et al., 2013, Gärdenäs et al., 2011, Six and Jastrow, 2002, Dungait et al., 2012, Plante et al., 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, 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).

Stable aggregate occluded carbon considered the stabilisation of the labile carbon stock held within the WSA fraction (**Section 5.4.4**), due to the physical protection offered by these aggregate structures inhibiting the breakdown and decomposition of the carbon stored within; while conversely unstabilised carbon considered the labile carbon that was not contained within the WSA fraction (**Section 5.4.4**), and thus with greater potential for degradation. Additionally, recalcitrant carbon (**Section 5.4.3**), was considered stabilised regardless of the soil aggregate pool in which it was contained 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  $3.64 \text{ g kg}^{-1} \text{ C}$  to  $10.99 \text{ g kg}^{-1} \text{ C}$  in the control and year 7 soil) (**Figure 5.5**). 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  $3.64 \text{ g kg}^{-1} \text{ C}$  in the control to  $7.66 \text{ g kg}^{-1}$  in the year 7 soil (**Figure 5.5**). 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  $2.64 \text{ g kg}^{-1} \text{ C}$ , before rebounding in years 5 and 7 (**Figure 5.5**). 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 (**Figure 5.5**).

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  $6.4 \text{ g kg}^{-1} \text{ C}$  and  $6.7 \text{ g kg}^{-1} \text{ C}$ , compared with  $4.2 \text{ g kg}^{-1}$  in the control soil (**Figure 5.5**). 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  $6.6 \text{ g kg}^{-1}$  (in the year 5 soil) relative to  $4.2 \text{ g kg}^{-1}$  in the control soil (**Figure 5.5**). 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 (**Figure 5.5**).



**Figure 5.5:** Labile SOC split by ocluded (hashed) and unprotected (plain) carbon pools ( $n = 5$ ) in the alley (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 alley and bush regimes.

#### 5.4.6 Carbon Stability at Field Scale

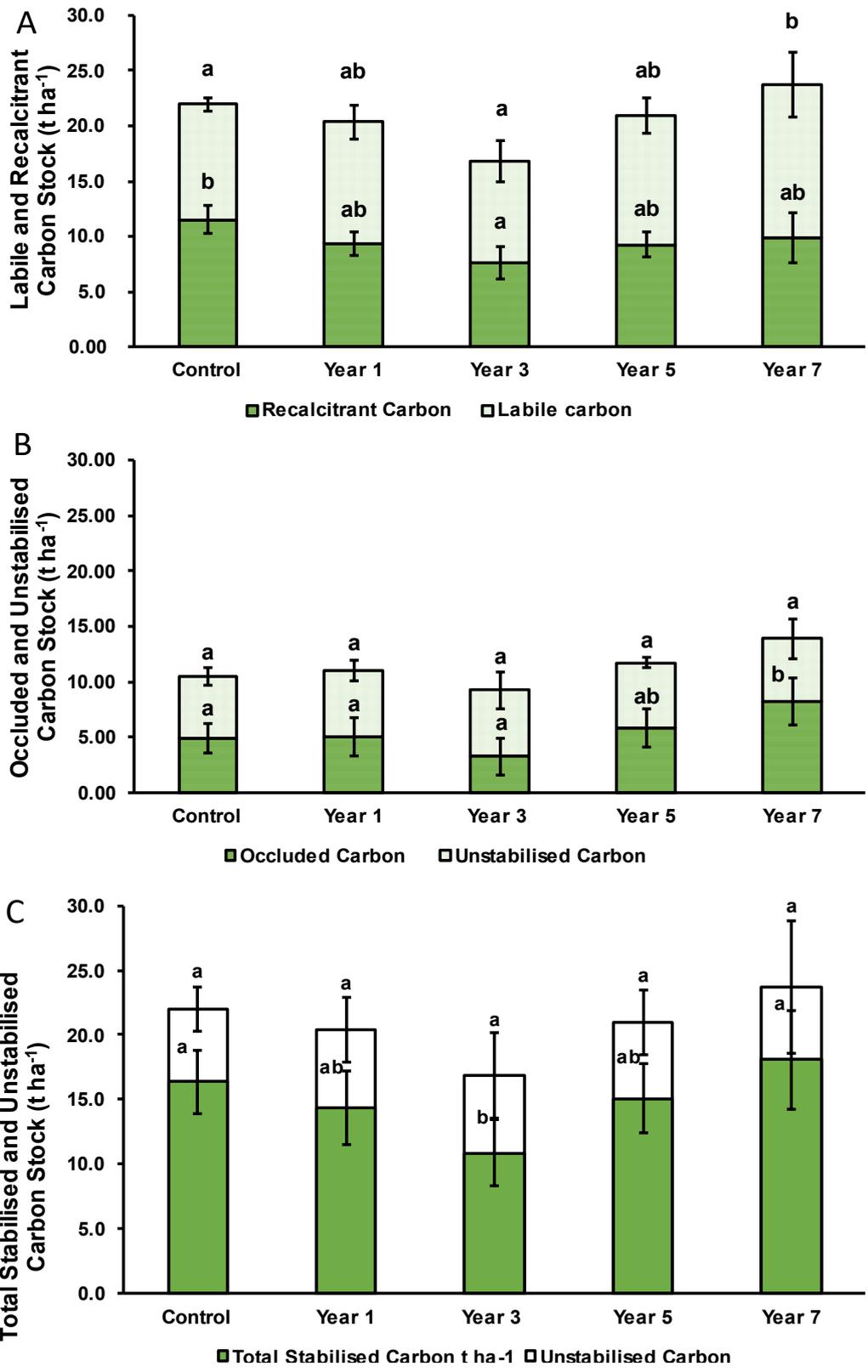
Acknowledging proportions of alley and bush soils (60% and 40% of field area, respectively) and accommodating the influence of SBD (**Section 5.4.1; Figure 5.1**), soil carbon contents (in  $\text{g C kg}^{-1}$ ) (**Section 5.4.3; Figure SI 5.2**) were converted to carbon stocks ( $\text{t C ha}^{-1}$ ). Field scale soil carbon stocks were observed to increase (not significantly ( $p \geq 0.05$ )) by  $1.75 \text{ t C ha}^{-1}$  over the 7-year period relative to the control soil (from  $21.98 \text{ t C ha}^{-1}$  to  $23.72 \text{ t C ha}^{-1}$ ) (**Figure SI 5.3**).

When viewed as a split between labile and recalcitrant carbon pools were observed (between the control and year 3 soils), with the majority of this decrease occurring in the recalcitrant carbon pool, decreasing significantly ( $p \leq 0.05$ ) from  $11.54 \text{ t C ha}^{-1}$  to  $7.62 \text{ t C ha}^{-1}$ , while labile carbon was observed to decrease less sharply (not significantly ( $p \geq 0.05$ )) from  $10.44 \text{ t C ha}^{-1}$  to  $9.22 \text{ t C ha}^{-1}$  (**Figure 5.6A**). 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, while the recalcitrant carbon had not fully recovered (**Figure 5.6A**). Over the full 7-year period recalcitrant carbon was observed to decrease (not significantly ( $p \geq 0.05$ )) to  $9.85 \text{ t C ha}^{-1}$ , while labile carbon stocks were observed to increase significantly ( $p \leq 0.05$ ) to  $13.87 \text{ t C ha}^{-1}$ . These results highlight that the increase observed in soil carbon stock over the 7-year period was comprised entirely of labile carbon (**Figure 5.6A; Figure SI 5.3**). It is likely that the initial decreases observed in both carbon pools related to soil disturbance and changing inputs 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., 2018a). Additionally, it has been observed that significantly increasing labile carbon inputs to the soil can somewhat undermine the stability of recalcitrant carbon due to the enhanced priming effect (De Graaff et al., 2010), potentially causing the recalcitrant carbon loss initially observed. While recalcitrant carbon stocks were observed to increase in latter years, this rate of increase was significantly less than that of the labile carbon pool (**Figure 5.6A**). 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.

Evaluating the field scale stabilisation of labile carbon showed a similar trend in the occluded carbon pool, with a (non-significant ( $p \geq 0.05$ )) decrease observed between the control and the year 3 soils, before increasing in years 5 and 7 (not significantly ( $p \geq 0.05$ ) and significantly ( $p \leq 0.05$ ) respectively); with an overall significant ( $p \leq 0.05$ ) increase in the occluded carbon pool between the control and year 7 soils, almost doubling from  $4.81 \text{ t C ha}^{-1}$  to  $8.21 \text{ t C ha}^{-1}$  (**Figure 5.6B**). While unstabilised carbon was observed to remain broadly consistent across all soils, ranging from  $5.63 \text{ t C ha}^{-1}$  in the control to  $6.02 \text{ t C ha}^{-1}$  in the year 1 soil, with no significant differences ( $p \geq 0.05$ ) observed between the different regeneratively managed soils and the control (**Figure 5.6B**). 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 ( $3.40 \text{ t C ha}^{-1}$  and  $3.42 \text{ t C ha}^{-1}$  respectively)(**Figure 5.6A/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. 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. When viewed as total stabilised carbon (recalcitrant carbon and occluded carbon) an increase (not significant ( $p \geq 0.05$ ) of  $1.7\text{t C ha}^{-1}$  was measured after 7 years relative to the control (**Figure 5.6 C**).



**Figure 5.6:** 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.

### 5.4.7 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 et al., 2004, Smith, 2008, Smith et al., 2020a, Soussana et al., 2019b, Baveye et al., 2020b, Keenor et al., 2021, Lal, 1997, Lal, 2004c). 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 (Schrumpp et al., 2013, Gärdenäs et al., 2011, Dungait et al., 2012, Six and Jastrow, 2002, Plante et al., 2011, McLauchlan and Hobbie, 2004)(**Section 5.4.4; Section 5.4.5**). 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).

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., 2020a). 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 ( $\text{g kg}^{-1}$ ) must increase at a greater rate than bulk density decreases.

In this research, soil bulk density (**Section 5.4.1**), was observed to decrease with length of time under regenerative practices, meanwhile soil carbon content (**Section 5.4.2**) was observed to increase with time. However, when changes in carbon stocks were considered on a  $\text{t C ha}^{-1}$  basis (with a prescribed soil depth of 30cm), carbon stocks did not increase incrementally with increasing time (**Section 5.4.6; Figure SI 5.3**). 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  $16.6 \text{ g kg}^{-1}$  in the control to  $27.5 \text{ g kg}^{-1}$  after 7 years (alley and bush soil collectively)), the total field scale increase in carbon stock was only ~8% (increasing from  $22.0 \text{ t ha}^{-1}$  to  $23.7 \text{ t ha}^{-1}$ )( **Figure SI 5.3**). 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 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 SBD, potentially sequesterable soil carbon was measured to increase over the 7-year period by 1.7 t C ha<sup>-1</sup> (**Section 5.4.6; Figure 5.6C**); 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<sub>2</sub>e 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 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<sub>2</sub>e after 7 years of continuous management, with the potential for further increases over a longer time period. Whilst this total sequestration after 7 years offers only a small improvement at a nationwide scale, this could be achieved with minimal changes to current soft fruit production management practice.

## **5.5 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; creating a trade-off where soil carbon stocks are calculated to a standard prescribed depth. Further 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 of SOC, and iii) the proportion of SOC physically protected by association with soil aggregates.

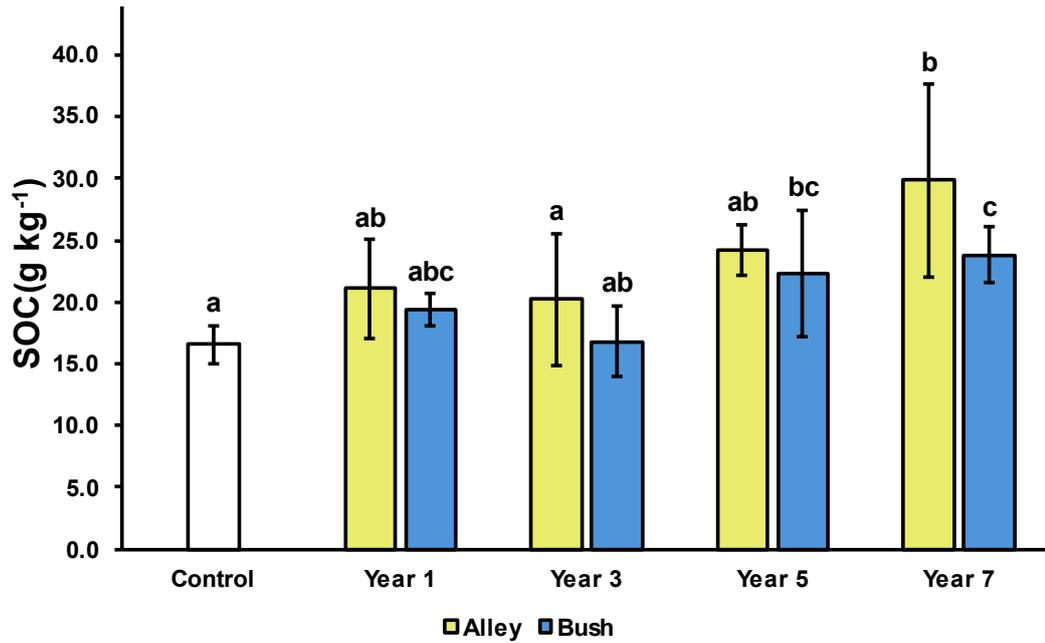
## 5.6 Supporting Information

	2014/15	2015/16	2016/17	2017/18	2018/19	2019/20	2020/21
Control Field	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat
Year 1 Field	Wheat	Barley	Wheat	Barley	Wheat	Barley	Blackcurrant
Year 3 Field	Blackcurrant			Wheat	Blackcurrant		
Year 5 Field	Blackcurrant		Blackcurrant				
Year 7 Field	Blackcurrant						

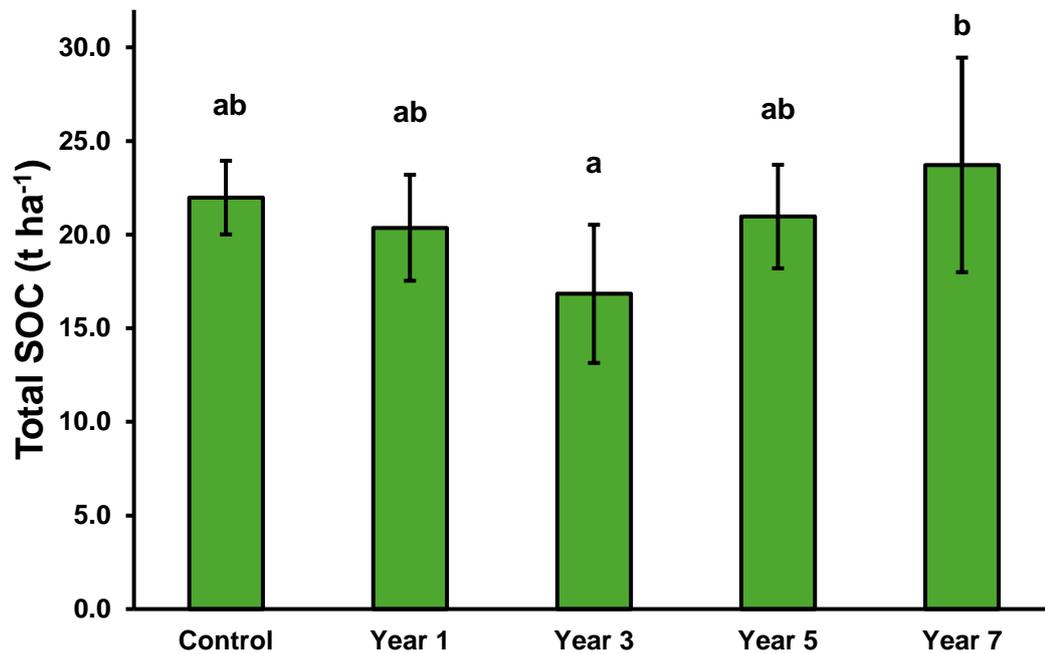
**Figure SI 5.1:** Field cropping history for the arable control, and regenerative blackcurrant fields (2014-2021). Discrete Boxes represent one full cropping cycle and where applicable re-planting of new bushes.

**Table SI 5.1:** Soil texture classification and relative proportion of sand, NWSA and WSA (%) ( $n=5$ ) of alley (white) and bush (grey) 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.

Field Site	Sand %	NWSA %	WSA %	Soil Texture Classification
Control Alley	66.5 $\pm$ 1.7 a	27.6 $\pm$ 1.8 a	5.8 $\pm$ 0.7 a	Sandy Loam
Control Bush	66.5 $\pm$ 1.7 a	27.6 $\pm$ 1.8 a	5.8 $\pm$ 0.7 a	Sandy Loam
Blackcurrants Year 1 Alley	74.4 $\pm$ 1.2 b	16.5 $\pm$ 1.0 b	9.2 $\pm$ 1.0 ab	Sandy Loam
Blackcurrants Year 1 Bush	76.4 $\pm$ 2.8 b	15.2 $\pm$ 1.3 c	8.5 $\pm$ 3.9 ab	Sandy Loam
Blackcurrants Year 3 Alley	76.5 $\pm$ 3.1 b	15.6 $\pm$ 1.0 b	7.8 $\pm$ 2.8 ab	Sandy Loam
Blackcurrants Year 3 Bush	77.4 $\pm$ 1.9 b	17.1 $\pm$ 2.0 bc	5.4 $\pm$ 2.2 a	Sandy Loam
Blackcurrants Year 5 Alley	73.8 $\pm$ 3.1 b	15.9 $\pm$ 1.4 b **	10.3 $\pm$ 2.5 b	Sandy Loam
Blackcurrants Year 5 Bush	70.2 $\pm$ 2.9 a	18.8 $\pm$ 0.9 bc **	11.0 $\pm$ 2.2 bc	Sandy Loam
Blackcurrants Year 7 Alley	71.4 $\pm$ 2.6 ab	12.6 $\pm$ 1.6 c **	16.0 $\pm$ 1.2 c	Sandy Loam
Blackcurrants Year 7 Bush	69.5 $\pm$ 1.9 a	16.1 $\pm$ 0.8 bc **	14.4 $\pm$ 1.7 c	Sandy Loam



**Figure SI 5.2:** SOC (n=5) of alley (yellow) and bush (blue) regimes with increasing years of establishment. Error bars represent  $\pm 1$ 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 alley and bush regimes.



**Figure SI 5.3:** Total carbon field stocks (n=5), Error bars represent  $\pm 1$ SD. Dissimilar lower-case letters indicate significant ( $p \leq 0.05$ ) differences across the timeseries



**Chapter 6:**  
**Changes to Soil Bacterial and Fungal  
Diversity and Abundance Under a Transition  
to Regenerative Agriculture**

## 6.1 Abstract

Regenerative agricultural principles have been proposed as effective means of improving agricultural productivity and enhancing soil properties. Noted improvements include enhancement of soil structural properties, hydrological functions and the sequestration of carbon, critical for ecosystem health and agricultural productivity. However, for a complete view of the potential transformations regenerative agriculture principles may have upon the soil environment, we must consider the impacts upon soil biodiversity. In this research, soils from blackcurrant fields under regenerative management (0 to 8 years) were analysed with respect to soil properties: soil moisture, soil pH, SOM, TOC, TON, C:N, and carbon stability (recalcitrant and labile carbon stock); and microbial biodiversity: bacterial (16S) / fungal (ITS) diversity and abundance. Metagenomic evaluation was undertaken at family level to consider the interactions between soil properties and relative upregulation or downregulation of microbial abundance. Results indicated significant ( $p \leq 0.05$ ) increases in SOM and soil moisture, alongside additional non-significant increases ( $p \geq 0.05$ ) in TOC and TON with time. Both recalcitrant and labile carbon stocks were measured to increase over time. However, neither increase was significant ( $p \leq 0.05$ ). No significant differences ( $p \leq 0.05$ ) were observed for either 16s or ITS diversity at the species level. Soil properties were observed to significantly ( $p \geq 0.05$ ) influence the upregulation or downregulation of specific bacterial and fungal families. This research provides valuable insight into the influence that different soil properties exert upon soil microbial biodiversity, and the influence regenerative management can have in the shaping of microbial habitat through changes to soil properties.

## 6.2 Introduction

Soil carbon, and soil biodiversity are foundational to ecosystem health, quality and function, through the myriad ecosystem services they underpin and regulate within the soil and wider environment (Wagg et al., 2014, Adhikari and Hartemink, 2016b, Creamer et al., 2016, Power, 2010).

Soils store and maintain the world's largest terrestrial carbon stock, containing approximately 3 times as much carbon as is held within the atmosphere - playing a pivotal role in the global carbon cycle and regulation of greenhouse gas emissions, while providing key structure and function to the soil (Paustian et al., 2016, Smith et al., 2020a). While the soil organisms are an essential driving force that catalyse the delivery of aggregate ecosystem services (Brussaard, 2012). Greater soil biodiversity contributes toward enhanced provision and maintenance of multiple goods and services, including organic matter decomposition, nutrient cycling, improved plant and soil health, and mediation of soil carbon stabilisation and storage (Thiele-Bruhn et al., 2012, Geisen et al., 2019, Delgado-Baquerizo et al., 2020). Collectively, these ecosystem services are fundamental to the structure and function of the environment, through the production of natural capital goods and facilitation of ecological services (De Groot et al., 2002a, Dominati et al., 2010, Lal, 2013, Adhikari and Hartemink, 2016b, Latawiec et al., 2020a). Indeed, soils are estimated to underpin up to 80% of all ecosystem services, in turn largely influenced by management practices that drive changes in soil properties (Dominati et al., 2010, Lal, 2013, Adhikari and Hartemink, 2016b, Bai et al., 2019, Bateman and Muñoz-Rojas, 2019).

Given the historic intensification of agriculture and land use practice, soils have been subjected to degradation and carbon loss on a global scale, (estimated to impact

approximately 20% of all soils globally and 50% of all agriculturally managed soils), significantly impacting ecosystem service provision and contributing to climate change and environmental dysregulation (Lal, 2001, Stavi and Lal, 2015, Bateman and Muñoz-Rojas, 2019). Much of this damage has been observed to relate to intensive seasonal cultivation of soils, the input of agrochemicals (such as pesticides, herbicides, and fertilisers), and the widespread adoption of mechanised farming practices (Lal, 1993, Janvier et al., 2007, Geisen et al., 2019). These actions can destroy soil structures by breaking soil aggregates and exposing previously stabilised occluded carbon to vectors of decomposition, accelerating the overall rate of carbon decay (Smith, 2008, Dungait et al., 2012, Schrumpf et al., 2013, Wagg et al., 2014, Baveye, 2020). With significant damage to soil biodiversity occurring as a result of changes to habitat and soil properties, soil disturbance and the direct impact agrochemical use has on microbial communities (Thiele-Bruhn et al., 2012, Geisen et al., 2019).

Collectively, the reductions in the diversity and abundance of soil microorganisms, alongside destruction of soil carbon stocks, are observed to link with commensurate declines in ecosystem service provision to reduce ecosystem stability over time - further compounding the ecosystem service disfunction (Bardgett and van der Putten, 2014, Wagg et al., 2014, Geisen et al., 2019). Thus, proactive change is required to recarbonise soils, increase soil biodiversity and improve soil properties to restore and preserve ecosystem service functions.

By adopting policies and practices which seek to redress carbon loss, the negative effects of soil degradation may be minimised or reversed, culminating in increased soil carbon stocks, enhanced ecosystem service provision, and improved physical, chemical, hydrological and biological properties of soil (Doran, 2002, Lal, 2016,

Vereecken et al., 2022, Bünemann et al., 2018). Yet, providing the necessary protections to, and improving upon soil biodiversity is also essential for long term soil sustainability and ecosystem service enhancement (Geisen et al., 2019). Thus, aligning practices and policies with interventions which deliver soil biodiversity enhancement in addition to physical soil improvements can thus offer a significant environmental dividend: improved soil health, enhanced environmental function and resilience, and climate change mitigation. Towards these ends, regenerative agriculture may offer significant potential.

Regenerative agriculture emphasises environmental and soil health as the cornerstone of a sustainable agricultural practice, through the adoption and integration of holistic ecological management practices which restore and enhance ecosystem services rather than damaging them (Schreefel et al., 2020, Lal, 2020a, Moyer et al., 2020). Regenerative practices have been observed to significantly improve soil structures and aggregate stability, increase water retention and hydrological function, enhance soil biodiversity and sequester carbon in soils (Gosnell et al., 2019, O'Donoghue et al., 2022). However, despite the well documented improvements regenerative agriculture exerts upon soil properties, a comprehensive understanding of the impact these interventions have upon soil biodiversity, especially bacterial and fungal community composition, remains limited and context dependant, with further work needed (Szoboszlay et al., 2017, Wagg et al., 2014).

To evaluate the influences on soil microbial assemblage of transitioning agricultural management from a conventional to a regenerative approach, a field experiment was conducted on a commercial blackcurrant farm in Norfolk, UK. The experiment investigated the effects of regenerative agriculture adoption (no till, reduced and

organic only agrochemical input, cover cropping and year-round soil cover, and applications of compost and compost tea) upon the soil in blackcurrant fields of increasing age under regenerative management (1 year to 8 years). The research provided a preliminary assessment of changes to soil bacterial and fungal abundance and diversity. Data was subsequently used to assess the impact that increased time under regenerative management had upon soil microbial biodiversity and the relative upregulation or down-regulation of specific family groups with commensurate changes to soil properties.

### **6.3 Materials and Methods**

#### **6.3.1 Field Experiment**

This research was conducted at Gorgate Farm in Norfolk, UK (52°41'58"N, 0°54'01"E), a part of the larger Wendling Beck Environment Project (WBNRP, 2024), a regenerative farming and landscape management initiative spanning approximately 750 hectares. This field experiment included four blackcurrant plots established in 2014, 2018, 2020, and 2021, representing 1, 2, 4, and 8 years since the last soil disturbance and a conventionally managed arable field serving as a control (reflecting 0 years since disturbance). The blackcurrant fields were established using a conservation strip tillage method, with bushes arranged in long strips, leaving alleyways approximately 2 meters wide. The blackcurrant bushes covered approximately 40% of the total field area, while the alleys made up the remaining 60%. After planting, the blackcurrant crops required minimal intervention, limited to annual harvesting, pruning, cover crop sowing in the alleys, and fertilization in line with the regenerative practices of limiting soil disturbance and agrochemical input.

Furthermore, the fields remained covered year-round with diverse cover crops planted in the autumn and winter months, while a summer fallow cover crop was utilized in the spring and summer. Cover crops were established using direct drilling and received applications of compost tea and organic fertilizer. In contrast, the control field was managed conventionally, cultivated annually, and planted with winter wheat, with stubble re-incorporated into the soil. Sampling occurred in late June, just prior to the harvest of both crop types.

### **6.3.2 Soil Sampling**

Soil samples (0 – 10 cm; n = 4) were collected from the centre of the alleyways at random points within each field using a soil auger, additionally further soil samples were collected randomly from the conventionally managed arable control field (n = 4). Soil samples were sealed and retained in cold storage ( $\leq 4$  °C) prior to laboratory analysis, the subsequently oven dried (40 °C for 24hrs) and soil moisture content calculated (n = 4).

### **6.3.3 Biodiversity Sampling**

Microbiome analysis was carried out externally (at the Quadram Institute of Bioscience, Norwich Research Park) with the following methods. Briefly: Biodiversity soil samples (n = 6) were collected with a sterilised micro-auger (top 10cm of the soil surface) from each field and stored in sterile sample tubes. Sample tubes were sealed and then transferred to the laboratory for immediate processing.

In the laboratory, samples were processed for DNA extraction using the MPBio FastDNA Spin it for Soil, following manufacturer's instructions. 16S and ITS regions of

the DNA were amplified for bacterial and fungal profiling respectively, using the following primers: Bacterial DNA was amplified using the forward primer, S-D-Bact-0341-b-S-17, 5' CCTACGGGNGGCWGCAG 3' and reverse primer S-D-Bact-0785-a-A-21, 5' GACTACHVGGGTATCTAATCC 3'. Fungal DNA was amplified using the forward primer ITS1F, 5' CTTGGTCATTTAGAGGAAGTAA 3' and the reverse primer ITS2, 5' GCTGCGTTCTTCATCGATGC 3'. The amplified DNA samples were quantified using the Qubit Fluorometer and merged at equimolar concentrations separately for 16S and ITS and sequenced in NextSeq2000 sequencing machine. The raw data was pre-processed using the LotuS2 amplicon data analysis pipeline (Özkurt et al., 2022).

#### **6.3.4 Soil Properties**

Soil organic matter (SOM) content was measured as loss on ignition (ISO, 1995). Briefly, soil (10 g; n = 4) was dried (74 °C for 16 h) and then combusted (470 °C for 24 h). Dry bulk soil, and soil fractions, were milled to produce a fine powder and 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 carbon (TOC) and nitrogen (TON). Instruments were pre-treated within conditioning samples (acetanilide 1900µg), a blank sample (empty capsule) and an organic blank sample (benzoic acid 1700µg) prior to sample analysis, and standard reference materials (acetanilide 1500µg) were run alongside samples (every 6<sup>th</sup> run) for QA/QC (a precision threshold of ± 1SD of the mean from the standard reference material) (Hemming, N.D.). Thermal stability of soil was assessed using a Thermo-gravimetric analyser (Mettler Toledo TGA/DSC 1). Samples (n=4) were contained in 70 µl platinum crucibles and heated, in an inert atmosphere, at a rate of 10°C min<sup>-1</sup> from 25°C to

1000°C. TGA data was subsequently used to ascribe stable/not-stable carbon and inorganic carbon content of the soil samples. Data was split into 4 distinct phases by temperature range according to organic matter attrition windows as stated in Mao *et al.* (2022): i) 25°C – 125°C (moisture evaporation), ii) 125°C – 375°C (labile components), iii) 375°C – 700°C (recalcitrant components), iv) 700°C – 1000°C (inorganic carbon), with both labile and recalcitrant phases being reported. Soil pH (n = 5) was measured (ISO, 1994) in 1:10 soil/water suspension using a pH electrode (Mettler Toledo Pro pH) and pH meter (Mettler Toledo 5 Easy).

### **6.3.5 Statistical Analysis**

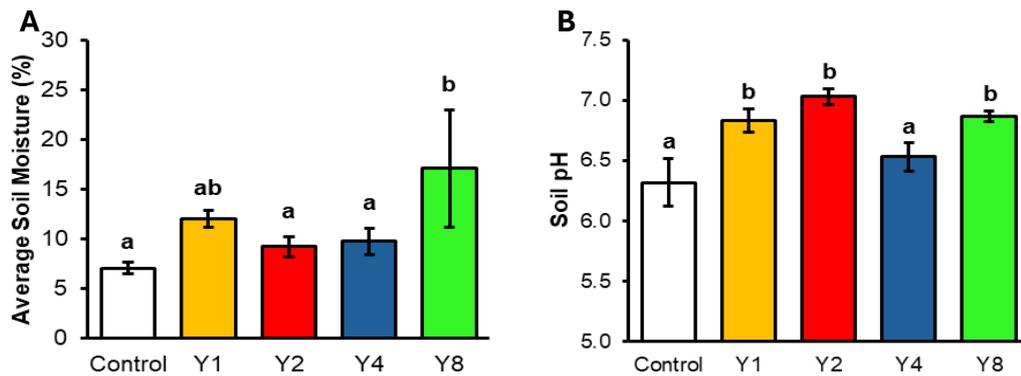
Soil properties and Simpson's diversity index of species level data utilised a one-way analysis of variance (One-way ANOVA) to test the results from the different field sites against each other for statistical significance. Significance level was set to 95 % ( $p \geq 0.05$ ) and determined by a *post hoc* test with Tukey's HSD comparison in IBM SPSS 28. Statistical analysis results are displayed in bar charts along with mean values and standard deviation. Family level biodiversity was assessed at a level of  $\geq 1\%$  of total abundance measured in any individual site. Family level biodiversity data was subsequently normalised and ordered through hierarchical clustering using a gap statistic for heatmaps to determine the number of significantly different clusters. Family biodiversity was also correlated against soil properties using Spearman's rank, with a significance level set to 95% ( $p \geq 0.05$ ), and subsequently clustered. Statistical analysis results are displayed as asterisks on the Spearman's rank heatmaps.

## 6.4 Results and Discussion

### 6.4.1 Soil Properties

Soil moisture content was observed to increase in a stepwise manner with time under regenerative agriculture management, rising significantly ( $p \leq 0.05$ ) from 7.0% in the control soil to 17.1% (more than doubling soil moisture content) in the year 8 soil (**Figure 6.1 A**). While soil moisture was also observed to increase in the other regeneratively managed soils, no significant differences ( $p \geq 0.05$ ) were observed relative to the arable control soil. However significant differences ( $p \leq 0.05$ ) were observed between the smaller increases in the years 2 and 4 soil moisture relative to the year 8 soil moisture content (**Figure 6.1 A**). Increases in soil moisture content likely related to commensurate increases in soil organic matter and soil carbon contents, with even small increases in these stocks having significant impacts upon water holding capacity of the soil (Libohova et al., 2018, Lal, 2020b).

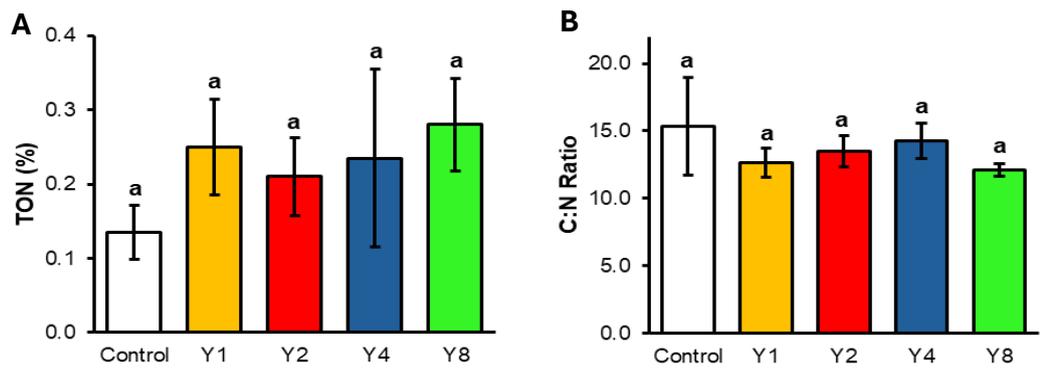
Soil pH was observed to be higher in all regeneratively managed soils compared to the conventional control, with these levels being significantly greater ( $p \leq 0.05$ ) in the year 1, year 2 and year 8 soils (6.8, 7.0, and 6.9, respectively) relative to the control soil (6.3) (**Figure 6.1 B**). Higher pH was also measured in the year 4 soil. However, this was not significantly different ( $p \geq 0.05$ ) to the control soil, additionally, no significant differences ( $p \geq 0.05$ ) were measured between the year 1, 2 and 8 soils (**Figure 6.1 B**).



**Figure 6.1:** Average soil moisture (n = 4) (a); Soil pH (n = 4) (b); of arable control and regeneratively managed soils. Letter values shown above the bars indicate the significance test (ANOVA) between the data, bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

TON was observed to increase in a similar pattern to that of the SOM and carbon measures, increasing markedly in year one before reducing slightly and then following a stepwise increase relative to the control soil (**Figure 6.2 A**). Following 8 years of regenerative agriculture practice TON was observed to double from 0.14% in the control to 0.28% (**Figure 6.2 A**). However, no significant differences ( $p \geq 0.05$ ) were measured between the control soil and the regeneratively managed soils. These increases in TON content likely related to deposition of crop residues and applications of nitrogen rich compost teas increasing direct nitrogen input and availability within the soil, alongside potential nitrogen sequestration resulting from the cover crops sown within the alleyways between crops.

Soil C:N ratios were observed to reduce (not significantly ( $p \geq 0.05$ )) in all regeneratively managed soils with time relative to the arable control soil, decreasing from 15.3:1 in the control to range between 14.3:1 in the year 4 soil to 12.1:1 in the year 8 soil (**Figure 6.2 B**). No significant differences were measured between the regeneratively managed soils at each time point (**Figure 6.2 B**), with this relative stability in C:N measures relating to broadly proportionate increases in carbon and nitrogen contents of the different soils.

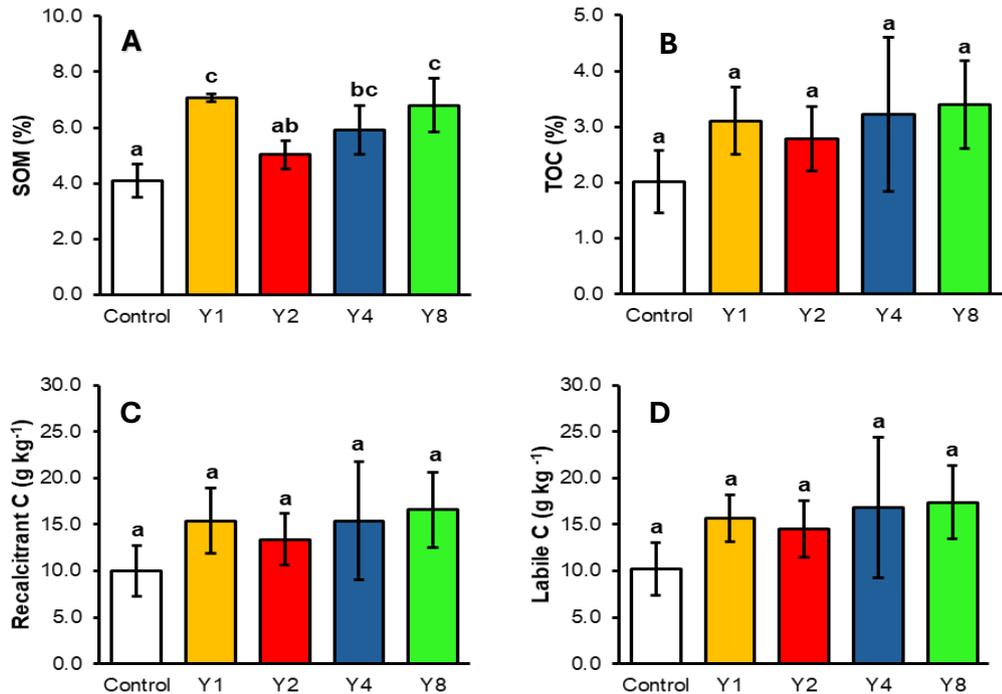


**Figure 6.2:** TON (n = 4) (a); C:N ratio (n = 4) (b); of arable control and regeneratively managed soils. Letter values shown above the bars indicate the significance test (ANOVA) between the data, bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

SOM was observed to increase in a stepwise manner relative to the control, with significant ( $p \leq 0.05$ ) increases in the year 1, 4 and 8 soils (increasing from 4.1% in the control soil to 6.8% in the year 8 soil); this change was not however significant ( $p \geq 0.05$ ) (Figure 6.3 A). Similarly to that observed with TON data, such increases in SOM were likely a consequence of crop residue deposition.

In terms of soil carbon stocks more specifically: TOC was observed to increase with time, from 2.0% in the control soil, to 3.4% in the year 8 soil. However, no significant differences ( $p \geq 0.05$ ) were measured between the control soil and the regeneratively managed soils, nor between the regeneratively managed soils at each time point (Figure 6.3 B). Additionally, both labile and recalcitrant carbon stocks were observed to increase with time under regenerative management, with labile carbon increasing slightly more than recalcitrant carbon (total  $7.2 \text{ g kg}^{-1}$  increase vs.  $6.6 \text{ g kg}^{-1}$  increase). (Figure 6.3 C; D). Labile carbon stocks were observed to increase over the 8 year period of regenerative management, increasing from  $10.2 \text{ g kg}^{-1}$  to  $17.4 \text{ g kg}^{-1}$  (Figure 6.3 C), while recalcitrant carbon was observed to increase from  $10.0 \text{ g kg}^{-1}$  to  $16.6 \text{ g kg}^{-1}$  between the control soil to year 8 soil, respectively (Figure 6.3 D). No significant differences ( $p \geq 0.05$ ) were observed between either labile or recalcitrant carbon

stocks when comparing regeneratively managed soils against the conventional control, or between the regeneratively managed soils at each time point (**Figure 6.3 C; D**).



**Figure 6.3:** SOM (n = 4) (a); TOC (n = 4) (b); Recalcitrant carbon (n = 4) (c); Labile carbon (n = 4) (d); of arable control and regeneratively managed soils. Letter values shown above the bars indicate the significance test (ANOVA) between the data, bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

Overall increases in soil carbon stocks (TOC, SOM, and both labile and recalcitrant carbon) with time emphasise the potential for regenerative transition to deliver increases to soil carbon content. Such increases can help to deliver enhanced ecosystem service outcomes through increases in biodiversity and microbial activity; and through carbon priming (increased labile carbon and organic matter), and long term carbon sequestration and storage (increased recalcitrant carbon) (De Graaff et al., 2010, Lal, 2018b, Ogle et al., 2012, Paustian et al., 2020, Dell'Abate et al., 2003, Martin and Sprunger, 2022, Paustian et al., 2016).

It is highlighted however, that despite overall increases in TOC, recalcitrant carbon, and labile carbon contents, considering a field scaled carbon content (rather than

proportional (% based)) may reflect carbon stocks differently, with potentially significant ( $p \leq 0.05$ ) increases or decreases in the overall carbon stock. Indeed, it is likely that changes in land management and increases in carbon content had commensurate impacts upon soil structuring and bulk density – thus changing the total volume that the soil occupies and hence carbon content on the spatial scale (Ruehlmann and Körschens, 2009, Smith et al., 2020a, Li et al., 2020). Additionally, considering the shallow soil sampling depth and the large root structures of the perennial crops and cover crops, further carbon enrichment may be present at greater depth within the regeneratively managed fields relative to the conventionally managed control – it may be more appropriate to base soil carbon measures upon a standardised sample depth or potentially a horizon based sampling approach for greatest accuracy when considering regenerative approaches.

#### **6.4.2 Bacterial and Fungal Diversity**

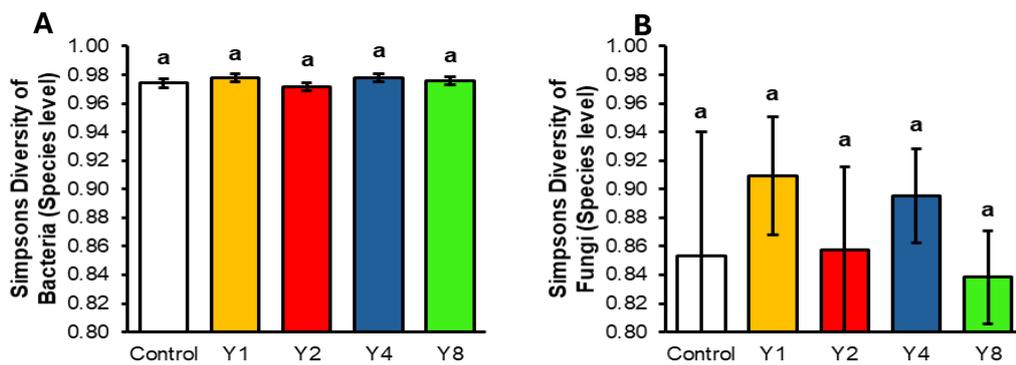
Adoption of regenerative agriculture methods and the subsequent changes in soil properties observed culminated in significant changes to bacterial and fungal community abundance and composition. Species diversity for both bacterial and fungal communities were measured using a Simpsons' diversity index, accounting for both species' evenness and richness within the different sample sites. This data highlighted substantial differences between the bacterial and fungal communities measured across the different sites – reflecting the changes to soil properties with time.

Bacterial species were measured to have a high degree of diversity in all sites, ranging between 0.917 in the year 2 soil, and 0.978 in the year 4 soil (**Figure 6.4 A**).

Species diversity was generally observed to be greater under regenerative management relative to the control soil, with slightly increased diversity observed in the years 1, 4 and 8 soils (0.977, 0.978 and 0.977, respectively) relative to the control (0.974) however, no significant differences ( $p \geq 0.05$ ) were measured between bacterial diversity at any site (**Figure 6.4 A**).

Fungal species diversity showed greater variance with time, as well as within sample sites, with diversity ranging between 0.838 in the year 8 soil and 0.909 in the year 1 soil (**Figure 6.4 B**). While species diversity was generally observed to be greater in the regeneratively managed soils relative to the control (0.853), fungal diversity was observed to peak in year 1 before reducing over time (**Figure 6.4 B**). Overall, however, no significant differences ( $p \geq 0.05$ ) in fungal diversity were observed across the time points (**Figure 6.4 B**).

These disparities between the bacterial and fungal diversity indexes suggest greater diversity, abundance and a more even distribution of bacterial species than fungal species within the different samples, likely influenced by soil property changes. However, the greater fungal diversity variation may have been a result of low resolution fungal species data artificially reducing diversity as multiple species may have only been assessed at a higher clade. This variance within the communities was further evidenced by the number and distribution of bacterial and fungal phyla comparatively: 51 different bacterial phyla, with the top  $\geq 1\%$  showing relatively even abundance over the 8 year transition period; Vs. 20 different fungal phyla, with the top  $\geq 1\%$  showing a more variable abundance and the domination of certain phyla in individual years relative to one another (i.e. relative dominance of *Mortierellomycota* in the year 8 sample) (**Figure SI 6.1; SI 6.2**).



**Figure 6.4:** Simpson's' diversity indices of species level data for bacterial (a) and fungal (b) communities (n= 6) of arable control and regeneratively managed soils. Letter values shown above the bars indicate the significance test (ANOVA) between the data, bars that share a lower-case letter indicate no significant differences ( $P \geq 0.05$ ).

Overall differences between regeneratively managed soils and the conventionally managed control could be seen in the NMDS plots of site vs. bacterial/fungal abundance (Figure SI 6.3 A;B). With a large shift from the control to year 8 data highlighting significant ( $p \leq 0.05$ ) differences between the conventionally managed control soil relative to all regeneratively managed soils of all ages, for both bacterial and fungal communities (Figure SI 6.3 A;B). Additionally, further significant differences ( $p \leq 0.05$ ) were observed between the year 2 and year 8 soils relative to all other sites within the bacterial communities (Figure SI 6.3A), and the year 1 soil relative to all other sites within the fungal communities (Figure SI 6.3B).

### 6.4.3 Microbial Assemblage and Abundance with Time Under Regenerative Agriculture

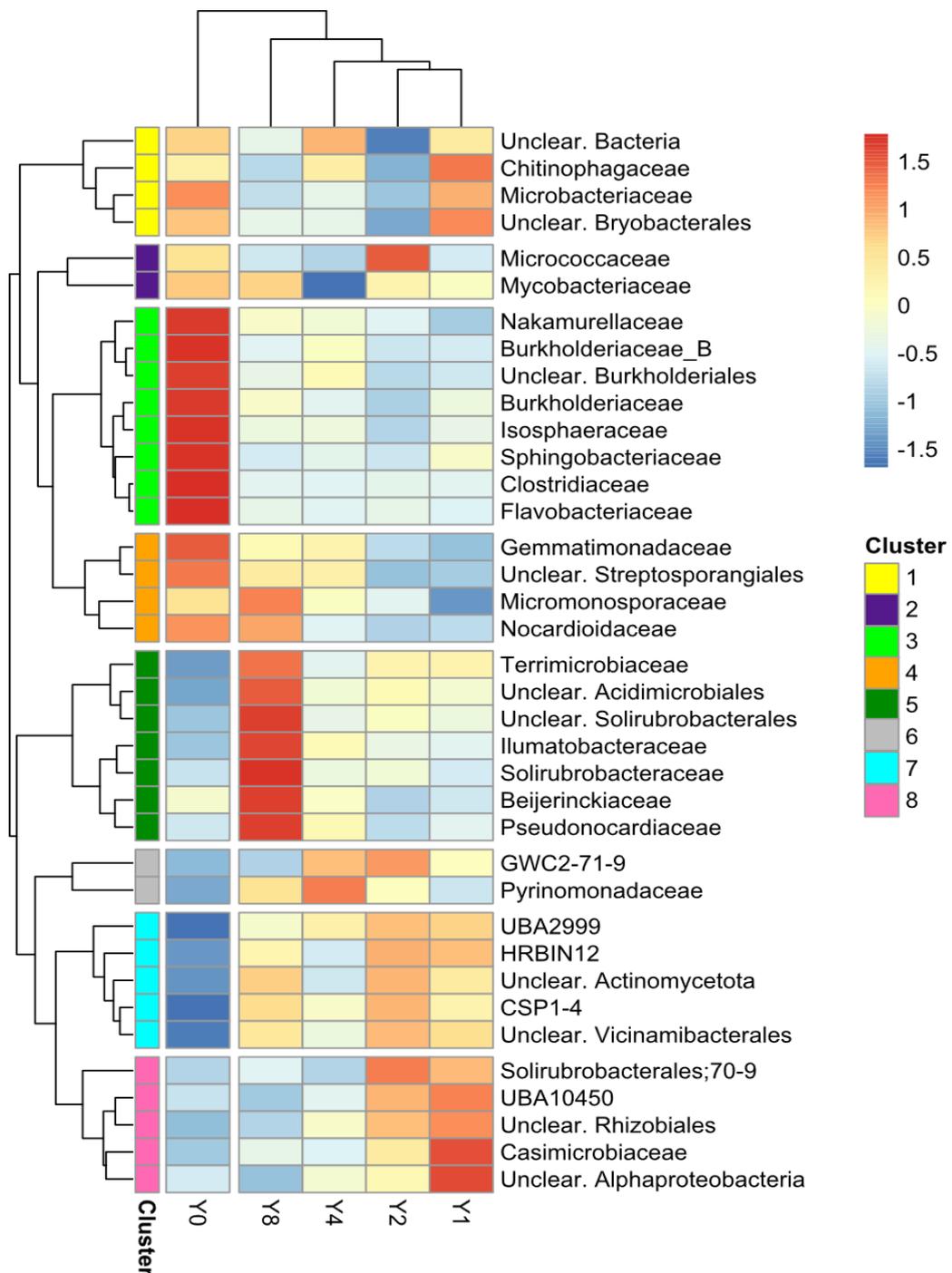
#### 6.4.3.1 Bacteria

A total of 51 different bacterial (16S) phyla were detected across all sites, with the most common (exhibiting a relative abundance of  $\geq 1\%$  at any given site) consisting of; *Actinobacteriota*, *Proteobacteria*, *Bacteroidota*, *Firmicutes*, *Acidobacteriota*,

*Chloroflexi*, *Gemmatimonadota*, *Myxococcota*, *Planctomycetota* and *Verrucomicrobiota* (**Figure SI 6.1**). Among these, *Actinobacteriota* and *Proteobacteria* were the dominant phylae, representing approximately 60% of the total assemblage at each site. No large shifts in bacterial phyla abundance were observed between the selected groups between the conventional control and regeneratively managed soils (**Figure SI 6.1**).

At the family level, heatmap analysis showed the top 37 most abundant bacterial families (relative abundance of  $\geq 1\%$  at any given site) split into 8 discrete clusters as a function of their similarity (**Figure 6.5**). Communities in different clusters were observed to be significantly different ( $p \leq 0.05$ ) from each other.

Changes to bacterial family assemblage were observed with time under regenerative management. In the control soil, the majority of the community was observed in clusters 1 – 4, with the greatest abundance in cluster 3, while families in clusters 5 – 8 were observed to be less abundant, and the lowest abundance in cluster 7 (**Figure 6.5**). Following 8 years of regenerative agriculture practice, family abundance was observed to shift substantially between the different clusters, showing relative enrichment in families of clusters 5 and 7, and reductions in clusters 1 and 3 with time (**Figure 6.5**). Families in cluster 4 were initially observed to decrease following conversion to regenerative agriculture practice in years 1 and 2, before increasing in relative abundance to converge with the control soil (**Figure 6.5**). Additionally, cluster 8 showed a substantial increase in family abundance following 1 year after regenerative agriculture conversion, reducing slowly in the subsequent years to converge with the control soil over time (**Figure 6.5**).



**Figure 6.5:** A heatmap of bacterial family abundance across the different sites (n = 6). Heatmap considers all bacterial families that met or exceeded a threshold of  $\geq 1\%$  of total abundance at any individual site, representing 37 individuals. Data was subsequently normalised and clustered reflecting a hierarchical clustering model using the gap statistic. Clusters represent families with statistically similar community structures at each site, while the heatmap represents relative increases or decreases in standard deviations of the mean abundance of these families.

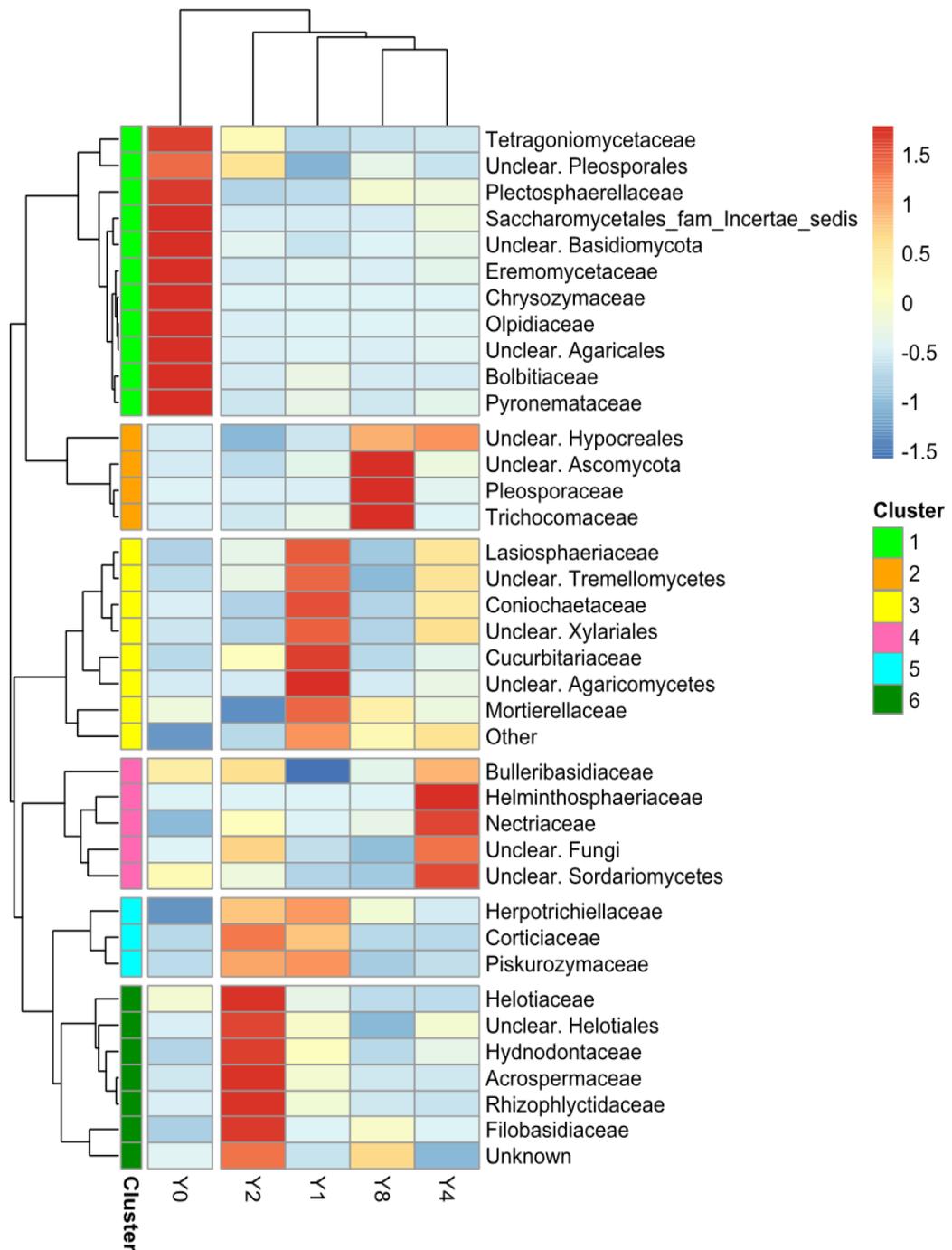
### 6.4.3.2 Fungi

A total of 20 different fungal (ITS) phyla were detected across all sites, with the most common (exhibiting a relative abundance of  $\geq 1\%$  at any given site) consisting of; *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota*, *Olpidiomycota*, *other fungi* and *unclear. fungi/ unknown fungi* (**Figure SI 6.2**). Among these fungal phyla, *Ascomycota* and *Basidiomycota* were observed to be the most abundant, appearing in substantial quantities in all soil samples and representing approximately 60% of total fungal phyla abundance. Substantial differences between the control soil and the year 8 soil were observed for both the *Basidiomycota* and *Mortierellomycota* phylae, with *Basidiomycota* decreasing from 24.1 % of the total community in the control to 4.6% in the year 8 soil, while *Mortierellomycota* was observed to increase over this time from 1.8% to 13.7% (**Figure SI 6.2**). No further large shifts in community composition were observed (**Figure SI 6.2**).

Heatmap analysis of family level data highlighted the 38 most abundant fungal families (relative abundance of  $\geq 1\%$  at any given site) split into 6 discrete clusters as a function of their similarity (**Figure 6.6**). Communities in different clusters were observed to be significantly different ( $p \leq 0.05$ ) from each other. Changes to fungal family assemblage were observed with time under regenerative management. However, the dominance of certain clusters in sites of different ages was more evident for fungal analysis than bacterial (**Figure 6.5; 6.6**).

In the control soil, the majority of the community was held within cluster 1, measuring the highest overall abundance, comparatively, clusters 2 – 6 observed lower and approximate mean phyla abundances. However no specific cluster stood out as the least abundant (**Figure 6.6**). After 1 year of regenerative practice, the

abundance of families in cluster 3 and 5 were observed to increase substantially from slightly below mean abundance toward relative enrichment, with the greatest increases observed in cluster 3 (**Figure 6.6**). Additionally, cluster 1 was observed to decrease substantially from the most abundant in the control soil, to below mean abundance (**Figure 6.6**). In the year 2 soil Cluster 6 was observed to be the most abundant, while cluster 3 reduced substantially from dominance in the year 1 soil to relative depletion (**Figure 6.6**). In the year 4 soil, cluster 4 was observed to become the most dominant assemblage, increasing from slightly below mean abundance toward enrichment, additionally, small increases in abundance were measured in cluster 3 relative to the year 2 soil, while cluster 6 was measured to deplete substantially (**Figure 6.6**). After 8 years of regenerative management cluster 2 was observed to contain the most abundant phyla, with relative reductions to below mean abundance measured in all other clusters (**Figure 6.6**).



**Figure 6.6:** A heatmap of Fungal family abundance across the different sites ( $n = 6$ ). Heatmap considers all bacterial families that met or exceeded a threshold of  $\geq 1\%$  of total abundance at any individual site, representing 38 individuals. Data was subsequently normalised and clustered reflecting a hierarchical clustering model using the gap statistic. Clusters represent families with statistically similar community structures at each site, while the heatmap represents relative increases or decreases in standard deviations of the mean abundance of these families.

#### 6.4.4 Microbial interactions with Soil Properties

When considering the influence of changes in specific soil properties upon microbial family abundance, bacterial and fungal groups were seen to either, significantly ( $p \leq 0.05$ ) positively or negatively correlate, or have no significantly observable influence ( $p \geq 0.05$ ). Spearman's rank correlation heatmap analysis highlighted these differences, showing the potential links between bacterial and fungal family abundance and change in soil properties over time as a function of regenerative management. Families observed to correlate positively suggested that changes in soil properties were beneficial to their health and function, while those correlated negatively suggested changes were detrimental.

##### 6.4.4.1 Bacterial Families and Soil Properties

Spearman's rank correlation of bacterial families with soil properties split the 37 bacterial families observed at  $\geq 1\%$  of total abundance into 5 distinct clusters based on their interactions with soil properties over the 8 year period. Soil properties were also clustered by their likeness creating 6 different soil property groups, of C:N; pH; SOM; soil moisture and TON; recalcitrant carbon content; and labile carbon content and TOC.

Cluster 1 consisted of 4 different bacterial families (**Figure 6.7**). No significant differences in correlation ( $p \geq 0.05$ ) were observed between soil C:N (positively correlated), soil pH (mixed correlations), SOM (positively correlated), soil moisture and TON (positively correlated) (**Figure 6.7**). TOC, recalcitrant carbon and labile carbon stocks were observed to correlate positively with all cluster 1 bacterial families, with significant positive correlation ( $p \leq 0.05$ ) observed with all three soil properties for the

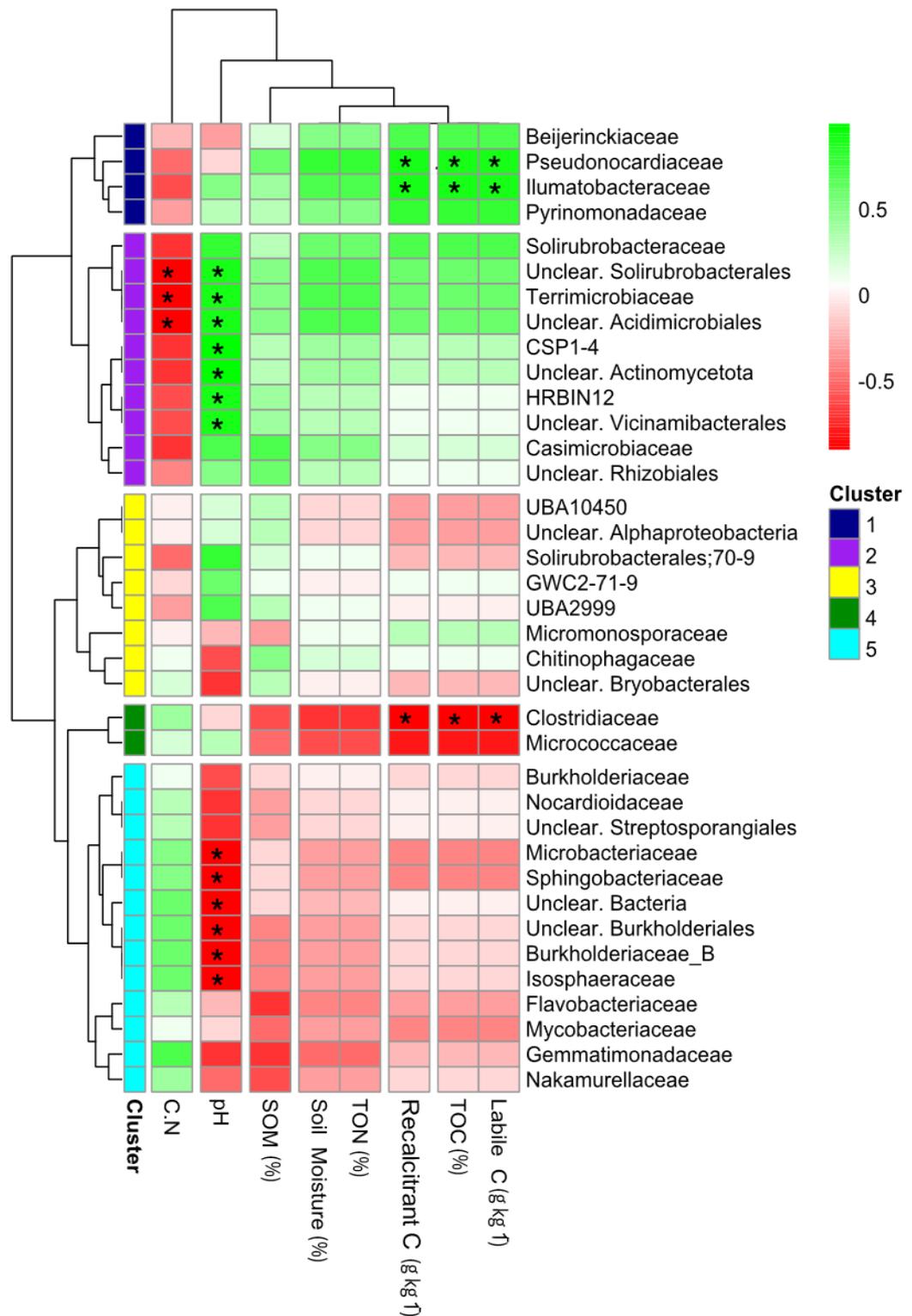
*Pseudonocardiaceae* and *Ilumatobacteraceae* families (**Figure 6.7**).

Cluster 2 consisted of 10 different bacterial families (**Figure 6.7**). No significant correlations ( $p \geq 0.05$ ) were observed between labile carbon stocks and TOC (positively correlated or uncorrelated), recalcitrant carbon (positively correlated or uncorrelated), soil moisture and TON (positively correlated), and SOM (positively correlated) (**Figure 6.7**). Soil pH was observed to correlate positively with abundance of all cluster 2 families, with significant positive correlation ( $p \leq 0.05$ ) in the *unclear Solirubrobacterales*, *Terrimicrobiaceae*, *unclear Acidimicrobiales*, *CSP1-4*, *unclear Actinomycetota*, *HRBIN12*, and *unclear Vicinamibacterales* families (**Figure 6.7**). Additionally, soil C:N ratio was observed to correlate negatively with all cluster 2 families, with significant negative correlation ( $p \leq 0.05$ ) in the *Unclear Solirubrobacterales*, *Terrimicrobiaceae*, and *unclear Acidimicrobiales* families (**Figure 6.7**).

Cluster 3 consisted of 8 different bacterial families; however, no significant correlations ( $p \geq 0.05$ ) were observed between any of the soil properties and bacterial abundance (**Figure 6.7**). Soil C:N ratios were observed to weakly negatively correlate or show no correlation, while all other soil properties showed mixed positive and negative weak correlations with family abundance (**Figure 6.7**).

Cluster 4 consisted of 2 different bacterial families (**Figure 6.7**). No significant differences ( $p \geq 0.05$ ) were measured between soil C:N ratios (weak positive correlation), soil pH (weak mixed correlation), SOM (negative correlation), and soil moisture and TON (negative correlation) (**Figure 6.7**). Recalcitrant, labile and TOC contents were observed to negatively correlate with both bacterial families in cluster 4, with this being significant ( $p \leq 0.05$ ) in the *Clostridiaceae* family (**Figure 6.7**).

Cluster 5 consisted of 13 different bacterial families (**Figure 6.7**). No significant differences ( $p \geq 0.05$ ) were observed between soil C:N ratios (positively correlated or uncorrelated), SOM (negatively correlated), soil moisture and TON (negatively correlated), recalcitrant carbon content (negatively correlated or uncorrelated), and labile carbon content and TOC (negatively correlated or uncorrelated) (**Figure 6.7**). Soil pH was observed to correlate negatively with all families in cluster 5, with significant ( $p \leq 0.05$ ) negative correlation in the *Microbacteriaceae*, *Sphingobacteriaceae*, *unclear Burkholderiales*, *Burkholderiaceae\_B*, and *Isosphaeraceae* families, as well as the wider *unclear bacteria* group (**Figure 6.7**).



**Figure 6.7:** Spearman's rank correlation analysis of soil properties vs. the bacterial family abundance. Families were selected based on meeting or exceeding a threshold of  $\geq 1\%$  of total abundance at any individual site, representing 37 individuals and 8 soil properties. Spearman's rank data was clustered reflecting a hierarchical clustering model using the gap statistic. Clusters represent families with statistically similar community structures at each site, while the heatmap represents relative increases or decreases in standard deviations of the mean abundance of these families vs. soil property. Asterisks (\*) indicate soil properties exerted a significant impact ( $p \leq 0.05$ ) upon family abundance.

#### 6.4.4.2 Fungal Families and Soil Properties

Spearman's rank correlation of fungal families with soil properties split the 38 fungal families observed at  $\geq 1\%$  of total abundance into 5 distinct clusters based on their interactions over the 8 year period. Soil properties were also clustered by their likeness creating 6 different soil property groups, of C:N, soil moisture, SOM, labile carbon content and TOC, recalcitrant carbon content and soil pH and TON.

Cluster 1 consisted of 4 different fungal families (**Figure 6.8**). No significant correlations ( $p \geq 0.05$ ) were observed between soil C:N ratios (weak mixed correlations), soil moisture (weak mixed correlations), SOM (weak mixed correlations), recalcitrant carbon stocks (negative and weak negative correlations), and soil pH and TON (negative correlations) (**Figure 6.8**). Both TOC and labile carbon content were observed to have negative correlations upon the different families in cluster 4, with both having a significant negative impact ( $p \leq 0.05$ ) upon the *Plectosphaerellaceae* family (**Figure 6.8**).

Cluster 2 consisted of 10 different fungal families, with each soil property having a significant impact ( $p \leq 0.05$ ) on one or more fungal families (**Figure 6.8**). Soil C:N ratios were observed to have a positive effect on all fungal families, with significant correlations ( $p \leq 0.05$ ) observed between C:N and the *unclear Basidiomycota* and *unclear Sordariomycetes* families (**Figure 6.8**). Soil moisture was observed to negatively correlate with all fungal families in cluster 2, with significant negative impacts ( $p \leq 0.05$ ) also measured on the *unclear Basidiomycota* and *unclear Sordariomycetes* families (**Figure 6.8**). SOM was observed to negatively correlate with all fungal families, with some weak and some strong negative correlations, 3 families were significantly negatively correlated ( $p \leq 0.05$ ) with SOM, including the

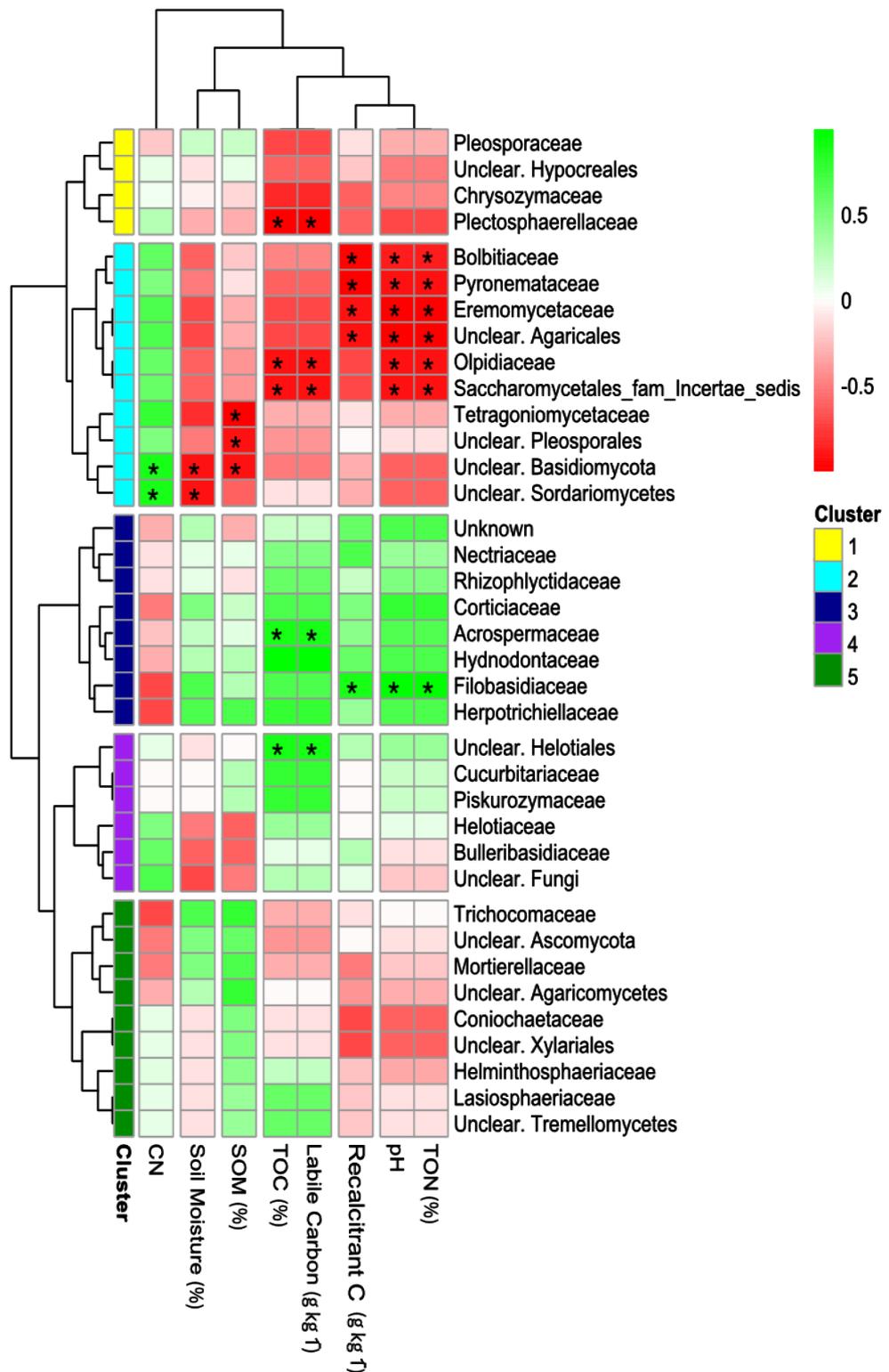
*Tetragonomycetaceae*, unclear *Pleosporales*, and unclear *Basidomycota* families (**Figure 6.8**). TOC and labile carbon content were observed to correlate negatively with all families, ranging from weak negative to strong negative, additionally, both *Oplidiaceae* and *Saccharomycetales\_fam\_Incertae\_sedis* families were observed to be significantly ( $p \leq 0.05$ ) impacted (**Figure 6.8**). Recalcitrant carbon was observed to negatively correlate or show no correlation with the fungal families in cluster 2, with significant negative correlation ( $p \leq 0.05$ ) with the *Bolbitaceae*, *Pyronemataceae*, *Eremomycetaceae* and unclear *Agaricales* families (**Figure 6.8**). TON and soil pH were found to negatively correlate with all families in cluster 2 ranging from weak correlations to strong correlations, additionally both soil properties were observed to significantly impact upon the *Bolbitaceae*, *Pyronemataceae*, *Eremomycetaceae* *Oplidiaceae*, and *Saccharomycetales\_fam\_Incertae\_sedis* families (**Figure 6.8**).

Cluster 3 consisted of 8 different fungal families (**Figure 6.8**). No significant correlations ( $p \geq 0.05$ ) were observed between the soil C:N ratios (weak negative correlation), soil moisture (weak positive correlation), and SOM (weak mixed correlations) (**Figure 6.8**). TOC and labile carbon positively (strong) correlated with all families; this was significant ( $p \leq 0.05$ ) in the *Acrospermaceae* family (**Figure 6.8**). Additionally, pH and TON, and recalcitrant carbon were observed to correlate positively (strong) in all families, with significant ( $p \leq 0.05$ ) positive correlation in the *Filobasidiaceae* family (**Figure 6.8**).

Cluster 4 consisted of 6 different fungal families (**Figure 6.8**). No significant correlations ( $p \geq 0.05$ ) were observed between the soil C:N ratios (weak positive or no correlation), soil moisture (weak negative or no correlation), SOM (mixed weak positive and weak negative correlations), recalcitrant carbon (weak positive or no

correlation) and soil pH and TON (mixed weak positive and weak negative correlations) (**Figure 6.8**). TOC and labile carbon were observed to positively correlate with all families, ranging from weak positive correlation to strong positive correlation, additionally this was significant in the *unclear Helotiales* family (**Figure 6.8**).

Cluster 5 consisted of 9 different fungal families (**Figure 6.8**). No significant impacts ( $p \geq 0.05$ ) upon correlation were observed between any of the soil properties and fungal families in this cluster (**Figure 6.8**). Weak positive correlations were observed between the families and SOM, while weak negative correlations were observed for recalcitrant carbon, soil pH and TON, additionally, soil C:N ratios, soil moisture and labile carbon and TOC observed mixed correlations (**Figure 6.8**).



**Figure 6.8:** Spearman's rank correlation analysis of soil properties vs. fungal family abundance. Families were selected based on meeting or exceeding a threshold of  $\geq 1\%$  of total abundance at any individual site, representing 38 individuals and 8 soil properties. Spearman's rank data was clustered reflecting a hierarchical clustering model using the gap statistic. Clusters represent families with statistically similar community structures at each site, while the heatmap represents relative increases or decreases in standard deviations of the mean abundance of these families vs. soil property. Asterisks (\*) indicate soil properties exerted a significant impact ( $p \leq 0.05$ ) upon family abundance.

Overall, changes in soil properties with regenerative agriculture management significantly influenced ( $p \leq 0.05$ ) the abundance of 16 different bacterial families, and 14 different fungal families, with some families significantly impacted ( $p \leq 0.05$ ) by the changes in multiple soil properties (**Figure 6.7; 6.8; Table SI 6.1**). Of these soil properties, changes in soil pH was observed to have the greatest overall impact upon significant ( $p \leq 0.05$ ) abundance change in both bacteria and fungi, highlighting soil pH as one of the foundational soil properties influencing soil microbial community composition and abundance (Rousk et al., 2010) (**Table SI 6.1**). Additionally, changes in SOM, soil moisture and TON were observed to only significantly ( $p \leq 0.05$ ) influence the abundance of fungal families, with much of this impact being negative (**Table SI 6.1**). TOC and labile carbon stocks were observed to significantly influence ( $p \leq 0.05$ ) the same bacterial and fungal families. Similarly, recalcitrant carbon stocks showed significant impacts ( $p \leq 0.05$ ) upon the same bacterial families as TOC and labile carbon, but impacting different fungal families. Given the similarities in the families influenced by these soil properties it is likely that they function as specialist decomposers, thus increasing in abundance with increased carbon input – however differences between the increased fungal communities of TOC/labile carbon and recalcitrant carbon suggest these families are specialised to different forms of carbon relative to their stability.

### **6.5 Linking Soil Biodiversity and Carbon to Land Management Practice**

Soil organisms play a crucial role in ecosystems, yet their contributions to soil health and function are often overlooked and poorly understood in agricultural management strategies (Bender et al., 2016, Tardy et al., 2015, Wagg et al., 2014, Rousk et al., 2010,

Jiao and Lu, 2020).

It is generally considered that greater biodiversity offers a greater potential for environmental benefit, given the specialisms of individual species, the ability to promote multiple ecosystem services simultaneously, and the effect of compounding community influences (Nielsen et al., 2011, Thiele-Bruhn et al., 2012, Bender et al., 2016). Furthermore, greater diversity affords an amount of redundancy, or functional equivalence in the system (Strickland et al., 2009, Jurgburg and Salles, 2015). Thus, where a community member is impaired/lost, another member can continue to deliver a given function. Soil biodiversity is responsible for catalysing ecosystem services such as: organic matter decomposition, carbon and nutrient cycling, soil formation, hydrological function, bioremediation, and plant-soil interactions (Nielsen et al., 2015, Nielsen et al., 2011, Zhang et al., 2020, Creamer et al., 2016). Biodiverse soils also offer significant potential from an agronomic perspective, exhibiting a greater nutrient use efficiency and plant disease suppression (Thiele-Bruhn et al., 2012). Furthermore, soil biodiversity contributes significantly toward soils carbon sequestration and storage potential (possibly accounting for up to 82% of soil carbon cycling variation), with much of this influenced by the proportion of fungi and overall microbial diversity and species richness (Bender et al., 2016, Creamer et al., 2016, Tardy et al., 2015, Six et al., 2006). As such, soil biodiversity is highlighted as a key determinant of soil health, quality and function (Creamer et al., 2016).

Yet, the degradation of soil carbon stocks as a result of intensive land management has culminated in significant damage to soils and the environment; impairing ecosystem service delivery and contributing towards declines in species diversity and abundance (Bender et al., 2016, Stavi and Lal, 2015, Aytenuw, 2021, Creamer et al.,

2016). Conventional agriculture management techniques such as soil disturbance and tillage can significantly impact the physical and biological properties of the soil and degrade soil carbon content, leading to declines in productivity (Khangura et al., 2023, Adhikari and Hartemink, 2016b, Lal, 1993). Additionally, such practices directly impact soil organisms by causing physical harm, death, or increased vulnerability to predation, with this effect especially pronounced in fungi due to the destruction of their hyphal networks (Thiele-Bruhn et al., 2012).

Considering the present dual crises of climate change and biodiversity loss, actions which redress these issues, through restoration and enhancement of soil carbon stocks, and improving outcomes for soil biodiversity, offer significant potential for environmental enhancement (Adhikari and Hartemink, 2016b, Benayas et al., 2009, Power, 2010). Herein, transition toward regenerative agriculture may offer significant opportunities, given the holistic and ecologically-focussed practices which seek to improve soil health and function, and thus enhance wider ecosystem service outcomes and improve soil biodiversity outcomes (Moyer et al., 2020, Paustian et al., 2016, Gosnell et al., 2019, Aytenuw, 2021, O'Donoghue et al., 2022, Schreefel et al., 2020).

Indeed, adopting practices such as minimum or no till, alongside reductions in agrochemical inputs have been observed to improve upon soil carbon stocks and carbon stability in the long term, delivering significant carbon sequestration potential (Gál et al., 2007, Ogle et al., 2012, Paustian et al., 2020). Furthermore, favouring regenerative practices which: minimise soil disturbance and tillage, are inclusive of cover crops and adopt organic farming methods, can help to improve soil biodiversity and promote a shift in community composition and structure, culminating in

enhanced soil carbon storage and stability, and subsequently sequestration potential (Six et al., 2006, Khangura et al., 2023).

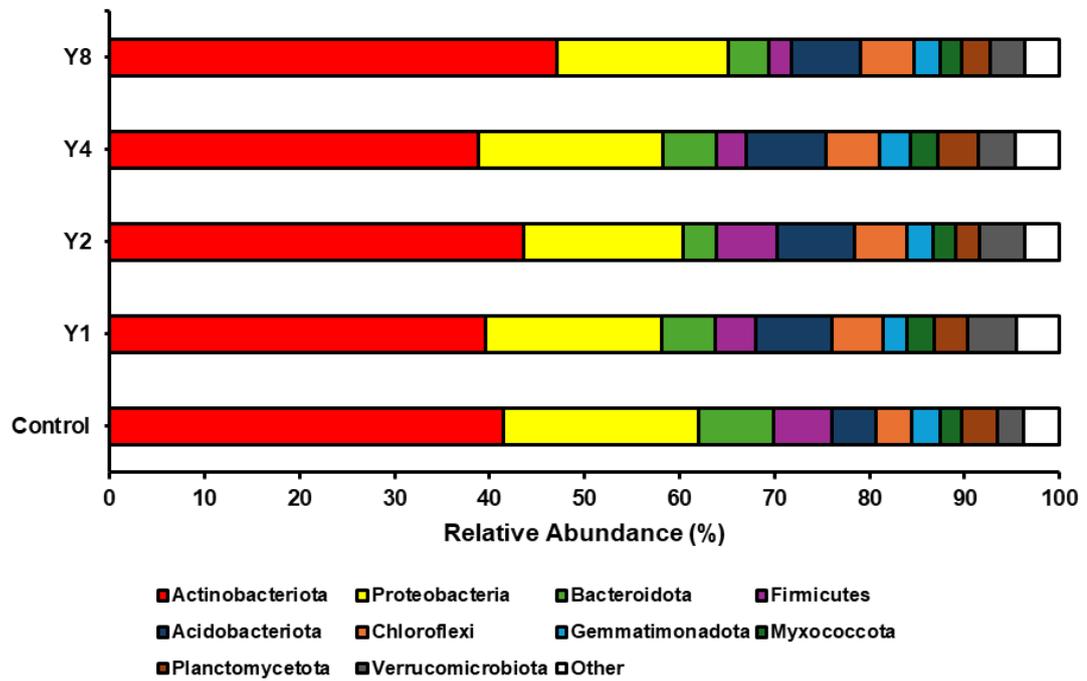
As such, improvements to soil biodiversity offer significant potential to further enhance agricultural, environmental and soil sustainability (Bender et al., 2016). However, for this to be effectively achieved a greater understanding of management processes and soil properties which drive microbial community composition is required (Rousk et al., 2010, Jiao and Lu, 2020, Tardy et al., 2015). Thus, by placing a focus on measuring and assessing these potential links, we will be better equipped to determine which practices are most beneficial, or what actions may be taken to achieve specific soil property and biodiversity outcomes, and delivering these through targeted interventions (Bender et al., 2016). Such an outcome will help to discern and subsequently deliver optimum soil management techniques that achieve soil property improvements, biodiversity net-gain and carbon sequestration for overall soil ecosystem service enhancement.

## **6.6 Conclusions**

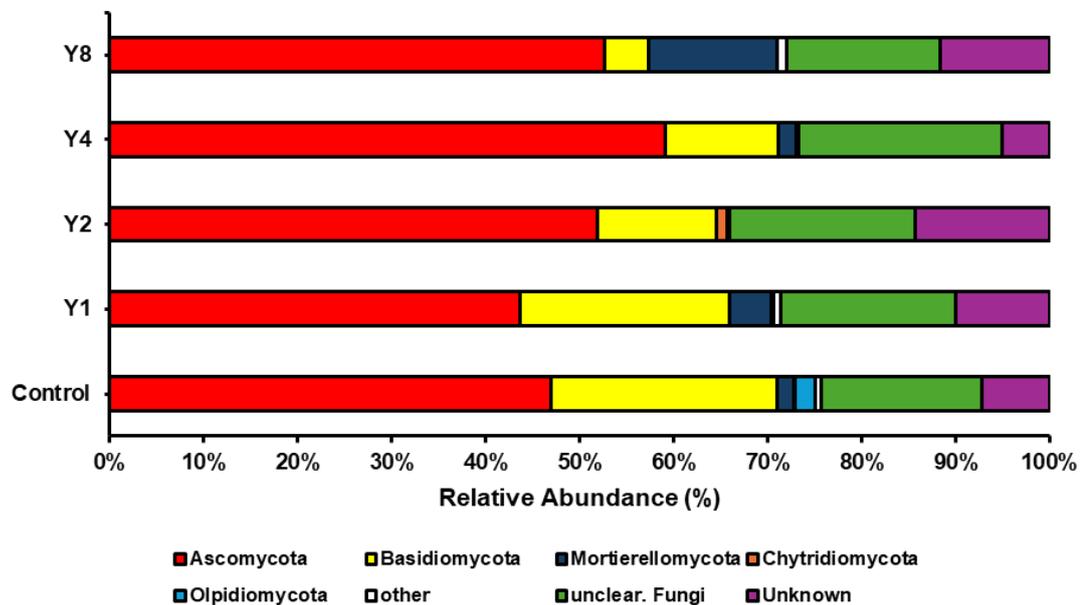
Transition from conventional management towards regenerative agriculture practice highlights the potential of these more holistic methods to deliver beneficial changes to soil properties and carbon stocks, alongside the commensurate effects of these changes upon soil microbial community composition and abundance. Bacterial and fungal communities responded to this transition with significant ( $p \leq 0.05$ ) shifts in community structure, abundances and functions after 8 years of regenerative management relative to conventional management. These community shifts were observed despite no significant changes ( $p \leq 0.05$ ) in the overall diversity or evenness

of species. By bridging the gap between soil health, soil property improvements, carbon uplift and impacts upon biodiversity, regenerative systems offer a promising pathway toward sustainable agriculture and environmental stewardship. However, given the highly complex nature of soil biodiversity dynamics further research will be required to better link these seemingly disparate subjects. Yet, nurturing this approach may highlight and provide significant opportunities to improve delivery of a range of environmental and ecosystem services, soil carbon sequestration, climate change adaptation and mitigation and biodiversity net-gain outcomes - providing manifold benefits and delivering win-win environmental outcomes.

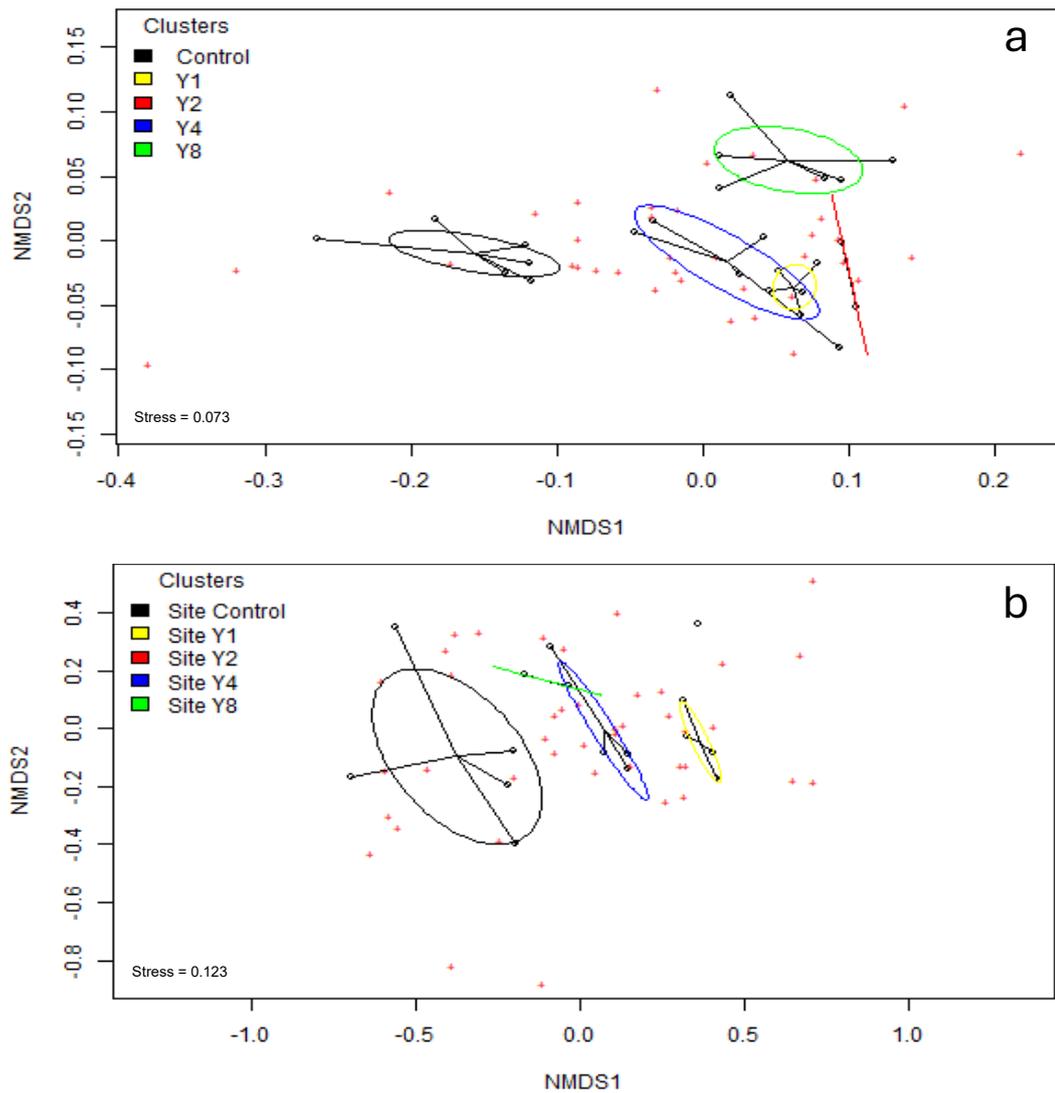
## 6.7 Supporting Information



**Figure SI 6.1:** Relative abundance (%) of the main bacterial communities at phylum level across arable control and regeneratively managed soils. Bar chart considers all bacterial Phyla that met or exceeded a threshold of  $\geq 1\%$  of total abundance at any individual site, representing 11 phyla



**Figure SI 6.2:** Relative abundance (%) of the main Fungal communities at phylum level across arable control and regeneratively managed soils. Bar chart considers all fungal phyla that met or exceeded a threshold of  $\geq 1\%$  of total abundance at any individual site, representing 8 phyla



**Figure SI 6.3:** NMDS plots of bacterial diversity vs. site (a), and fungal diversity vs. site (b) ( $n = 6$ ). NMDS plotted using Bray-Curtis distance, spider plots connect individual sample data points of the same site to the average position, while ellipses represent the confidence region of the average position ( $p \leq 0.05$ ). Ellipses that overlap are not significantly different ( $p \geq 0.05$ ).

**Table SI 6.1: Bacterial and fungal families significantly impacted ( $p \leq 0.05$ ) by changes to soil properties under regenerative management**

Soil property	Significant Change ( $p \leq 0.05$ )	Significantly Increased Abundance ( $p \leq 0.05$ )		Significantly Decreased Abundance ( $p \leq 0.05$ )	
		Bacteria	Fungi	Bacteria	Fungi
CN	None		<i>Unclear Basidiomycota</i> <i>Unclear Sordariomycetes</i>	<i>Unclear Solirubrobacterales</i> , <i>Terrimicrobiaceae</i> <i>Unclear Acidimicrobiales</i>	
pH	Increase	<i>Unclear Solirubrobacterales</i> , <i>Terrimicrobiaceae</i> <i>Unclear Acidimicrobiales</i> CSP1-4 <i>Unclear Actinomycetota</i> HRBIN12 <i>Unclear Vicinamibacterales</i>	<i>Filobasidiaceae</i>	<i>Microbacteriaceae</i> <i>Sphingobacteriaceae</i> <i>Unclear Burkholderiales</i> <i>Burkholderiaceae_B</i> <i>Isosphaeraceae</i> <i>Unclear Bacteria</i>	<i>Bolbitaceae</i> <i>Pyronemataceae</i> <i>Eremomycetaceae</i> <i>Oplidiaceae</i> <i>Saccharomycetales_fam_Incertae_sedis</i>
SOM	Increase				<i>Tetragonomycetaceae</i> <i>unclear Pleosporales</i> <i>unclear Basidiomycota</i>
Moisture	Increase				<i>unclear Basidiomycota</i> <i>unclear Sordariomycetes</i>
TON	None		<i>Filobasidiaceae</i>		<i>Bolbitaceae</i> , <i>Pyronemataceae</i> , <i>Eremomycetaceae</i> <i>Oplidiaceae</i> , <i>Saccharomycetales_fam_Incertae_sedis</i>
Recalcitrant	None	<i>Pseudonocardiaceae</i> <i>Ilumatobacteraceae</i>	<i>Filobasidiaceae</i>	<i>Clostridiaceae</i>	<i>Bolbitaceae</i> , <i>Pyronemataceae</i> , <i>Eremomycetaceae</i> <i>unclear Agaricales</i>
TOC	None	<i>Pseudonocardiaceae</i> <i>Ilumatobacteraceae</i>	<i>Plectosphaerellaceae</i> <i>Acrospermaceae</i> <i>unclear Helotiales</i>	<i>Clostridiaceae</i>	<i>Oplidiaceae</i> <i>Saccharomycetales_fam_Incertae_sedis</i>
Labile	None	<i>Pseudonocardiaceae</i> <i>Ilumatobacteraceae</i>	<i>Plectosphaerellaceae</i> <i>Acrospermaceae</i> <i>unclear Helotiales</i>	<i>Clostridiaceae</i>	<i>Oplidiaceae</i> <i>Saccharomycetales_fam_Incertae_sedis</i>



## **Chapter 7: Conclusions and Suggestions for Further Work**

The research presented in this thesis contributes information towards the understanding and application of agricultural methods with potential to recarbonise soil, thus supporting uplifts in soil carbon stocks, improvements to soil properties and enhanced provision of soil ecosystem services.

Within this research four field experiments were conducted to evaluate the potential of two soil recarbonisation methods in their efficacy of soil carbon uplift and ecosystem service enhancement. These experiments aimed to acknowledge some of the potential differences between site, soil type, environment and agricultural context as well as carbon permanence and influence upon soil properties, to provide a more insightful and nuanced view of soil recarbonisation potential. Outcomes regarding the two methods evaluated (PC soil amendment application, and regenerative agriculture practice) are summarised and discussed below with respect to hypotheses set (**Page 27**) and recommendations for further research.

### **7.1 PC soil amendment application**

PC was applied to the soils of two separate fields of differing soil typologies, clay soil (**Chapter 3**) and sandy soil (**Chapter 4**), in varying quantities of 50 to 200 t ha<sup>-1</sup>, and was evaluated for its influence upon soil carbon stock, long term carbon storage potential and ability to enhance a variety of ecosystem services.

In line with the hypotheses proposed (**Chapters 3 and 4**), PC application was observed to offer an effective means of significantly improving ( $p \leq 0.05$ ) soil carbon stocks in both the short and long term, as well as significantly enhancing ( $p \leq 0.05$ ) soil bulk density and soil hydrological properties on both clay and sandy soil types. In addition to this, PC applications were observed to increase soil nutrient levels and act

as a fertilising agent. PC was also observed to afford significant ( $p \leq 0.05$ ) improvements to soil hydrological function, specifically, water infiltration and water storage potential. These outcomes are particularly significant as they evidence resilience and tolerance in drought prone soils (**Chapter 4**). In general, higher application doses of PC delivered greater improvements to soil properties on both clay and sand rich soils. However, it was acknowledged that even the standard treatment dosage of  $50 \text{ t ha}^{-1}$  delivered many significant ( $p \leq 0.05$ ) improvements to the amended soils. When analysed for long term carbon stability, treatment with PC was observed to offer substantial long term carbon storage and stability on both clayey and sandy soils, highlighting opportunities for soil carbon sequestration and climate change mitigation.

Given the potential for increased amendment feedstock size, little requirement for change to standard operating procedures, and benefits accrued for edaphic outcomes, the increased use of soil amendments such as PC represent a significant win-win outcome for more sustainable agricultural soil management practice, ecosystem service delivery and climate change mitigation. Further work to expand upon the results of these studies could include:

- i) Continuing the study and examination of the PC soil amendment for its influence upon additional ecosystem services such as biodiversity and crop yield potential/productivity
- ii) Investigation of a range of other soil amendments for their capacity to improve soil carbon stocks, considering their carbon prognoses and influence upon ecosystem service provision
- iii) Further consideration of the differing types of PC product relating to their

production processes, and subsequent evaluation of their respective edaphic impacts

## 7.2 Regenerative Agriculture Practice

Transitioning land management practice toward regenerative agriculture principles specifically focussed upon the use of; no-till methods, significant reductions in agrochemical inputs, the use of perennial crops, and year-round soil cover. These practices were examined for their influence upon soil properties, carbon storage and stability, soil aggregate formation (**Chapter 5**), and soil microbial biodiversity (**Chapter 6**), to enhance the delivery of ecosystem services, with increasing time under regenerative management (up to 8 years).

In line with the hypotheses proposed (**Chapters 5 and 6**); adoption of regenerative agriculture principles was found to have significant ( $p \leq 0.05$ ) influence upon soil carbon stocks, soil aggregate fractions and stability, soil bulk density, and soil biodiversity community structures.

Conversion of conventionally managed arable land to a regenerative no-till system culminated in the significant reduction ( $p \leq 0.05$ ) of soil bulk density, indicating improved soil structure, corroborated by significant shifts ( $p \leq 0.05$ ) in the proportion of stable and non-stable soil aggregates with time. When assessed for carbon stability, recalcitrant carbon was observed to decrease (not significantly ( $p \geq 0.05$ )) over the 7 year period, while labile carbon increased significantly ( $p \leq 0.05$ ). Additionally, it was observed that the amount of carbon held within the stable aggregate fractions shifted significantly ( $p \leq 0.05$ ) with time, with this shift associated with enhanced storage of labile carbon fractions. These outcomes highlighting the potential for occlusion and

stabilisation of carbon within soil structures that are promoted by regenerative approaches to agriculture, and hence providing carbon stability. When considering the impacts upon soil biodiversity; adoption of regenerative principles was observed to have no significant impact ( $p \geq 0.05$ ) on overall species diversity and evenness, but significant impacts ( $p \leq 0.05$ ) upon the abundance of specific bacterial and fungal groups as a function of changing soil properties. Such results suggested shifts in community composition and structure linked to environmental function, and highlighting how specific microbial family groups may be increased or decreased in their abundance given changes in individual soil properties.

Overall, the adoption of these regenerative agriculture practices and reversion away from conventional agriculture was seen to provide significant benefits ( $p \leq 0.05$ ) to soil properties, carbon stocks and carbon stability, while also significantly ( $p \leq 0.05$ ) influencing soil microbial community composition. Such results highlight the potential of these regenerative agriculture practices to deliver soil recarbonisation and ecosystem service enhancements – whilst emphasising the importance of soil biodiversity and the need for this to be better considered in decision making given the influence biodiversity exerts over wider ecosystem service functions. Further work to expand upon the results of these studies could include:

- i) Examine regenerative agriculture practices for their influence on additional ecosystem services such as soil hydrology, fertility and crop yield potentials/productivity,
- ii) Examine the extent to which these regenerative practices may influence soil carbon stock growth and prognosis, and aggregate stability in an annual cropping context

### **7.3 Comparison of Outcomes with PC and Regenerative Agriculture**

When considered together, these two methods can offer significant potential for soil recarbonisation and ecosystem service enhancement. The findings from both sets of trials highlight the potential to deliver upon these goals in a variety of different agricultural and edaphic contexts for a variety of different ecosystem services. Together, both soil amendment application (PC) and regenerative agriculture practice offer significant scope for wider adoption, from the individual farm scale to national scale projects. The amount and variety of different soil amendments available to land managers is increasing as a result of efforts to create a more circular economy and improve resource efficiency. While opportunities to adopt regenerative agriculture principles are increasing as a result of the growing popularity of these methods and exposure as an economically and environmentally viable alternative to conventional agriculture. Furthermore, there exists the opportunity to link both regenerative practices and soil amendment use together. This potential for synergistic management may present in the form of using a mix of both practices together.

### **7.4 Intended Outcomes**

To date, analysis of field trials which examine soil amendment additions and regenerative agriculture have become somewhat commonplace. However, significant gaps in the research have remained, pertaining to practical soil recarbonisation potential and the impact of soil property changes upon ecosystem service delivery. This is particularly true for differing agricultural and soil contexts, understandings of the potential longevity and prognosis of carbon stocks and impacts upon soil biodiversity. Resulting from this lack of clarity, there persists reservation in the support

for recarbonisation and ecosystem service enhancement methods from both a policy and direct-action point of view. These likely reducing their uptake at scale. Thus, actions which improve confidence in these methods (improving the body of research, disseminating research to land practitioners and policymakers, adopting standardised approaches to soil property determination, and providing economic incentives) will help to establish soil recarbonisation as a credible method for soil and ecosystem service enhancement. Key to this however is the establishment of robust, reproducible and standardised analytical methods for soil carbon evaluation, with an appreciation that, when viewed through an ecosystem service lens, not all carbon is equal.

Considering this, the research presented in this thesis has explored the importance of methods beyond the fundamental assessment of soil carbon content/stock. Supporting the need for stability-based soil carbon measurements, delineating between labile carbon (to support soil biodiversity enhancements), and recalcitrant carbon stocks (to support carbon sequestration), given their different residence times within the soil. Providing these more accurate carbon determination methods and tethering the measurement of soil carbon to robust carbon stability prognoses will increase confidence and trust in soil carbon sequestration as a credible option for climate change mitigation and improvement of ecosystem services. It is hoped that this increased trust will result in the creation of additional soil recarbonisation and sequestration projects, helping us to meet our climate targets, and presenting opportunities for manifold environmental enhancements. Furthermore, it is hoped that the results of these experiments may be used to help facilitate the creation and integration of land management practice and policy recommendations; such that

defined environmental outcomes (such as recarbonisation of degraded soils, hydrological improvement of drought prone soils, or enhancement of soil biodiversity), might be achieved. Such an outcome would greatly enhance the potential for sustainable management of the wider soil resource and allow for targeted and specific improvements to soil properties and ecosystem services where they are most needed or would provide the greatest benefit to the environment.

Thus, it is hoped that the insights provided through this research will help to promote *soil carbon capture for ecosystem service enhancement*, and delivery of ancillary benefits for wider environmental services and climate change mitigation.



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# Appendices

# The Case for Increasing Deployment Rates of Paper Crumble to Agricultural Land

## 1.1 Executive Summary

Currently GreenWorld typically deploy paper crumble (PC) 25 - 50 t ha<sup>-1</sup>. Presented herein is a case to increase (PC) deployment to 75 t ha<sup>-1</sup> (*and 65 t ha<sup>-1</sup> in nitrogen vulnerable zones (NVZ)*).

Following the positive results of the VALCRUM and PANEZA PC field trials, established with the University of East Anglia, GreenWorld seeks to increase their maximum permissible deployment threshold from 50 t ha<sup>-1</sup> to 75 t ha<sup>-1</sup> (or 65 t ha<sup>-1</sup> for NVZs to remain within acceptable N-limits). Such increases may significantly increase soil carbon storage potential and provide agricultural benefit.

PC amendment use was investigated for its efficacy as a soil improving agent for agricultural and environmental benefit in two field trials, on silty clay soil (VALCRUM) and on loamy sand soil (PANEZA). PC was applied at 50 t ha<sup>-1</sup>, 100 t ha<sup>-1</sup>, 150 t ha<sup>-1</sup>, 200 t ha<sup>-1</sup> and 250 t ha<sup>-1</sup> in the VALCRUM trial and, 50 t ha<sup>-1</sup>, 100 t ha<sup>-1</sup> and 200 t ha<sup>-1</sup> in the PANEZA trial.

Under field trial conditions, PC applications were observed to enhance soil physical properties (reduce soil bulk density and penetration force, suggesting reductions in soil compaction (**Section 1**)), improve soil hydrological capacities (uplifts in water holding capacity and infiltration rates (**Section 2**)), alter soil pH and improve soil cation exchange capacity (**Section 3**), provide a significant increase in soil organic matter and carbon contents (**Section 4**), enhance crop yields (**Section 5**) and provide a source of essential and trace soil nutrients (**Section 6**). Furthermore, no significant negative effects were observed when applying PC to the fields at any quantity of application.

In this report, to assess soil property changes under an increased PC treatment rates of 65 and 75 t ha<sup>-1</sup> results have been calculated through the interpolation of real-world trial data. Increasing maximum permissible deployment rates from 50 t ha<sup>-1</sup> to 75 t ha<sup>-1</sup> shows potential to enhance beneficial soil properties, crop outcomes and carbon sequestration, while also increasing business and environmental efficiencies for GreenWorld.

### Key points:

- Increasing deployment rates could enhance operational efficiency and reduce GreenWorld carbon emissions
- Increased deployment rate of PC could defray costs associated with conventional fertiliser due to nutrient inputs
- Increased deployment rate of PC could increase soil organic carbon content by 6 t ha<sup>-1</sup>
- Increased deployment rate of PC could improve winter wheat yields by 7% and rye yields by 10%
- Increased deployment rate of PC could improve soil bulk density, soil aggregation and compaction
- Increased deployment rate of PC could improve soil drainage and water holding capacity
- Increased deployment rate of PC does not significantly change soil pH
- Increased deployment rate of PC does not raise concerns regarding potentially toxic elements

**Table 1. Paper Crumble properties, table adapted from Mao et al., 2022.**

<b>Parameter</b>	<b>Unit</b>	<b>Value</b>
OM	% dry mass	29.9 ± 0.3
TC	% dry mass	24.4 ± 1.9
TN	% dry mass	0.55 ± 0.07
C:N		45:1
WHC	%	131 ± 10.8
Bulk density	g cm <sup>-3</sup>	0.39 ± 0.02
CEC	me/100g	89.4 ± 2.7
pH		6.94 ± 0.08
Moisture content	%	~35
<b>Essential major elements</b>		
Na	mg kg <sup>-1</sup> dry mass	781 ± 18
K	mg kg <sup>-1</sup> dry mass	67.4 ± 3.8
P	mg kg <sup>-1</sup> dry mass	5.61 ± 0.84
Mg	mg kg <sup>-1</sup> dry mass	142 ± 3.9
<b>Essential trace elements</b>		
B	mg kg <sup>-1</sup> dry mass	0.37 ± 0.01
Ni	µg kg <sup>-1</sup> dry mass	0.11 ± 0.01
Mo	µg kg <sup>-1</sup> dry mass	0.14 ± 0.01
Cu	µg kg <sup>-1</sup> dry mass	0.26 ± 0.01
Zn	µg kg <sup>-1</sup> dry mass	BD
<b>Non-essential elements</b>		
Cr	µg kg <sup>-1</sup> dry mass	0.01 ± 0.002
Cd	µg kg <sup>-1</sup> dry mass	BDL
Hg	µg kg <sup>-1</sup> dry mass	BDL
Pb	µg kg <sup>-1</sup> dry mass	BDL

## 2.1 Background

Paper crumble (PC) is a co-product in the paper recycling processes. PC is comprised primarily of wood pulp fibres although other components of the paper feedstock (e.g., kaolinite clay and other elements) will also be present.

A number of scientific publications have reported the use and effects of PC spreading to land in a variety of different agricultural and environmental contexts (Chantigny et al., 1999, Zibilske et al., 2000, Foley and Cooperband, 2002, Chow et al., 2003, EA, 2005, Abiven et al., 2009, Gallardo et al., 2012, Powlson et al., 2012, Rasa et al., 2021, , Mao et al., 2022)

In general, these publications have reported manifold benefits to soil physical, chemical, and hydrological properties. Such benefits have been reported to manifest in, for example, the reduction of surface water run-off following rainfall events and mitigated soil erosion.

## 3.1 GreenWorld Operations and Field Trials

The PC discussed within this report relates to recycled newsprint originating from the Palm Paper Ltd. Facility in Kings Lynn, Norfolk. This PC is considered a type 1 crumble (*non-virgin, de-inked*) and undergoes treatment with bio-digestate and ink sludge reintroduction post processing, enriching the crumble with nitrogen and essential elements. The typical PC deployment rate used by GreenWorld is between 25-50 t PC ha<sup>-1</sup>. The properties of this PC are listed in **Table 1**.

Field trials at and beyond these “normal” applications rates were undertaken through GreenWorld-UEA collaborations. These trials were made possible with funding from UKRI that supported two projects: VALCRUM and PANEZA. These field trials were established to determine the effects of varying PC application rates for soil carbon storage and climate change mitigation potential, and their influence on soil properties.

In both trial, PC was applied to the field in specified quantities prior to ploughing. PC was spread in crumb form and subsequently incorporated to a depth of approximately 5cm by flat-lifting and culti-pressing the soil.

The VALCRUM project was established in 2017 and ended in 2020, this trial took place on a silty clay soil. PC amendment at rates of 50 t ha<sup>-1</sup>, 100 t ha<sup>-1</sup>, 150 t ha<sup>-1</sup>, 200 t ha<sup>-1</sup> and 250 t ha<sup>-1</sup> were applied to an arable field to evaluate soil property changes and soil carbon storage potentials under different PC application regimes. The primary focus of this experiment was to determine the technical potential for soil carbon storage using high carbon soil amendments. Control measures were taken from the unamended field margins to provide a zero-treatment benchmark for comparison. The VALCRUM data presented was collected in 2018/19. The results of the VALCRUM trial have been published (Mao et al., 2022) In the journal *Science of the Total Environment*, highlighting the potential of PC to improve soil properties and provide significant soil carbon storage capability.

The PANEZA trial was established in 2019 as the successor trial to VALCRUM and is still ongoing, this trial took place on a loamy sand soil. PANEZA considered PC application rates 50 t ha<sup>-1</sup>, 100 t ha<sup>-1</sup> and 200 t ha<sup>-1</sup> PC, applied to an arable field to evaluate the same property changes as investigated in the VALCRUM project (complimenting the heavy soil with a light soil comparison), with the addition of more in-depth hydrology measurement. Control blocks were randomly allocated within the trial field to minimise potential sources of data error and

bias. The results of the PANEZA project are currently being used to prepare further publications, and further data collection will continue in the coming years.

Significant changes to soil properties were observed between the different PC treatment rates in each trial, with higher rates generally being associated with improved soil properties, and thus by extension, benefit to agriculture. No negative effects to soil properties nor crop yield were observed following PC amendment, including treatments of 250 t ha<sup>-1</sup> (*5x the normal application rate*).

The information contained within this report focuses on the agriculturally relevant soil property characteristics and changes when increasing the standard treatment rate from 50 t ha<sup>-1</sup> to 100 t ha<sup>-1</sup> and the effects such an increase in treatment may have upon the agricultural environment.

#### 4.1 Motivation to increase PC deployment rates

Following the positive results of the VALCRUM and PANEZA trials GreenWorld seeks to increase their maximum deployment threshold from 50 t ha<sup>-1</sup> to 75 t ha<sup>-1</sup> (or 65 t ha<sup>-1</sup> for NVZs to remain within acceptable N-limits).

Increasing the PC addition rate to such levels would significantly enhance GreenWorld operational efficiency whilst having positive benefit to both the end user (*farmer*) and the environment. This would also provide customers with a greater flexibility in their amendment choices, with higher variable rates potentially changing the frequency of PC application to rotations more suitable to the farmer/crop.

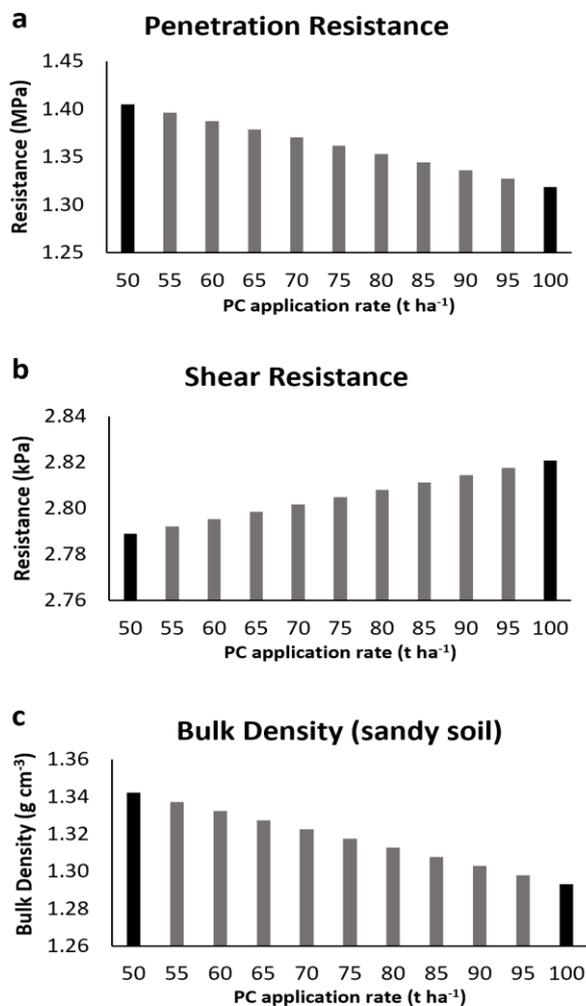
In the subsequent section of this report evidence is presented to support an increase in PC deployment rate to 75 t ha<sup>-1</sup>. This evidence is presented in sub-sections relating to; physical property responses (**section 1.**), hydrology responses (**section 2.**), chemical property responses (**section 3.**), organic matter responses (**section 4.**), crop yield responses (**section 5.**), PC nutrient additions to soil (**section 6.**) and potentially toxic elements (**section 7.**). Finally, a summary is provided regarding the overall environmental and agronomic benefits (**sections 8 & 9.** respectively).



In presenting this data measured values obtained under the field trials has been highlighted (black bars), while non-measured (interpolated) values have been added to illustrate data trends (grey bars) and to define outcomes at 65 and 75 t ha<sup>-1</sup> application rates. Control data (PC-absent regimes) is reported within the discussion.

## 5.1 Soil Physical Responses

Management practices that influence soil structure and aggregation facilitate root growth and improve the ingress and storage of air and water in soil pores, supporting crop success. Soil penetration resistance was measured in the silty clay soil, and shear resistance and soil bulk density within the loamy sand soil.



**Figure 1:** Soil physical property changes under increasing PC application rates, Penetration resistance (VALCRUM), shear stress resistance and soil bulk density (PANEZA).

of PC influences the formation and cohesion of soil aggregates. Changes in the physical properties projected from the increased application rate of 75 t ha<sup>-1</sup> support a mild increase in soil aggregate formation (where PC acts as an aggregate binding agent within the soil sphere). Such predictions of soil physical property changes support the use of PC as a soil enhancing amendment for broad soil structural benefit. Such structures may help mitigate issues with impeded plant rooting and water ingress, while also physically protecting soil from erosive forces.

## 6.1 Hydrology Responses

The soil water infiltration rate and water holding capacity (WHC) are key in mitigating flood and drought events. Generally, where a soil has high infiltration and WHC water will be able

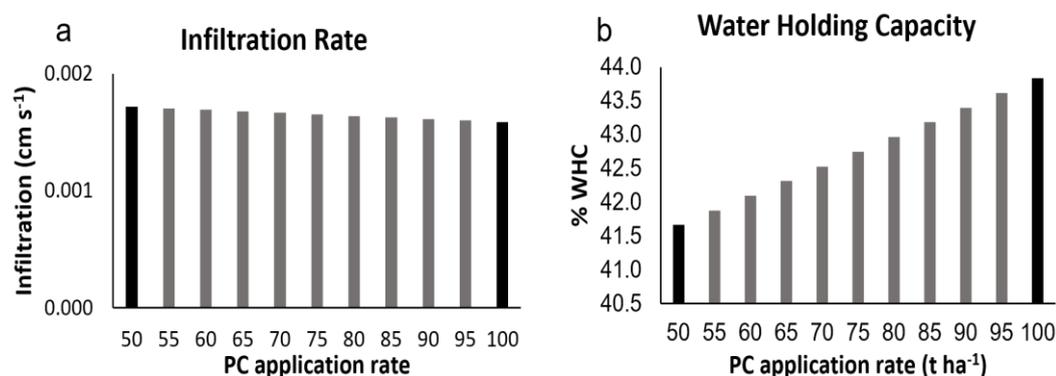
Increasing PC application from 50 to 75 t ha<sup>-1</sup> shows a projected reduction in penetration resistance, from 1.41 MPa to 1.36 MPa (**Figure 1A**). Penetration resistance was found to reduce significantly when treatments of 50 t ha<sup>-1</sup> were applied *in situ* (control value 2.21 MPa)(**Table 4**).

No consequential changes were projected in the soil shear resistance when increasing treatments from 50 to 75 t ha<sup>-1</sup>, 2.79 kPa and 2.81 kPa, respectively (**Figure 1B**). A small reduction was noted between the 50 t ha<sup>-1</sup> PC treatment and control soil (control value 2.90 kPa)(**Table 4**).

Soil bulk density also projected no consequential changes when increasing application rates from 50 to 75 t ha<sup>-1</sup>, projected reduction from 1.34 g cm<sup>-3</sup> to 1.32 g cm<sup>-3</sup> (**Figure 1C**). Treatments of 50 t ha<sup>-1</sup> PC measured a small reduction in soil bulk density compared to the control soil (control value 1.36 g cm<sup>-3</sup>)(**Table 4**).

From experimental observations, changes in soil penetration, shear, and bulk density, indicate that the presence

to flow more easily into the soil, minimising surface run off and associated erosion. These soils will also be capable of holding on to more water than a comparable soil with lower infiltration and WHC, further mitigating issues of flooding alongside improved drought tolerance. Infiltration rate and WHC were measured in the loamy sand soil.



**Figure 2:** Soil hydrological property changes (infiltration rate and water holding capacity) under increasing PC application rates (PANEZA)

Increasing PC treatment rates from 50 to 75 t ha<sup>-1</sup> was not projected to alter the rate of water infiltration (**Figure 2A**). Substantial increases in soil infiltration rate were observed experimentally however following a 50 t ha<sup>-1</sup> PC treatment relative to untreated soil, increasing from 41% to 43% (**Table 4**).

WHC of soil was projected to increase from 41.6% to 42.7% when increasing the treatment rate from 50t to 75 t ha<sup>-1</sup>. Such increases would significantly enhance the water holding capacity of the soil (by up to 4.6%) relative to an untreated soil (control value 38.1%).

Soil hydrological properties benefit from the application of PC in general, showing enhanced infiltration rates and WHC, improving the soil from both an agronomic and an environmental standpoint. Despite no projected significant changes to infiltration rates when increasing PC application rates from 50 to 75 t ha<sup>-1</sup>, the calculated increases observed in soil WHC suggest substantial hydrological benefits may be seen.

## 7.1 Chemical Responses

Soil pH and cation exchange capacity (CEC) regulate soil chemical functions, thus directly impact soil fertility, biological activity, and agricultural productivity. Changes in soil pH can affect the availability of soil nutrients, with some major and trace elements becoming more or less available in different pH ranges, with ideal soil pH broadly ranging between approximately 5.5-8 for peak nutrient availability. Soil CEC provides a direct measure of the soil's ability to absorb, hold and exchange cations within the soil matrix; these ions support crop growth, and may also assist in buffering soil pH. Soil pH was measured in both the loamy sand and silty clay soil, and CEC was measured in the silty clay soil.

No significant change in pH was projected when increasing the PC application from 50 to 75 t ha<sup>-1</sup> in either soil type; small projected pH increases from 7.58 to 7.64 in the loamy sand soil (**Figure 3A**), and 8.54 to 8.55 in the silty clay soil (**Figure 3B**). Experimentally a significant

increase in soil pH was measured in the loamy sand soil trial when 50 t ha<sup>-1</sup> PC was applied, raising soil pH from 6.48 (Table 4), while a small decrease (not significant) in soil pH was observed in the silty clay trial at application rates of 50 t ha<sup>-1</sup>, reducing from 8.59 (Table 4).

CEC is projected to increase slightly when increasing PC treatment from 50 to 75 t ha<sup>-1</sup>, however such increases are small, rising from 89.9 me 100g<sup>-1</sup> to 91.2 me 100g<sup>-1</sup> (Figure 3C). Treatment with PC experimentally at a rate of 50 t ha<sup>-1</sup> was found to increase CEC from a base level of 86.2 me 100g<sup>-1</sup> in the untreated control soil.

PC additions can enhance soil CEC, boosting the fertility and functionality of the treated soil. Application of PC has a minor liming influence upon soils, raising the pH. This can be beneficial in a range of soil types that are too acidic in nature. Conversely applications of PC to already alkaline soils may reduce the pH, converging towards neutral. It is unlikely that PC applications will be detrimental to soil pH levels (raising too high) due to the circumneutrality of the amendment (pH approximately 7 (Table 1)).

### 8.1 Organic Matter Responses

Soil organic matter (SOM) and soil organic carbon (SOC) are often underpinning factors of desirable soil properties. SOM/SOC influence soil infiltration rates and WHC, may enhance physical properties and aggregate formation, buffer against changes in pH and facilitate the release and uptake of essential soil nutrients. SOM and SOC were measured on the loamy sand soil.

Increasing the application rate of PC from 50 to 75 t ha<sup>-1</sup> projects an increase in the total SOM input of by 0.8%, from 3.1% to 3.9% (Figure 4A), and the total SOC by 0.4% from 1.6% to 2.0% (Figure 4B). Such increases correspond to an increased input of approximately 4.6 t C ha<sup>-1</sup>, a significant soil carbon stock increase.

Untreated soil by contrast, experimentally measured an SOM content of 2.9% and SOC content of 1.5%. As such the projected increased addition rate shows significant increases in the SOM and SOC storage potential of the soil. Assuming an incorporation depth of 10cm and receiving soil bulk density of 1.3 g cm<sup>-3</sup>, a 75 t ha<sup>-1</sup> PC amendment is projected to increase soil carbon stock (SOC) by a total 0.5%, corresponding to a 6 t ha<sup>-1</sup> carbon stock increase. Increasing the treatment rate of PC offers significant potential to improving soil carbon stocks and underpins the provision and enhancement of all other soil properties.

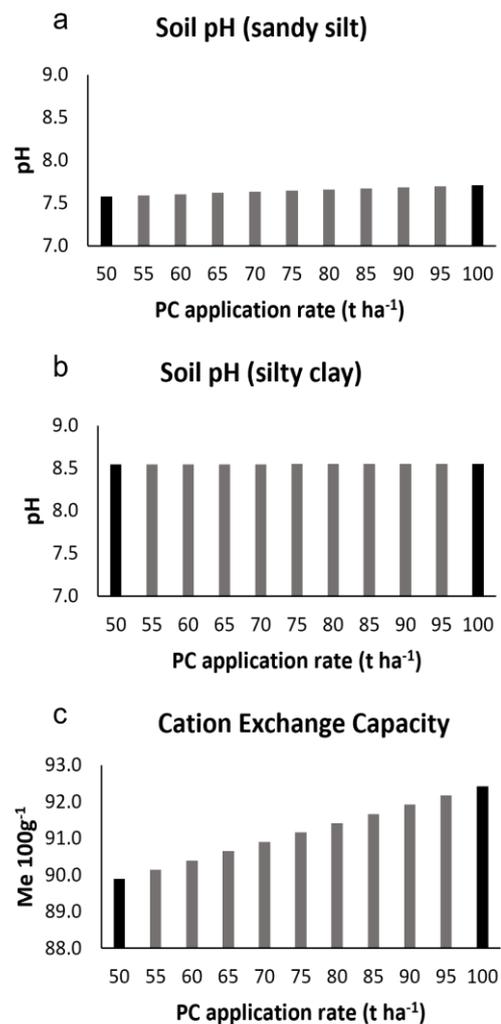
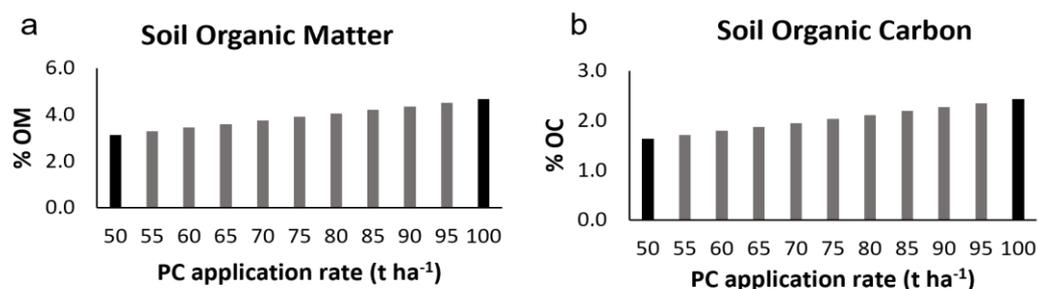


Figure 3: Soil pH changes in a sandy silt soil (PANEZA) and a silty clay soil (VALCRUM); and cation exchange capacity (CEC) of soil (VALCRUM), under increasing PC application rates

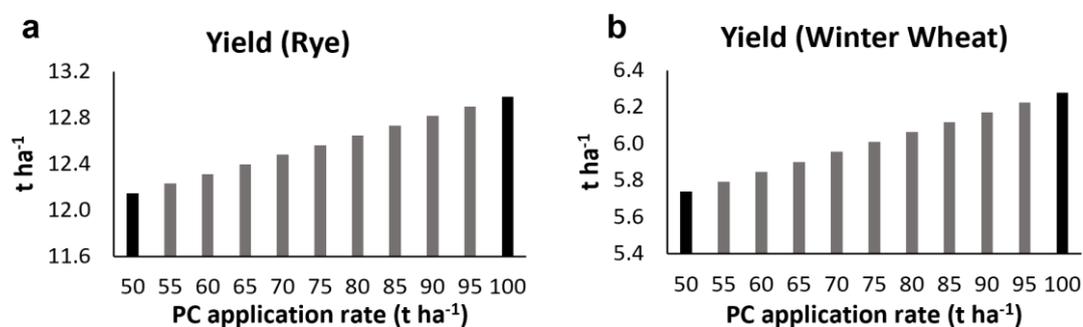


**Figure 4:** Soil organic matter (SOM) and soil organic carbon (SOC) changes under increasing PC application rates (PANEZA).

## 9.1 Crop yield Response

Enhancing crop yields can increase profits. Small variations in crop yield, when extrapolated across a farm, can be extremely beneficial or detrimental. Crop yield data was measured on the crops harvested from both the loamy sand (*rye*) and the silty clay soils (*winter wheat*).

Yield increases were projected in both crops when PC application rates are increased from 50 to 75 t ha<sup>-1</sup>. Total increases of 0.5 t ha<sup>-1</sup> of rye (rising from 12.1 t ha<sup>-1</sup> to 12.6 t ha<sup>-1</sup> (**Figure 5A**)); and 0.3 t ha<sup>-1</sup> of winter wheat (rising from 5.7 t ha<sup>-1</sup> to 6.0 t ha<sup>-1</sup> (**Figure 5B**)), were projected. Such increases, equivalent to a 4% and 5% yield improvement for rye and winter wheat, respectively. Total potential yield increases projected for a 75t ha<sup>-1</sup> treatment relative to zero PC application can be as much as 1.1t ha<sup>-1</sup> rye (10% increase), and 0.4t ha<sup>-1</sup> winter wheat (7% increase).



**Figure 5:** Crop yield changes of rye crop (PANEZA), and winter wheat crop (VALCRUM), under increasing PC application rates

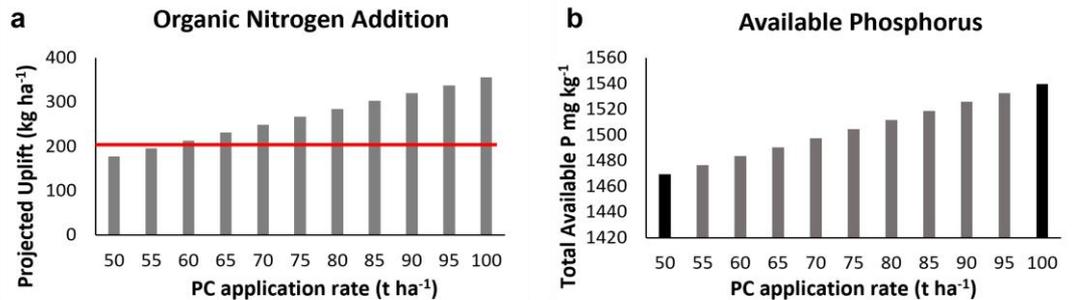
## 10.1 PC Nutrient Additions

Application of PC offers potential to increase the concentrations of essential and trace elements within the soil, as well as key nutrients such as phosphorus and nitrogen. Nitrogen data presented has been calculated as an uplift based upon the nutrient values of the pure paper crumble amendment (**Table 1**) when applied at the defined rate, while phosphorus data was interpolated from experimental Olsen-P measured in loamy sand soil.

Increasing PC application rates from 50 to 75 t ha<sup>-1</sup> is projected to add an additional 89 kg N ha<sup>-1</sup>, rising from 178 kg N ha<sup>-1</sup> to 267kg N ha<sup>-1</sup> (**Figure 6A**). Due to this calculated addition exceeding the 250 kg N ha<sup>-1</sup> limit, a 75 t ha<sup>-1</sup> PC application rate cannot be used in NVZ areas. As such, in NVZs a maximum permissible application rate of 65 t ha<sup>-1</sup> PC is proposed:

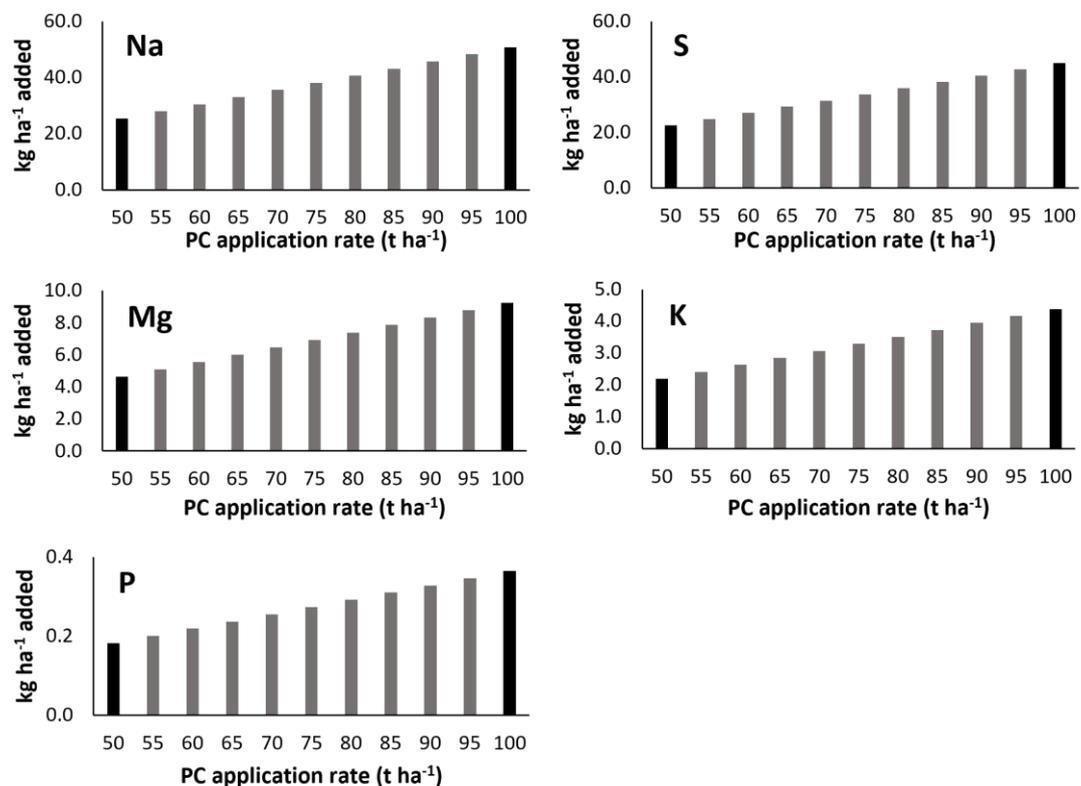
Amendment with 65 t ha<sup>-1</sup> PC is projected to add a total 231 kg N ha<sup>-1</sup>, below the NVZ threshold.

Total available P was projected to increase by 35 mg kg<sup>-1</sup>, from 1469 mg kg<sup>-1</sup> P to 1504 mg kg<sup>-1</sup> P, when increasing application rates from 50 to 75t ha<sup>-1</sup>(**Figure 6B**). Experimentally the application of PC at 50 t ha<sup>-1</sup> was found to significantly enhance the levels of available P in the soil, increasing from 1184 mg kg<sup>-1</sup> in the untreated control soil.



**Figure 6:** Total organic nitrogen additions (VALCRUM) and total available phosphorus (PANEZA) from increased PC application rates. Red line in figure a) shows nitrogen application limit in nitrogen vulnerable zones (NVZs).

Increasing application rates of PC from 50 to 75 t ha<sup>-1</sup> is projected to increase the input of essential elements (**Figure 7**), and trace elements (**Table 3**) required for plant growth. Key increases of note include uplifts to abundance of potassium (**K**), magnesium (**Mg**) and sulphur (**S**), all projected to increase in significant quantities from increased PC addition. Such increases in nitrogen, phosphorus, and trace elements, may help minimise conventional fertiliser needs, (potential to reduce NPK fertiliser use).



**Figure 7:** Essential element additions from increasing PC application rates. Elements in order of abundance (Na, S, Mg, K, P)(VALCRUM and NRM laboratory data).

## 11.1 Potentially toxic elements

Potentially toxic elements (PTE) are those, which if introduced to soil in excessive quantities have the ability to cause damage to the soil environment, plant health or human health, due to their inherent toxicity. PTE's are discussed relative to the concentrations measured in the PC product calculated to a kg ha<sup>-1</sup> addition basis. Maximum permissible average annual PTE additions, outlined in *DEFRA Sewage sludge in agriculture: code of practice for England, Wales, and Northern Ireland (2018)*, are shown in **Table 2**. Total PTE concentrations in application rates between 50 – 100 t ha<sup>-1</sup> are recorded in **Table 3**.

**Table 2.** Maximum permissible average annual rate of PTE addition over 10 years (kg ha<sup>-1</sup>)

	Zn	Cu	Ni	Cd	Pb	Hg	Cr	Mo
Maximum yearly PTE addition	15	7.5	3	0.15	15	0.1	15	0.2
Maximum PTE's added (10 yrs.)	150	75	30	1.5	150	1	150	2
75 t ha <sup>-1</sup> PC PTE accumulation (yearly application 10 yrs.)	0	0.12	0.05	0.09	4.4	0.04	0.005	0.07

Increasing PC application rates from 50 – 75 t ha<sup>-1</sup>:

- Zn additions are not projected to change following increased PC addition (0 kg ha<sup>-1</sup> yr<sup>-1</sup> added).
- Cu additions are projected to increase by 0.004 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.008 to 0.012 kg ha<sup>-1</sup> yr<sup>-1</sup>)
- Ni additions are projected to increase by 0.002 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.004 to 0.005 kg ha<sup>-1</sup> yr<sup>-1</sup>)
- Cd additions are projected to increase by 0.003 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.006 to 0.009 kg ha<sup>-1</sup> yr<sup>-1</sup>)
- Pb additions are projected to increase by 0.15 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.29 to 0.44 kg ha<sup>-1</sup> yr<sup>-1</sup>).
- Hg additions are projected to increase by 0.001 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.003 to 0.004 kg ha<sup>-1</sup> yr<sup>-1</sup>)
- Cr additions are projected to increase by 0.0002 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.0003 to 0.0005 kg ha<sup>-1</sup> yr<sup>-1</sup>)
- Mo additions are projected to increase by 0.002 kg ha<sup>-1</sup> yr<sup>-1</sup> (rising from 0.005 to 0.007 kg ha<sup>-1</sup> yr<sup>-1</sup>)

Increasing PC application rates from 50 to 75 t ha<sup>-1</sup> is projected to increase the total addition of PTEs to the soil (**Table 3**). However, such additions remain within the maximum permissible limits for sewage sludge material application to arable soils.

## 12.1 Conclusion Regarding Agricultural and Environmental Benefits

Based on evidence gathered in relation to two field trials using PC up to a deployment of 250 t ha<sup>-1</sup> it was the general conclusion that the greatest agricultural benefits were observed in treatments of approximately 100 t ha<sup>-1</sup>. Furthermore, no negative effects were observed with respect to the soil properties. However, due to the organic nitrogen content in PC, additions of 100 t ha<sup>-1</sup> PC would exceed permissible N-additions in NVZs. Given this constraint a revised maximum deployment amount of 65 t ha<sup>-1</sup> in NVZs is suggested. Out with NVZs, an application

of 75 t ha<sup>-1</sup>, as based upon the content of this report, is suggested as a revised upper limit for PC deployment.

Treatment with PC can enhance many soil properties for both environmental and agronomic benefit: improving soil aggregate structure, reducing compaction/bulk density (**Section 1**), improving water infiltration and WHC (**Section 2**), regulating soil pH, and soil chemical properties (**Section 3**), substantially increasing organic matter and carbon stocks (**Section 4**) and providing a source of nutrients for crops (**Section 6-7**).

PC offers a significant source of nitrogen (**Figure 6A**), phosphorus (**Figure 6B**) and trace elements (**Figure 7**). Thus, increasing the maximum deployment amount to 75 t ha<sup>-1</sup> may offer particular benefit to farmers, by increasing the soil fertility, potentially reducing the reliance on conventional sources of fertiliser. Simultaneously the increased application of PC is projected to offer a variety of other more holistic soil benefits (increased SOM/SOC content and enhancement of soil physical and hydrological properties), which a conventional fertiliser would not provide.

Crop yields were projected to improve by 0.5 t ha<sup>-1</sup> in rye crops and 0.3 t ha<sup>-1</sup> in winter wheat crops following an increase in PC treatment from 50 to 75t ha<sup>-1</sup> (**Figure 5**) (a total increase of 1.1t ha<sup>-1</sup> and 0.4 t ha<sup>-1</sup> respectively, when compared with an untreated soil). Such increases in crop yield can be substantial at field and farm scale.

More broadly, increasing the maximum PC deployment from 50 to 75t ha<sup>-1</sup> would reduce the number of haulage journeys required. Thus, increasing efficiency while cutting the GreenWorld carbon footprint. Additionally, benefits such as reducing the total number of PC tip sites, minimising issues of eyesore, smell and farm vehicle traffic on the roads would likely result of increased spreading intensity on the selected fields.

### 13.1 Data sets

Data was collected from 3 sources, VALCRUM and PANEZA data sets (in collaboration with the University of East Anglia) and reports provided from professional analysis services (NRM Laboratories) used for QA/QC purposes for PC materials on GreenWorld sites.

**Table 3.** Paper Crumble (PC) spreading rates and associated nitrogen and elemental uplifts in kg ha<sup>-1</sup>. Adapted from VALCRUM data set (TON, Na, K, Mg, P, B Ni, Mo, Cr, Cu, Zn), PANEZA data set, and NRM laboratories analysis set (S, Pb, Cd, Hg).

PC Spreading Rate (t ha <sup>-1</sup> )	TON	Na	K	Mg	P	S	B	Ni	Mo	Cr	Cu	Zn	Pb	Cd	Hg
50	178	25.4	2.19	4.62	0.182	22.5	0.0000120	0.00358	0.00455	0.000325	0.00845	0	0.295	0.00575	0.00293
55	196	27.9	2.41	5.08	0.201	24.7	0.0000132	0.00393	0.00501	0.000358	0.00930	0	0.325	0.00633	0.00322
60	214	30.5	2.63	5.55	0.219	27.0	0.0000144	0.00429	0.00546	0.000390	0.0101	0	0.354	0.00690	0.00351
65	231	33.0	2.85	6.01	0.237	29.2	0.0000156	0.00465	0.00592	0.000423	0.0110	0	0.384	0.00748	0.00380
70	249	35.6	3.07	6.47	0.255	31.5	0.0000168	0.00501	0.00637	0.000455	0.0118	0	0.413	0.00805	0.00410
75	267	38.1	3.29	6.93	0.273	33.7	0.0000180	0.00536	0.00683	0.000488	0.0127	0	0.443	0.00863	0.00439
80	285	40.6	3.51	7.39	0.292	36.0	0.0000192	0.00572	0.00728	0.000520	0.0135	0	0.472	0.00920	0.00468
85	302	43.2	3.72	7.86	0.310	38.2	0.0000204	0.00608	0.00774	0.000553	0.0144	0	0.502	0.00978	0.00497
90	320	45.7	3.94	8.32	0.328	40.5	0.0000216	0.00644	0.00819	0.000585	0.0152	0	0.531	0.0104	0.00527
95	338	48.3	4.16	8.78	0.346	42.7	0.0000228	0.00679	0.00865	0.000618	0.0161	0	0.561	0.0109	0.00556
100	356	50.8	4.38	9.24	0.365	45.0	0.0000241	0.00715	0.00910	0.000650	0.0169	0	0.590	0.0115	0.00585

**Table 4.** Observed changes to soil properties following varied PC spreading rate. Data from VALCRUM (Penetration Resistance, Soil pH (silty clay), Yield (winter wheat), CEC), and PANEZA (Shear Resistance, Infiltration Rate, WHC, Soil moisture, Bulk Density, SOM, SOC, Soil pH (loamy sand), Yield (rye), Available P. Measured results for 50tha<sup>-1</sup> and 100 t ha<sup>-1</sup>, with other spreading rates interpolated.

PC Spreading Rate (tha <sup>-1</sup> )	Penetration Resistance	Shear Resistance	Infiltration Rate	WHC	Bulk Density	SOM	SOC	Soil pH (loamy sand)	Soil pH (silty clay)	Yield (Rye, tha <sup>-1</sup> )	Yield (Winter Wheat, t ha <sup>-1</sup> )	CEC	Available P.
Control	2.21	2.90	0.000673	38.1	1.36	2.91	1.51	6.49	8.59	11.5	5.65	86.2	1184
50	1.40	2.79	0.00172	41.7	1.34	3.15	1.64	7.58	8.54	12.1	5.74	89.9	1469
55	1.40	2.79	0.00170	41.9	1.34	3.30	1.72	7.59	8.54	12.2	5.79	90.1	1476
60	1.39	2.80	0.00169	42.1	1.33	3.45	1.79	7.60	8.54	12.3	5.85	90.4	1483
65	1.38	2.80	0.00168	42.3	1.33	3.60	1.87	7.62	8.55	12.4	5.90	90.7	1490
70	1.37	2.80	0.00166	42.5	1.32	3.76	1.95	7.63	8.55	12.5	5.95	90.9	1498
75	1.36	2.80	0.00165	42.7	1.32	3.91	2.03	7.64	8.55	12.6	6.01	91.2	1505
80	1.35	2.81	0.00164	43.0	1.31	4.06	2.11	7.66	8.55	12.6	6.06	91.4	1512
85	1.34	2.81	0.00163	43.2	1.31	4.21	2.19	7.67	8.55	12.7	6.12	91.7	1519
90	1.34	2.81	0.00161	43.4	1.30	4.37	2.27	7.68	8.55	12.8	6.17	91.9	1526
95	1.33	2.82	0.00160	43.6	1.30	4.52	2.35	7.70	8.55	12.9	6.23	92.2	1533
100	1.32	2.82	0.00159	43.8	1.29	4.67	2.43	7.71	8.55	13.0	6.28	92.4	1540

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# WBEP Soil Report – April 2022

## 1.1 Context and summary:

This report has been prepared to provide eCountability with soil information to complement their natural capital assessment of 8 sites on the Wendling Beck Environment Project. These sites representing a range of land use / habitat regimes.

Soil samples for physical and chemical assessment were collected on the 21st of February, and soil biodiversity samples were collected on the 1<sup>st</sup> of March 2023. These samples were subsequently assessed to determine soil pH, soil texture, soil water holding capacity, soil organic matter (by loss on ignition), soil carbon content (elemental analysis) and carbon stability (by thermal analysis). The results relating to these parameters are reported herein. Complementary samples were obtained to support soil biodiversity assessment (16s RNA and ITS, respectively directed towards bacterial and fungal biodiversity). Data relating to soil microbial biodiversity is not available at this time and will be reported in the final report in due course.

During soil sampling site 3 was instructed to remain unsampled due to issues of access, and site 7 was sampled in the adjacent orchard rather than arable field due to uncertainty on the sample day. This substitute site 7 has been since measured approximately 100m to the east of the desired point.

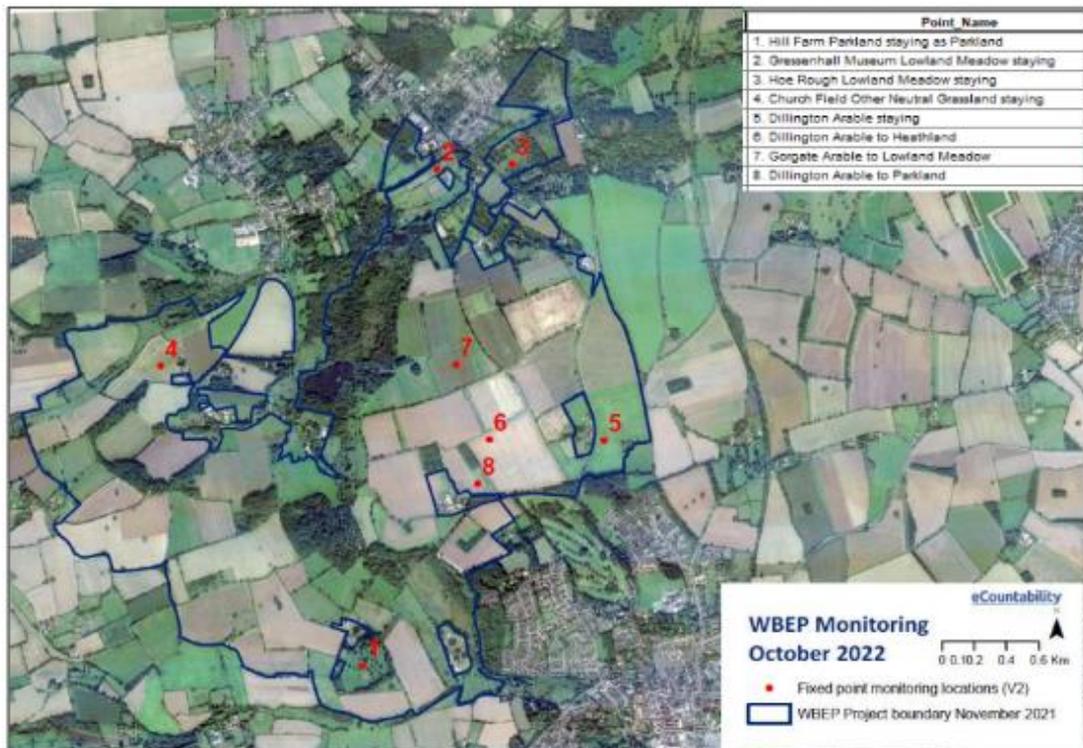
### Key points:

- All sites were of the same soil texture: loamy sand (**Table 2**).
- Soil field moisture varied from 10.4% to 14.5% (**Figure 3**), no significant differences ( $p > 0.05$ ) were observed between the different sites.
- WHC was observed to vary across the sites, 29.3% to 39.3% (**Figure 4**), it was noted that pastorally managed fields showed greater WHC relative to the arable field counterparts, with some significant differences ( $p < 0.05$ ) observed between sites.
- Soil pH was constrained, between 6.30 and 7.53 across all of the sites (**Figure 5**), with some significant differences ( $p < 0.05$ ) measured between sites, however, no clear patterns were observed between land use types.
- Soil organic matter (SOM) measured between 2.10% to 5.50% (**Figure 6**), with significantly ( $p < 0.05$ ) higher SOM observed between pasture fields and their arable counterparts.
- Organic carbon (OC) followed a similar trend to SOM, it is ranging between 1.75% and 4.78% (**Figure 7**), with generally significantly ( $p < 0.05$ ) higher values observed in pastorally managed fields when compared to their arable counterparts.
- Soil carbon stability was found to vary across the sites, with the total proportion of relatively stable carbon stored measuring between 32.5% and 42.6% (**Figure**

**8, Table 3, Table 4**). No significant difference ( $p > 0.05$ ) was observed for carbon stability between field sites, however, greater quantities of relatively stable were measured in soils with a higher total carbon content.

- Simpson's Index of diversity (D) was high for both 16S and ITS data (**Figure 11**), ranging from 0.786 to 0.982, with 16S D higher than ITS D at all sites.
- Species richness was considerably higher for 16S data than ITS across all sites (**Figure 12**), ranging from 786-1104 (16S) compared to 110-372 (ITS).
- Relative abundance of 16S and ITS 10 most abundant families (**Figure 13, Figure 14**) show that 16S were not dominated by their most abundant families under arable systems, but were in grazed systems. The most abundant ITS families were dominant at all sites, in line with lower overall fungal diversity.
- 16S D correlated with physicochemical soil properties indicated a correlation with WHC (**Figure 15**,  $R^2 = 0.883$ ), SOM (**Figure 17**,  $R^2 = 0.883$ ) and loosely with SOC (**Figure 18**,  $R^2 = 0.659$ ). No relationship was found with 16S D and pH (**Figure 16**,  $R^2 = 0.5006$ ).
- No correlations were found with ITS D and other soil properties. (**Figures 15-18**).

## 2.1 Sites investigated:



**Figure 1:** Sample location (1-8) on the WBEP where soil samples were obtained. The red point indicates the general location samples (further details are provided in Table 1)

Eight sites were identified by eCountability, and have been studied using other forms of environmental and ecosystem analysis. The locations of these sites are indicated in **Table 1**. These sites reflect a range of land use / habitat regimes as indicated in **Table 1**.

**Table 1:** Site names and numbers, land use types and location, detailing present and future land use at each sampling site. Colours indicate land use regime, carried forwards in data presentation.

Site Number	Field Name	Current Land Use	Future Land Use	GPS Co-ordinates
1	Hill Farm Parkland	Parkland (grazed)	Parkland (grazed)	052° 41' 4.97" N 000° 55' 54.26" E
2	Gressenhall Museum	Lowland Biodiversity Meadow (grazed)	Lowland Biodiversity Meadow (grazed)	052° 42' 47.76" N 000° 55' 20.59" E
3	Hoe Rough	Lowland Meadow (grazed)	Lowland Meadow (grazed)	052° 42' 48.14" N 000° 55' 43.62" E
4	Church Field	Other Natural Grassland (grazed)	Other Natural Grassland (grazed)	052° 42' 8.57" N 000° 53' 47.47" E
5	Dillington Arable 1	Arable Field	Arable Field	052° 41' 51.55" N 000° 56' 16.94" E
6	Dillington Arable 2	Arable Field	Heathland	052° 41' 52.21" N 000° 55' 36.36" E
7	Gorgate Arable 1	Arable/Orchard	Lowland Meadow/Orchard	052° 42' 5.96" N 000° 55' 29.45" E
8	Dillington Arable 3	Arable Field	Parkland (grazed)	052° 41' 41.45" N 000° 55' 28.81" E

### 3.1 Soil Sampling

Soil sampling was undertaken on the 21st of February and biodiversity sampling on the 1<sup>st</sup> of March 2023. Samples were obtained at five random sites selected within a 20m radius of i) the eCountability monitoring markers, or, where markers were not present, ii) the centre of the field (Table 1 defines the central point for sampling at a given location).

Soil samples (**n=5**; per site) to be used for physical and chemical measurements were collected using a soil auger (0-40cm). The auger was wiped clean prior to sample collection at each sampling point. Samples were returned to the laboratory and sieved (2mm). Samples

were dried (74 °C; 16 h), to obtain a field moisture value at time of collection. Dried samples were used in onward analysis.

Where samples (**n=5**; per site) were collected for eDNA assessment soil was collected with a clean and sterilised micro-auger from the top 10cm of the soil surface and stored in sterile sample tubes. The micro-auger was cleaned and sterilised prior to sample collection at each point. Sample tubes were sealed and then transferred to the laboratory where they were preserved by freezing at -80°C, ahead of eDNA extraction.

#### 4.1 Laboratory Analysis

##### Soil Texture

Soil texture (**n=5**) was measured by transferring soil (~25g) into a 50ml graduated measuring cylinder and filling the remainder of the cylinder with water. The cylinder was subsequently shaken vigorously, and the soil allowed to settle out and fractionate. Sand, silt and clay fractions were then estimated as % volume relative to the total soil volume after settling. The texture triangle (**Figure 2**) was then used to ascribe soil texture.

##### Soil Water Holding Capacity

Water holding capacity (**n = 5**) was assessed by transferring soil (~ 20 g) in a filter paper (Whatman No.1 filter paper) held in a funnel. The sample was then saturated with distilled water. Samples were allowed to drain until gravity release of water stopped.

Water holding capacity of soil was determined by drying wet soil (10 g) at 74 °C for 16 h.

##### Soil Organic Matter, Organic Carbon Content and Thermal Analysis

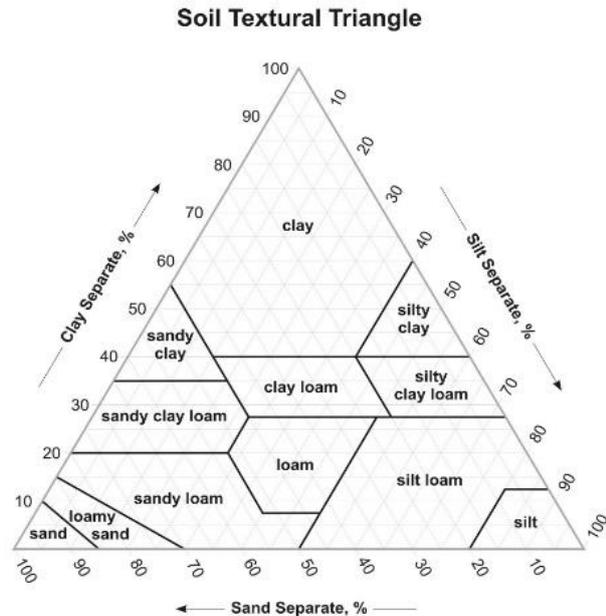
SOM content was measured as loss on ignition (ISO, 1995). Briefly, soil (10 g; **n = 5**) was dried (74 °C for 16 h) and then combusted (470 °C for 24 h).

For total carbon (OC), milled dry soil samples (2 mg; **n = 5**) were packed in tin capsules (8 × 5 mm) and measured using an elemental analyser (Exeter CHNS analyser).

The thermal stability a Thermo-gravimetric analyser (Trios TGA 550) was used to assess carbon stability. Briefly, milled, dry samples (**n = 3**) were heated in a nitrogen atmosphere, at a rate of 20 °C min<sup>-1</sup> from 25 to 1000 °C.

##### Soil pH

Soil pH (**n = 5**) was measured (ISO, 1994) in 1:10 soil/water suspension using a pH electrode (Mettler Toledo Pro pH) and pH meter (Mettler Toledo 5 Easy).



**Figure 2:** Soil texture triangle, used to measure the texture of a soil based upon the relative % of sand, silt, and clay. USDA Agricultural Research Service <https://www.ars.usda.gov/>

## Soil Microbial Diversity Assessment

Microbial eDNA analysis was carried out upon soil DNA extraction (**n=5**). 16S eDNA data was generated with 16S rRNA gene metabarcoding, an amplicon-based sequencing method directed towards assessment of bacterial diversity. DNA barcoding of fungi was completed with Internal transcribed spacer (ITS) amplicon sequencing of nuclear DNA.

### Statistics

On-way analysis of variance (One-way ANOVA) was used to test the WHC, field moisture, Soil pH, SOM, and SOC results from the different field sites against each other for statistical significance. Significance level was set to 95 % ( $p < 0.05$ ) and determined by a *post hoc* test with Tukey's HSD comparison. This procedure was completed using IBM SPSS 28. Statistical analysis results are displayed in bar charts along with mean values and standard deviation.

## 5.1 Results

### Soil Texture

Soils were found not to deviate from a sandy loam soil texture across the trial sites (**Table 2**). Small changes in sand and silt content were observed between soils across the study sites, however differences were small <10%. Clay content of all measured soils were often low.

**Table 2:** Soil texture classification and relative proportion of sand, silt, and clay (%)

Field Site	Land Use	Sand %	Silt %	Clay %	Soil Texture Classification
1	Parkland (grazed)	70.8 ± 4.3	27.3 ± 4.2	1.9 ± 0.1	Sandy Loam
2	Lowland Biodiversity Meadow (grazed)	69.9 ± 2.6	28.3 ± 2.6	1.8 ± 0.1	Sandy Loam
4	Other Natural Grassland (Grazed)	62.4 ± 3.7	36.5 ± 3.9	1.1 ± 0.9	Sandy Loam
5	Arable Field	65.9 ± 12.0	33.7 ± 11.7	0.4 ± 0.7	Sandy Loam
6	Arable Field	62.8 ± 6.1	36.1 ± 5.5	1.1 ± 0.9	Sandy Loam
7	Arable Orchard	65.8 ± 2.9	31.6 ± 3.0	2.6 ± 1.0	Sandy Loam
8	Arable Field	62.3 ± 4.4	37.7 ± 4.4	0.0 ± 0	Sandy Loam

### Field Moisture

Soil moisture content across the 8 sites varied from 10.4% (site 6 (*arable field*)) to 14.5% (site 7 (*orchard/arable field*)) (Figure 3). There were no significant differences ( $p > 0.05$ ) in soil moisture content across the 8 sites (Figure 3).

Land use across the site was observed to have a mild (non-significant ( $p > 0.05$ )) effect upon field moisture, with higher field moisture observed in the pastoral and orchard soils, relative to the

arable soils (Figure 3). Given proximity of field sites and similar environmental conditions, the greater field moisture observed in site 7 it is likely due to maintained permanent ground cover relative to the fairly bare soil conditions in site 5 where new arable crops are establishing following soil cultivation. It is also likely that the greater quantities of SOM/OC (Figure 7, Figure 8) found in the pasture sites relative to the arable sites is a contributing factor to increased soil field moisture.

### Water Holding Capacity

Soil WHC showed greater variation across the 8 sites than soil field moisture with significantly ( $p < 0.05$ ) greater WHC in some sites compared with others (Figure 4). WHC varied from 29.3% (site 6 (*arable field*)) to 39.3% (site 1 (*parkland*)) (Figure 4).

Sites 1, 2 and 4 (*noted to be pasture sites*) had nominally higher WHC than the other sites (*noted to be arable and orchard sites*) (Figure 4).

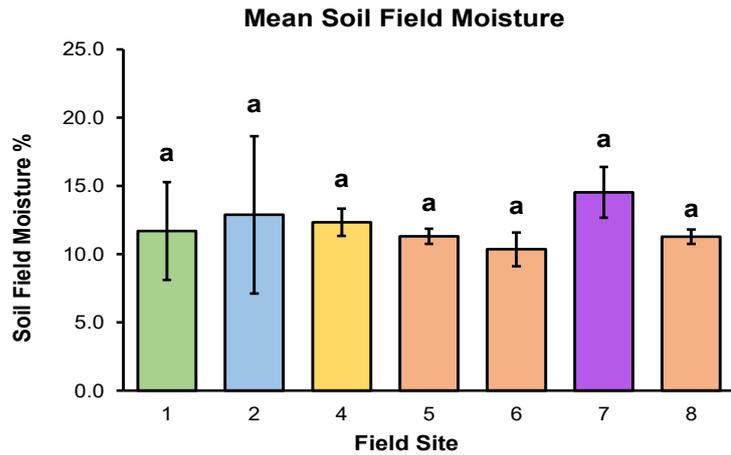


Figure 3: Mean soil field moisture. Error bars represent SD of the mean (n=5). Bars that share a lower-case letter are not significantly different ( $p > 0.05$ ).

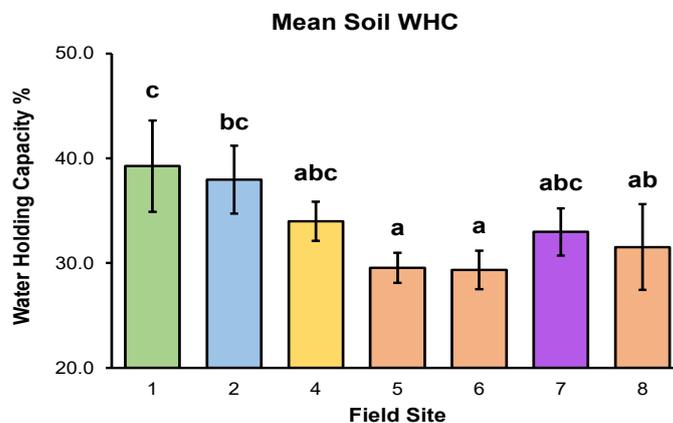
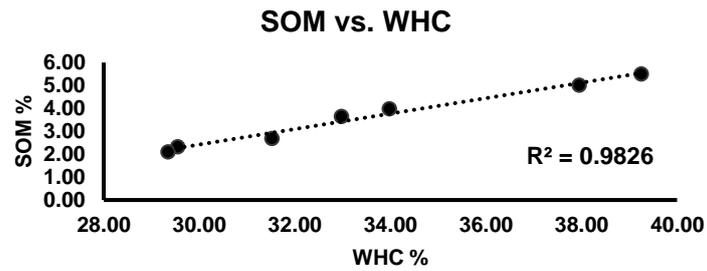


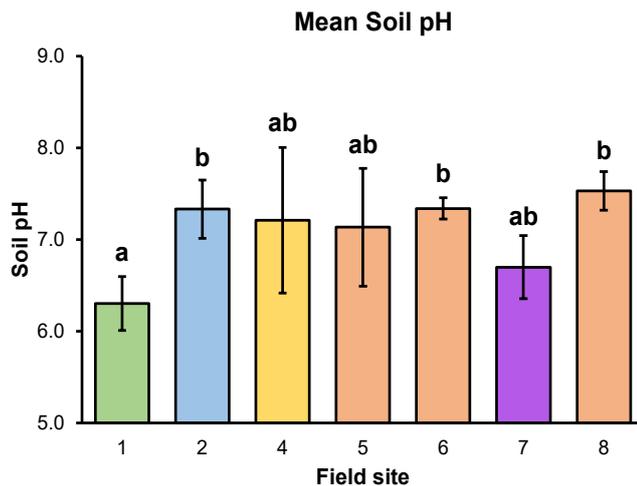
Figure 4: Mean soil water holding capacity (WHC). Error bars represent SD of the mean (n=5). Bars that share a lower-case letter are not significantly different ( $p > 0.05$ ).

Site 1 was observed to have significantly greater ( $p < 0.05$ ) WHC than all arable sites (sites 5,6, and 8), while site 2 was observed to have significantly greater ( $p < 0.05$ ) WHC than both sites 5 and 6, but not significantly different ( $p > 0.05$ ) to site 8 (Figure 4). WHC strongly correlated SOM content



**Figure 5:** Correlation between soil organic matter (SOM) and soil water holding capacity (WHC).

(Figure 7). It is likely that the SOM content (*potentially related to land use*) was the primary factor regulating WHC. Another potentially influencing factor supporting greater WHC of the pastoral land relative to the arable land may be soil structure (*potentially related to land use*); where the lacking soil disturbance in pastoral land relative to the ploughing and cultivation of the arable land may have allowed for the formation of stable soil aggregates and restructuring of the soil environment.

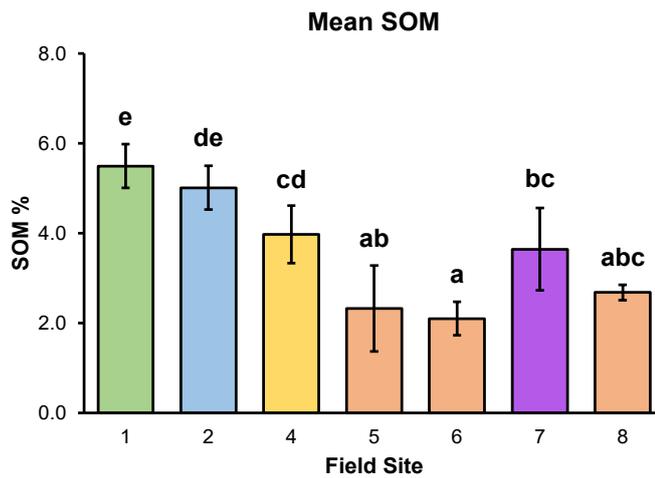


**Figure 6:** Mean soil pH. Error bars represent SD of the mean ( $n=5$ ). Bars that share a lower-case letter are not significantly different ( $p > 0.05$ ).

all measured on the higher end of the pH scale (Figure 6). It is possible that the arable fields have previously undergone some form of liming treatment to increase the soil pH, assisting in soil-plant nutrient availability, a treatment unlikely to have occurred recently within any of the pastoral fields. However, given the relative similarity in soil pH between many of the fields it is likely that soil pH relates more closely to soil textural properties (Table 2) and parent material than agricultural land use.

#### Soil pH

Soil pH varied between field sites and land use types with no clear patterns evident (Figure 6). Some field sites had significantly ( $p < 0.05$ ) higher or lower soil pH than others (Figure 6). Soil pH varied from mildly acidic pH 6.3 (site 1 (*parkland*)) to mildly alkaline pH 7.5 (site 8 (*arable field*)). However, the range of soil pH observed may be considered relatively neutral in soil terms. Significant differences ( $p < 0.05$ ) were observed between site 1 and sites 2,6, and 8, where the arable fields were



**Figure 7:** Mean soil organic matter (SOM). Error bars represent SD of the mean (n=5). Bars that share a lower-case letter are not significantly different ( $p > 0.05$ ).

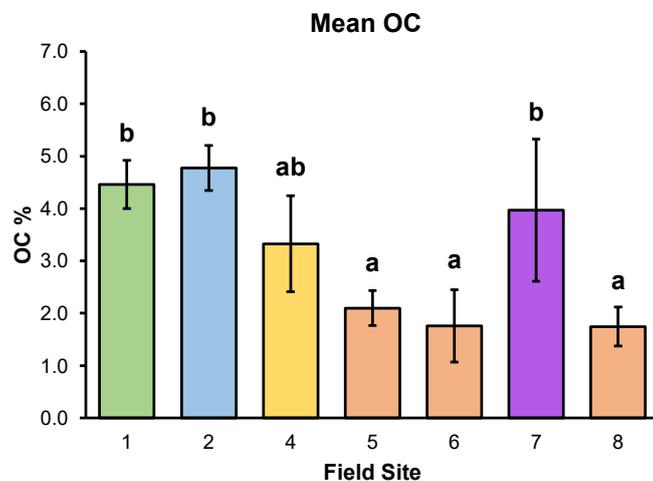
### Soil Organic Matter (SOM)

SOM was measured by loss on ignition method (ISO, 1995). SOM varied significantly ( $p < 0.05$ ) between the different sample sites (Figure 7). SOM content ranged between 2.1% in site 6 (*arable field*) and 5.5% in site 1 (*parkland*) (Figure 7). Land use was also seen to have a significant ( $p < 0.05$ ) impact on SOM content, with pastoral land containing significantly more ( $p < 0.05$ ) organic matter than arable land in all cases barring site 8, where no significant difference ( $p > 0.05$ ) was observed from site

4 (Figure 7). orchard/ arable soils also maintained a higher quantity of SOM relative to all standard arable fields, however this was only significant ( $p < 0.05$ ) compared to site 6 (Figure 7). Orchard/arable soil was also observed to be significantly lower ( $p < 0.05$ ) in SOM than the site 1 and 2 pastoral fields (Figure 7). It is likely that land use plays a significant role in underpinning the greater quantities of SOM observed in the pastoral soils relative to the arable soils, due to minimal soil disturbance (lack of tillage), permanent soil plant cover, and frequent input of organic matter from grazing excrement.

### Organic Carbon (OC)

OC was measured by instrumental analysis with Exeter CHNS analyser. SOC was observed to follow a similar trend to SOM content (Figure 7), where pastorally managed field sites contained more OC than arable field sites, with this difference significant ( $p < 0.05$ ) in many cases (Figure 8). SOC measurements ranged between 4.8% in



**Figure 8:** Mean soil organic carbon (SOC). Error bars represent SD of the mean (n=5). Bars that share a lower-case letter are not significantly different ( $p > 0.05$ ).

site 2 (*lowland meadow*), and 1.8% in site 8 (*arable field*) (Figure 8). Significant differences ( $p < 0.05$ ) were observed between sites 1,2, and 7 compared with all arable fields (Figure 8). Site 3 measured a higher OC content than the arable fields, however this was not a significant ( $p > 0.05$ ) difference when compared to either the arable fields or the other pasture fields (Figure 8). The orchard/arable field was observed to have a similar SOC to that of pasture sites 1 and 2 and was also observed to be significantly ( $p < 0.05$ ) different to the arable fields (Figure 8).

Similarly as with the trends observed in SOM, It is likely that the observed differences in OC content related to land use. Where permanent ground cover and no soil tillage would minimise OC attrition from oxidative soil carbon loss and microbial decomposition of liberated stable carbon (from destruction of stable soil aggregates), compared with the arable field sites. Furthermore, the presence of grazing animals would contribute to the delivery of more carbon to the soil.

Differences arising between SOM and OC show both the presence of other organic materials (SOM showing organic H,N,S etc.), and also the variability and inaccuracy of the LOI method for determining soil organic carbon (Figure 9). While OC determination by calculation from SOM

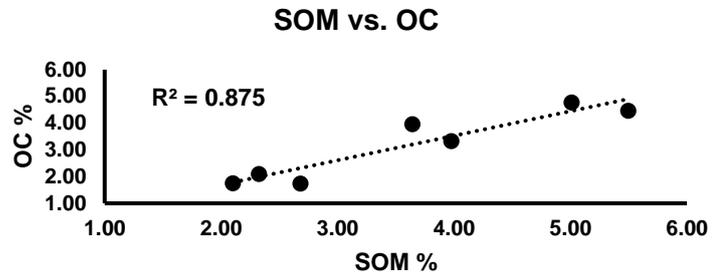


Figure 9: Correlation between soil organic matter (SOM (loss on ignition)) and organic carbon (OC) content.

content provides a good initial indication of OC content, it would be inappropriate to ascribe a soil carbon content from calculated OC % value.

### 6.1 Carbon Relative Stability

Carbon content was assessed using elemental analysis (CHN) and this data used to in conjunction with the TGA data to define relatively stable OC and relatively unstable OC loads in the soil samples. Unsurprisingly, given variations in OC (Figure 8), the amounts of relatively stable carbon and relatively unstable carbon varied between sites (Figure 10).

Relatively unstable OC varied between 6.9 g C kg<sup>-1</sup> at site 6 (*arable field*) and 24.1 g C kg<sup>-1</sup> at site 1 (*parkland*) (Figure 10). Despite these relatively large differences in OC content the relatively unstable OC as a % of total OC ranged

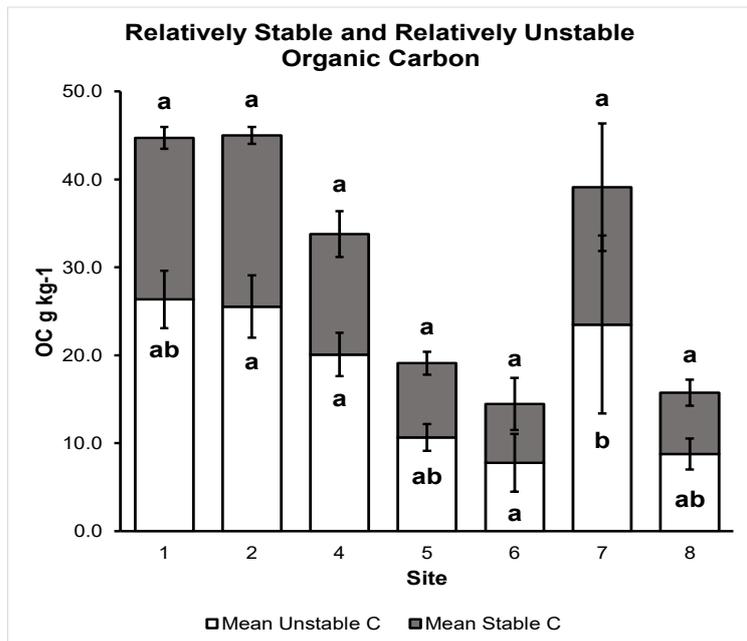


Figure 10: Total soil carbon shown as stable soil carbon and unstable soil carbon at each site. Error bars represent SD of the mean (n=3). Bars that share a lower-case letter are not significantly different ( $p > 0.05$ ), letters on the top represent significance for the mean stable C fraction and letters at the bottom represent significance for the mean unstable C fraction. Significance calculated as % of total carbon in the

between 49.5% (site 6 (arable field)) to 58.9% (site 7 (orchard/arable)) (Table 3). Significant differences ( $p < 0.05$ ) were observed between site 7 (orchard/arable) and sites 2, and 4 (both pastoral) as well as site 6 (arable), however no other significant differences ( $p > 0.05$ ) were observed between sites; furthermore, no significant difference ( $p > 0.05$ ) was observed between the different

**Table 3:** Unstable and stable soil carbon shown as relative % of total carbon.

Site	Unstable Carbon as % of Total Carbon	Stable Carbon as % of Total Carbon
1	54.0	38.5
2	50.4	39.7
4	51.2	34.8
5	53.9	42.8
6	49.5	42.5
7	58.9	38.5
8	52.5	41.8

land use types (pasture vs. arable) (Figure 10). Relatively stable OC varied between 6.01 g C kg<sup>-1</sup> at site 6 (arable field) and 17.7 g C kg<sup>-1</sup> in site 2 (lowland meadow) (Figure 10). Similarly, to the relatively unstable OC content, relatively stable OC content as a % of total OC fell within a tight range between 34.8% (site 4 (other natural grassland)) and 42.8% (site 5 (arable field)) (Table 4). No significant differences ( $p > 0.05$ ) were observed between relatively stable OC measurements, regardless of land use type (Figure 10).

## 7.1 Soil eDNA Biodiversity assessment

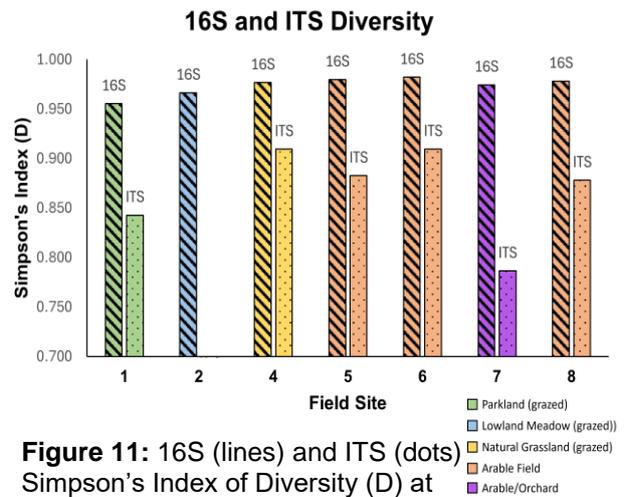
### 16S and ITS Analysis

Microbial diversity was assessed across the different sites with 16S RNA and ITS sequencing, directed towards bacterial and fungal communities respectively. These were sequenced to the species level to give Simpson's Diversity Index (D) (**Figure 11**). With this index, a highly biodiverse and stable environment was indicated by a high D value, ranging from 0 to 1. All sites showed high diversity for both 16S and ITS

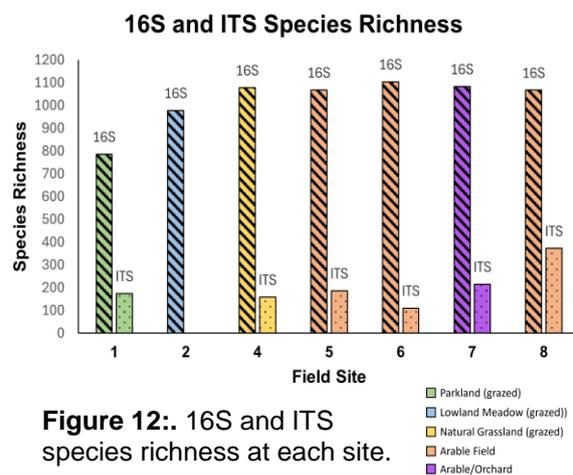
analyses, with higher bacterial than fungal diversity at all sites. Bacterial (16S) diversity stayed within a close range, from  $D=0.955$  (site 1 (*grazed parkland*)) to  $D=0.982$  (site 6 (*arable field*)), showing little difference to bacterial diversity with land use. Fungal (ITS) diversity was more variable, ranging from  $D=0.786$  (site 7 (*arable/orchard*)) to  $D=0.909$  (sites 4 (*grazed grassland*); and 6 (*arable*)), suggesting a greater interaction between fungal diversity and land use, in favour of arable land use relative to pastoral, perhaps due to increased vegetation turn over and decay.

Analyses of the 16S and ITS data was also completed to give species richness, i.e the number of different species at each site. The species richness of bacterial communities ranged from 786 (site 1 (*grazed parkland*)) to 1104 (site 6 (*arable*)) (**Figure 12**). The other sites remained within a close range, with a high count of different species across all sites. Species richness of fungal communities was lower for every site and ranged from 110 (site 6 (*arable*)) to 372 (site 8 (*arable*)).

These data for the microbial ecological communities indicate that further taxonomic analysis may be required to understand land use influence on soil biological assemblages. Additionally, with increased sampling efforts for eDNA analysis, statistical analysis could be conducted to assess significant differences between biodiversity and species richness measures for each site.



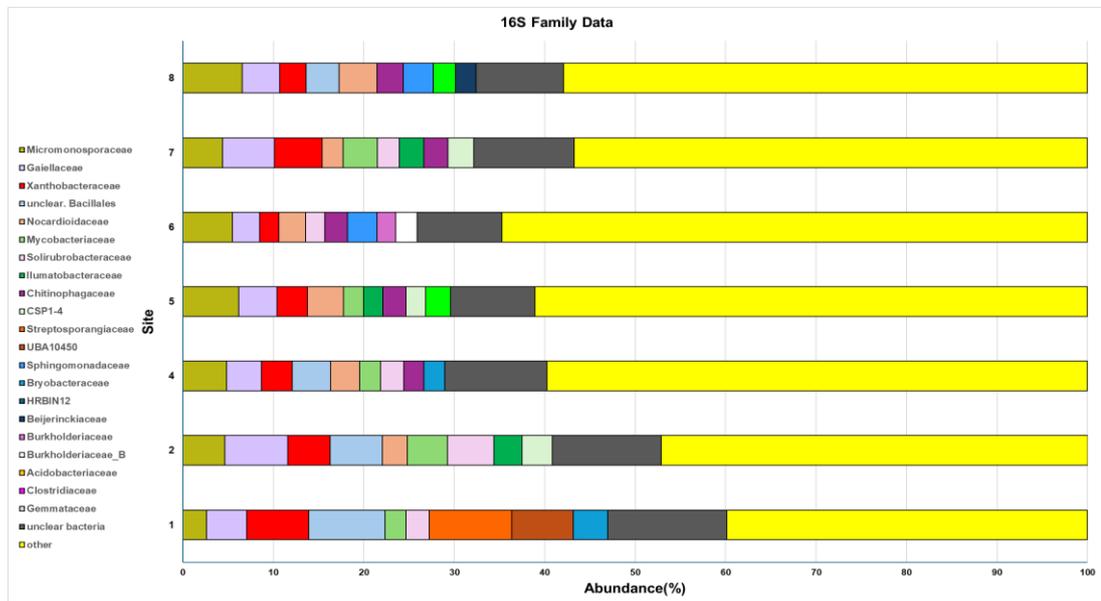
**Figure 11:** 16S (lines) and ITS (dots) Simpson's Index of Diversity (D) at each field site.



**Figure 12:** 16S and ITS species richness at each site.

## Family Abundance Data

Further taxonomic analysis was conducted on the eDNA datasets to give relative abundances of the 10 most abundant families at each site. This lends to detailed assessment of population differences between sites for 16S (**Figure 13**) and ITS (**Figure 14**) data.



**Figure 13:** 16S family data. 10 Families selected for each site to show their relative abundance with this land regime. Bacterial families are colour-coded in line with the key (left). All other families fall into the 'other' category in yellow

The bacterial family level data (**Figure 13**) indicates the 10 most dominant bacterial/fungal families in each sample site. These 10 families represented generally half of the total population, ranging from 39.1% in site 6 (arable field) to 63.6% in site 1 (*grazed parkland*). Pastorally managed land tended towards a greater enrichment of their respective 10 most abundant families relative to the arable systems, which were less dominated by their 10 most abundant families, with 'other' families making up 57.93-64.75% of total abundance.

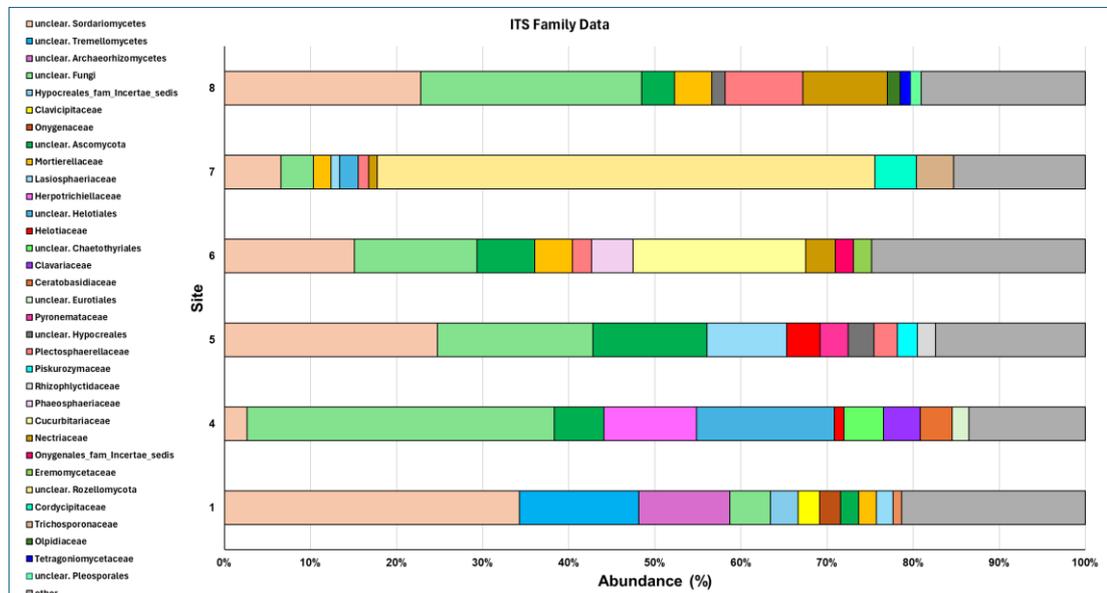
Sites were observed to generally contain similar bacterial assemblages, with Micromonosporaceae, Gaiellaceae, Xanthobacteraceae, Nocardiodaceae present in all sites, excluding site 1. Site 1 showed greater variance in the top family composition relative to the other sites, with Streptosporangiaceae and UBA10450 making up 9.15% and 6.79% of relative abundance respectively, with only site 2 also containing family UBA10450 in meaningful concentrations (1.98%), further to this these two bacterial groups were not detected at any of the other sites. This may indicate the influence of grazing, long term soil cover, or soil disturbance sensitivity on presence of these groups which were not seen in the other land regimes.

These data show that further taxonomic analysis to identify functional groups of bacteria present in pastoral compared to arable systems may be required as opposed to quantitative diversity indices.

Fungal family level data (**Figure 14**) also showed the 10 most abundant families at each site, showing that ITS families were dominated by fewer groups than the 16S data showed. This is indicated by a range of 75.16% (site 6 (arable)) to 86.45% (site 4 (*grazed grassland*)) dominance by the 10 most abundant families. This is tied to lower fungal diversity assessed at all sites compared to bacterial diversity.

Of the named families, Unclear Sordariomycetes was recorded at all sites, whilst the remaining groups show no clear patterns across the different land use systems. Site 7 (*arable orchard*) is dominated by a family in the Rozellomycota clade, suggesting this is a fungi distinctive to the orchard environment.

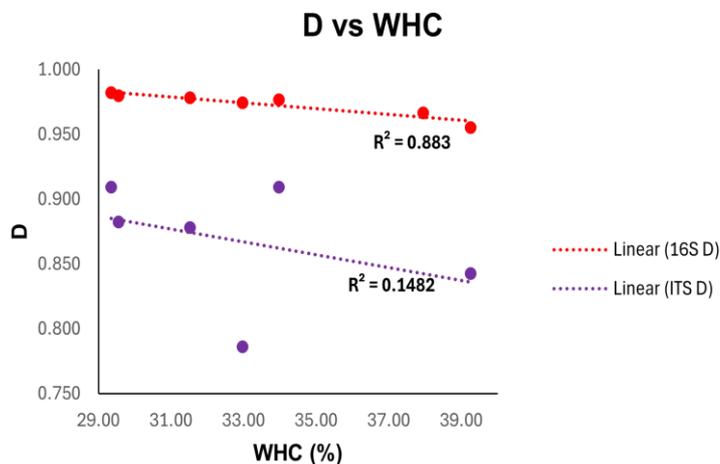
Increased eDNA sampling for ITS sequencing would provide clearer understanding of fungal assemblages and reduce ambiguity where unclear groups are present. As well as this, with improved reference databases for ITS sequencing clarity in the data would be improved.



**Figure14:** ITS family data. 10 Families selected for each site to show their relative abundance with this land regime. Fungal families are colour-coded in line with the key (left). All other families fall into the 'other' category in grey

## 8.1 Diversity and Soil Properties

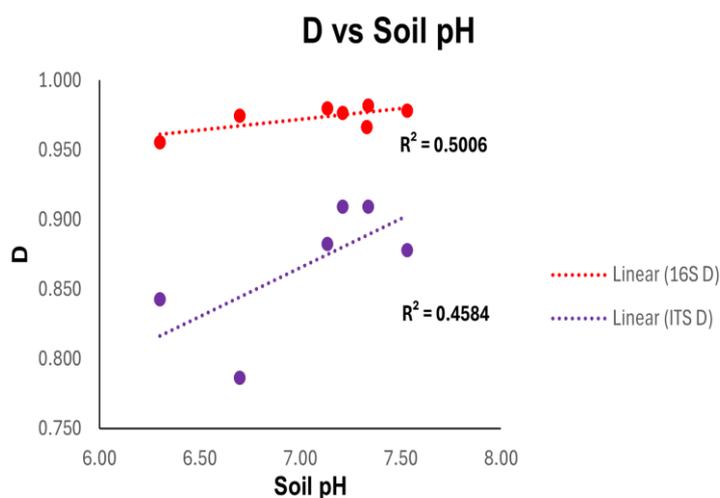
Physical and chemical soil properties were correlated with 16S and ITS Diversity to draw relationships between physicochemical and biological parameters. This was completed for WHC, soil pH, SOM and OC, identified as the dominant physical and chemical properties.



**Figure 15:** Correlation between Simpson's Diversity Index (D) and Water Holding Capacity (WHC (%)).

Diversity (D) and WHC shows a correlation between 16S D (**Figure 15**), wherein D decreases with increasing WHC ( $R^2 = 0.883$ ). It is generally expected that soil physical stability and bacterial community composition increase concurrently, however this result was not found for these data. Therefore, assessment

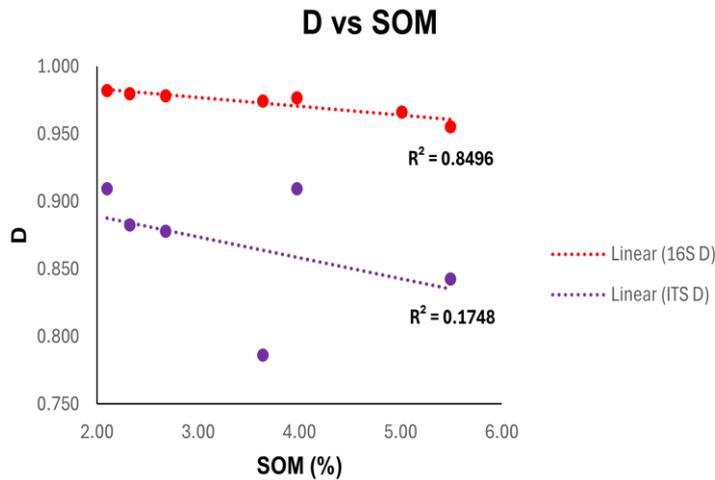
of functional groups may be required as opposed to overall D. There is no correlation between ITS D and WHC, so it cannot be inferred that soil physical structure influences fungal communities at these sites.



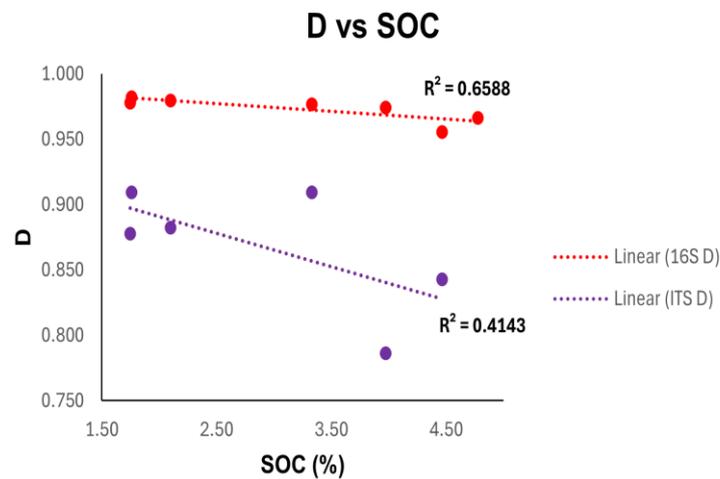
**Figure 16:** Correlation between Simpson's Diversity Index (D) and soil pH

Soil pH and D were observed to have a weak increasing correlation in both 16S and ITS ( $R^2 = 0.5006$  and  $R^2 = 0.4584$ , respectively) (**Figure 16**), where increases in soil pH up to an approximate pH 8, led to very small increases in bacterial abundance, and small increases in fungal abundance. However, a different

biodiversity measurement may be required to infer this relationship further, such as microbial biomass as opposed to D.



**Figure 17:** Correlation between Simpson's Diversity Index (D) and soil organic matter (SOM (%))



**Figure 18:** Correlation between Simpson's Diversity Index (D) and Soil Organic Carbon (SOC (%))

Compared to SOM (Figure 17) showed a strong negative correlation with the 16S data ( $R^2 = 0.883$ ). However, there was no correlation between ITS D and SOM. These results are in line with D relationships between the other soil properties WHC and SOC (Figures 15 and Figure 18), where there was also a mild negative correlation ( $R^2 = 0.659$ ;  $0.414$ ) between bacterial D and SOC, and fungal D and SOC respectively. It would be expected that SOM and SOC increase microbial community stability and diversity as these organisms are directly associated to the turnover of SOM in the soil.

Upon further analysis to give microbial biomass, these relationships may be more readily drawn. This is a limitation of the eDNA presence/absence data as this gives relative abundance instead of microbial biomass. Furthermore, different functional groups may be more variable based on these soil properties rather than the whole community.

In reference to all biodiversity data, however, it is highlighted that with increased development of sequence databases for soil microbes, richer data may be developed wherein there is reduced ambiguity of taxonomic groups and improved biodiversity indices to compare to the physical soil data, providing a stronger proxy of soil properties with varied land use.