The impact of cover crops on carbon cycling as part of an agricultural rotation

Mandy Stoker

This dissertation is submitted for the degree of

Doctor of Philosophy

March 2023

Lancaster Environment Centre, Lancaster University

Declaration

Except where reference is made to other sources, I declare that the work in this thesis is my own and has not been previously submitted, in part or in full, to any institution for any other degree or qualification.

Mandy Stoker

Abstract

Sustainably providing food for a growing population whilst maintaining a healthy soil is currently a large challenge for agriculture. Integral to the provision of a healthy soil is the input of carbon. The government's 25 Year Environment Plan (2018) includes measures to increase carbon in soil, and cover crops have been put forward as a sustainable solution for agricultural. A cover crop is a crop grown in between cash crops, either over winter or as part of a rotation. Typically, cover crops have been used as a green manure, allowing the plant to be turned into the soil to decompose, but their impact on the flow of carbon is not well understood, especially when used alongside other farming practices such as weed eradication using herbicides (glyphosate) and use of plant growth promoters such as microbial inoculants.

The overarching aim of this research was to understand the impact of cover crops on soil carbon and the soil microbial community. An in-field experiment was carried out over a three year period. A replicated experiment was used to investigate the impact of cover crops, glyphosate, and a commercial inoculant. The soil's microbial community and organic matter were analysed. Results showed that no treatments alone, or in combination, increased the soil organic matter above the pre-treatment baseline level. However, there was an increased presence of the phospholipid fatty acid (PLFA) fungal group of biomarkers. An increase in fungi could lead to an increase of stabilized carbon in soil. Inoculants used together with cover crops had a negative impact on microbial activity, and the yield of above ground biomass. Glyphosate showed an increase of gram-negative bacteria nearly 3 years after it was applied.

A pot trial using a solution of plant materials from 7 different plant species dosed into either a clay, or sandy soil, showed significant differences across all soil types and within each soil type. Turnips and mustard showed higher levels of microbes associated with high carbon:nitrogen, including fungal and gram-positive functional groups, whereas clover and phacelia (nitrogen fixing legumes), showed higher levels of gram-negative bacterial groups.

A survey on the use of cover crops by farmers, the barriers and difficulties encountered, and how farmers source information about this or any new farming techniques showed that of the farmers surveyed, 94 % of respondents used cover crops and many felt that although it was an additional expense, they were beneficial. The main issues arising were lack of knowledge about which crop species to plant, and the timing of sowing

and destroying the crop. Information gathering is a combination of word of mouth, agronomists, Farmers Weekly and the internet.

Although cover crops did not increase soil organic matter, other benefits were apparent. Further work needs to be carried out to get a better understanding of the long term effect of a mix of agricultural chemical and biological interventions over a harvest year. Specifically, an understanding about microbial activity in the rhizosphere of a standing cover crop, and the effect of the cover crop biomass as it is incorporated into the soil over several harvest. This information could assist farmers in deciding cover crop mixes and timings within the whole farm scheme.

Acknowledgements

I feel very lucky to have had the opportunity to study something new for 4 years and even luckier to have been given the support to do so, by so many people. I'm particularly grateful to my lovely husband Barry, who gave up a field for me, was my technician and farming mentor. A big thank you to Will and Hannah who are the best children in the World for putting up with an insane mother – although I know they're both grateful for extended access to the student discount card. I am of course, looking to have an upgrade on the old peoples home you'll be putting me in when I have "Dr" in front of my name.
Thank you to my parents, who have absolutely no idea what a PhD is but have been happy to listen to my ramblings. I'm so very grateful to Marie Harland and family for the fantastic accommodation in Potters Bar, and those fabulous breakfasts, jigsaw puzzles, gossip and excellent company.

Even though she refers to me as "the voice of doom in the corner" I have much to thank Deb Cairns for. She knows as much as me as she has had to listen to all my presentations and hear fascinating facts about bacteria and fungus for 4 years. Katy Jones, what a star. I can't believe you read through my work to check for typos etc but I'm truly grateful. Thanks to my other lovely friends at the Royal Hill that make life a joy. Or is that the beer?

Then there's my wonderful new friends who've been on the same path, all clever, bright people, briming with youth and encouragement. It's been wonderful to be around you all – you have made me feel very optimistic for the future of the planet. Hannah McGrath, the best company and an absolute legend, Roisin O'Riordan and Emily Masters-Clarke, my fellow microbe enthusiasts – I loved every ridiculously enthusiastic, excitable discussion we've had about these amazing little critters.

Then thank you to the professionals without whom I could not have got to where I am now. Prof. Carly Stevens, thank you for your kindness and endless patience. I have learnt so much from you and you always found my weaknesses and made me confront them, for which I am sure I'm a better person for

that. Dr Ian Shield who gave me this opportunity in the first place and gave great practical support and advise. Thank you Prof. Freddie Theodoulou for checking in on me and being the perfect mentor during the strangest of times during the Covid pandemic. Dr Annette Ryan and Dr Clare Bearskin, you have been amazing - great teachers, great wisdom and good sense of humour. I now have a thorough knowledge on stopping/starting and repairing a tower and GC. You both do a great job.

Finally, thank you to Prof. John Quinn and Dr Jeanette Whitaker for agreeing to read my work. I hope I offer something new and thought provoking.

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List of Abbreviations

ANOVA	Analysis of variance
AM	Arbuscular Mycorrhizae
BD	Bulk Density
C:N	Carbon:Nitrogen ratio
CO_2e	Carbon dioxide equivalent
F:B	Fungal:Bacterial ratio
MBC	Microbial biomass – Carbon
MBN	Microbial biomass – Nitrogen
PLFA	Phospholipid fatty acid
SOC	Soil organic carbon
SOM	Soil organic matter
%SOM	Percentage soil organic matter
SPM	Solution of Plant Material

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1 Introduction

Soil is vital to life. It is where life begins and ends, continually recycling elements to form organic and inorganic compounds. Soil provides us with one of our most basic needs -food. Without it we risk starvation. A significant challenge for famers is improving and maintaining the health of soil for future generations and to do that, it is important to understand the impact of current and new farm practices.

1.1 Big challenges for agriculture

Agriculture is facing a big challenge to produce enough nutritional food to meet the demands of the growing population. The Food and Agriculture Organisation (FAO) estimates that global crop production will grow by 18 % in the next ten years (DEFRA, 2021). By 2050 there needs to be an increase of crop production of between 25 % to 70 % above 2017 levels (Hunter et al., 2017). However, the land's ability to provide this food is diminishing (Porter et al., 2014), brought on by other demands including land use change, over reliance on chemicals and fertilisers, industrial farming and mono cropping (Giller et al., 2021; LaCanne & Lundgren, 2018). In the UK, DEFRA's Food Security Report highlights the potential threat on food supply from the effects of climate change and over exploitation of natural resources(DEFRA, 2021).

Climate change is having an impact on agriculture in many ways. Extreme and variable weather makes it difficult to predict seasonal norms making forward planning and day to day decisions about what and when to sow seeds and harvest, difficult. Environmental stress caused by drought and floods destroy crops creating global yield shocks. FAO report that 20 % - 40 % crops are lost to increased plant disease and pests which may be exacerbated by climatic change (DEFRA, 2021). Agriculture is also a major contributor to greenhouse gas (GHG) emissions causing climate change. In 2019 agriculture in the UK produced 46 million tonnes CO_2 equivalent of GHG (DEFRA, 2022a). This is a global problem and was addressed at the Climate Change Convention (COP21). COP21 aimed to hold the rise in global temperatures to "well below 2^{o}C above pre-industrial levels and limit the temperature increase to 1.5°C above pre-industrial levels" by establishing a universal agreement that is legally binding (UNFCCC, 2015). This resulted in the "Paris Agreement", which was adopted in 2015 and ratified by the UK in November 2016 (UNFCCC, 2015). The health of soil was identified as

an area of concern. For example, in 2017, a government Select Committee reported that 2.2 million tonnes of soil are eroded each year in the UK (Environment Audit Committee, 2017). Increased flooding contributed to the erosion of soil which, between 1978 and 2003 saw the carbon in soil decrease by 5g kg^{-1} from arable land (Soil Health First Report of Sessions 2016-17, 2017). The significant loss of carbon in soil was addressed at COP21, and within the Paris Agreement the 4/1000 initiative was officially launched which aims to increase the amount of carbon held in topsoil by 0.4% per annum (LPAA, 2015). Subsequently, the protection and improvement of soil was included in the Agricultural Act 2020 receiving support from stakeholders (Coe & Finlay, 2020; Soil Health First Report of House of Commons, 2017). More recently, the UK government has responded with the launch of its Soil Health Plan (SHAPE) which has ambitious goals for soil to be managed sustainably by 2030 (DEFRA, 2021).

Soil provides a vital interconnecting role in the activities associated with food production, provision of water and mineral resources, and can regulate the effects of climate change. Continual mismanagement of soil can lead to reduced fertility, increased erosion and ultimately, food scarcity and starvation (Brevik & Burgess, 2013; Ericksen, 2008; Graves et al., 2015; Hawkesworth et al., 2010). In agriculture, a healthy soil is regarded as one that is fertile and able to support the growth of crops (Kibblewhite et al., 2007). Besides offering nutrients, soil also supports several biological and ecological services which contribute to the fertility, biodiversity and sustainability of a soil (Doran, 2002). Soil needs to be well aerated, hold water and contain a rich microbial community of bacteria and fungi. Soil organic matter (SOM) is vital to the functioning of the soil and is an indicator of soil health (Finney et al., 2017). SOM can absorb and slow the flow of water (Bachmann et al., 2008; Lal, 2015; LPAA, 2015) thus reducing the loss of vital elements such as nitrogen and phosphorous to groundwater which can cause pollution to watercourses and contribute to global warming. It can also moderate the effects of drought and flooding, offering a stable habitat and a storage facility for nutrients, microbes, and other soil organisms (Amaranthus et al., 2008; Beed et al., 2018).

Applying regenerative agricultural techniques is one way to address the need to enhance SOM. The use of cover crops is one regenerative technique used to improve soil health and SOM.

1.2 Regenerative agriculture

It is recognized that the challenges to provide food, reduce GHG emissions and help mitigate climate change, require a shift towards less damaging agricultural practices. The need to practice agriculture in a way that satisfies global food requirements, and retains soil health and biodiversity, has resulted in a growing interest in regenerative farming (Giller et al., 2021; Toensmeier, 2016). Regenerative farming was highlighted by Gabel (1979), with the aim to farm in a way that is sympathetic to nature, working with it rather than against it, and seek ways to reduce the reliance on chemical inputs and fossil fuels. The main regenerative practices include a) the reduction of soil disturbance, b) never leaving soil bare c) increased plant diversity and d) integration of crops and livestock (LaCanne & Lundgren, 2018). Unlike organic farming, regenerative farming does not exclude the use of herbicides, fertiliser or additives.

Farming still relies heavily on some chemicals, and research is continually striving to invent new, sustainable products to replace synthetic chemicals. In particular there has been a growth in the manufacturing and use of various biological inoculants (Bhattacharyya & Jha, 2012; Santos et al., 2019; Suyal et al., 2016). These contain beneficial microbes that can stimulate plant growth (Bhattacharyya & Jha, 2012; Dasilva, 2022; Glick, 1995; Kundan & Pant, 2015) and provide resistance and resilience to disease and stress in crops (Beneduzi et al., 2012). The microbial species used and the ecosystem services they provide to improve soil health are discussed further in the literature review, Chapter 2. A regenerative technique that is becoming more widespread is the use of cover crops, which is a crop that is established between cash crops. It provides protection of bare soils as well as other benefits, including soil stability, improved soil health, and can provide an overwinter fodder (White et al., 2016). Further details of species of cover crops used, and the benefits that they offer are discussed below.

1.3 Use of cover crops in the UK and Europe

Catch crop and green manures are other interchangeable terms used to describe cover crops based on the service provided. A catch crop is specifically used to capture nutrients in the soil preventing them from being leached into water courses (Doltra & Olesen, 2013). Green manures are grown for the purpose of adding biomass to the soil (AHDB, 2018).

The uptake in the use of cover crops is growing, albeit slowly as it is still perceived as an added cost (Storr et al., 2019). In a survey carried out in the UK in 2016, 66% of the 117 farmers that responded, used cover crops following harvest in 2016 (Storr et al., 2019). In the US there has been a 50 % increase in cover crops from 10.3 million acres in 2012 to 15.4 million acres in 2017, (Wallander et al., 2021) . It is primarily used on maize silage with a smaller amount in wheat. The Federal State and private organisations have financially assisted farmers to adopt the use of cover crops since 2018 (Wallander et al., 2021). In Europe the uptake of cover crops varies amongst the member states. In a survey of 600 farmers (150 per country) carried out in 2019, cover crop use in Spain was 12 %, in France 84 %, in the Netherlands 99 % and in Romania 46 % (Smit, 2019). As in the US, cover crops are mainly used after silage maize, wheat and barley. Drivers to use cover crops include legislation linked with Government subsidies and the Nitrate Directive (Smit, 2019).

In the UK, under the terms of the governments basic payment scheme (BPS), a rural grant that supports farmers, a cover crop has to include at least two plant species and be established by the 1st October, remaining until the 15th January. The Agriculture Act 2020 sets out new areas where financial assistance may be provided. The Government's policy paper "Arable and horticultural standards 2022" offers a sustainable incentive scheme for arable and horticultural soils (DEFRA, 2021a). The standard provides a financial reward for farmers for the establishment of an over winter green cover. The standard provides a financial payment to 3 levels – introductory, intermediate, and advanced based on the total coverage of cover crops (DEFRA, 2021a)

Table 1.1 The levels and indicative annual payments for the use of cover crops under the arable and horticultural soils standard

Level	Payment per hectare (ha)	Actions
Introductory	£26	Complete a basic soil assessment Establish green cover over winter (5% area) Increase soil organic matter (10% area)
Intermediate	£41	Complete a basic soil assessment Establish green cover over winter (10% area) Increase soil organic matter (15% area) Use no, low or min tillage techniques (25% area)
Advanced	£60	Complete a basic soil assessment Create a soil management plan Establish green cover over winter (15% area) Increase soil organic matter (20% area) Use no, low or min tillage techniques (25% area)

Research has demonstrated the long-term benefits of cover crops in terms of ecosystem services including increased food production, climate regulation, water and soil regulation, and weed control (Calonego et al., 2017; Dutta et al., 2019; Groff, 2015a; Kaye & Quemada, 2017; Kruger et al., 2013). Commonly, cover crops are used to capture nitrogen, improve soil structure, weed suppression and pest control. Their role in the addition of soil organic carbon (SOC), which plays a major part in the delivery of various ecosystem services, is of particular interest in this research and discussed further in Chapter 2. The plant species used depends on the purpose, the site conditions, and the crop rotation.

1.3.1 The roles of cover crop to capture nitrogen

The agricultural sector relies heavily on nitrogen inputs to increase yields yet cover crops can be used to add nitrogen to the soil. Cover crops can be identified under two groups: legumes (such as hairy vetch and clovers) that fix atmospheric nitrogen making it available in soil, and non-legumes (such as grasses and brassicas) or cereals and non-cereals that scavenge or catch nitrogen in the soil and keep it in situ preventing its loss into groundwater. Once the cover crop is incorporated into the soil, the nitrogen is made available for the next cash crop.

Nitrogen fixation and release can be affected by soil type, climate, and plant species (Doltra & Olesen, 2013; Li et al., 2015; Sparrow et al., 1995), and legumes fix more atmospheric nitrogen in soils that are poor in nitrogen (Oldroyd, 2013). As cover crops are often established over winter, the effectiveness of the legume can vary depending on the time it was established. Warm soil and longer daylight hours allow for photosynthesis, which is conducive to high levels of nitrogen fixed (White, Holmes, & Morris, 2016). Biological nitrogen fixing requires energy which comes from glucose stored by the host plant. It is estimated that 22.8 g of glucose is required to produce 1 g of N_2 which equates to 25-33 % of the carbon fixed in photosynthesis (Li et al., 2015; Pate, 1985). It has been determined that there is a positive correlation between nitrogen fixation and dry matter production (White, Holmes, & Morris, 2016). A mix of legumes and grasses can enhance the fixing of nitrogen (Li et al., 2015). The grasses scavenge nitrogen from the soil which forces the legume to take nitrogen from the atmosphere (Rasmussen et al., 2012). Non legumes such as mustard (*Brassica hirta*), rye (*Sicate*

cereale), black oats (*Avena strigosa*) , radish (*Raphanus sativus)* , phacelia (*Phacelia tanacetifolia)* and buckwheat (*Hircum triticum*) mine soil nitrogen, mopping up any residual mineral nitrogen and mineralized nitrogen from easily degradable organic nitrogen(Grinsven HJM, 2015). Such cover crops are used to prevent leaching by retaining nutrients in situ. An average maximum uptake of 30 kg ha^{-1} N was recorded in cover crops planted in the UK between August and October and destroyed between December and March (Harrison 1998, cited in an ADAS report to Defra (ADAS, 2007). Table 1.2 shows the quantity of over winter nitrogen

Table 1.2 Commonly used nitrogen fixing cover crops giving uptake and release (Reproduced from White, Holmes, Stobart, 2016)

Species	Typical autumn / winter N uptake (kg N/ha)	Typical N release for following crop (kg N/ha)	C:N ratio	References
Hairy vetch (*Vicia villosa)*	154	132	11 (8-15)	Reeves (1994) (Clark 2012; Ranells and Wagger 1996)
Rye (*Secale cereale*)	30-61	24	82	Patoja *et al.* (2016), (Clark 2012; Odhiambo and Bomke 2008; Ranells and Wagger 1996)
Crimson clover (*Trifolium incarnatum*)	28	60	11 - 25	Reeves (1994) (Odhiambo and Bomke 2008; Ranells and Wagger 1996)
White senf mustard (*Brassica hirta*)	57 - 116	30-40	Total plant 14; Leaves-9; Stems-19	(Collins et al. 2007; Bugg et al. 2011; Stivers-Young 1998; Silgram et al. 2015)
Oilseed radish (*Raphanus sativus)*	70 -127	10-50	Stem 13; bulb 20	(Dean and Weil 2009; Silgram et al. 2015; Stivers-Young 1998)

fixed by commonly used cover crop species and the total made available to the following cash crop. The role of bacteria in nitrogen fixation and how it affects the cycling of carbon is discussed further in the literature review, Chapter 2.

1.3.2 Weed suppression

The suppression of weeds with cover crops has been demonstrated in several field (Brust et al., 2014; Florence et al., 2019; Soti & Racelis, 2020; Wallander et al., 2021) and laboratory experiments (Bezuidenhout et al., 2012). Cover crops offer both a physical and chemical solution to weed control. Physically, the establishment of cover crops over winter can provide a canopy which denies potential weeds of light (Brust et al., 2014). Chemically, particular cover crop species, such as white mustard (*Sinapis alba*), turnips (*Brassica rapa*), radish (*Raphanus sativus*) and hairy vetch (*Vicia villosa*) produce weed control chemicals that are released through the roots, volatilized through leaves or leached into the soil by decomposition (Brennan et al., 2020; Dutta et al., 2019; Kruger et al., 2013). It is considered that the competitive, physical (elimination of light) mechanism has the advantage of working relatively quickly giving sufficient short-term benefits, (Finney et al., 2016). The chemical, allelopathic mechanism is slower but provides longer lasting benefits of weed control (Bezuidenhout et al., 2012). Particular brassicas such as turnip, white mustard, and radish can produce glucosinolates that break down into nematotoxic isothiocyanates (ITC) that fumigate the soil killing or reducing pests such as nematodes (Brennan et al. 2020; Dutta, Khan, and Phani 2019; Brust, Claupein, and Gerhards 2014; Kruger, Fouri, and Malan 2013). ITC's are released when the plant cell walls are damaged by decomposition or maceration (Kruger et al., 2013). In work carried out using a mustard cover crop as a biofumigant on root rot in beet, there was a reduction of the transmission of the primary infection (Motisi et al., 2013).

1.3.3 Soil structure

The physical attributes of a soil can determine its effectiveness in supporting a crop. Soil structure depends on the soil type, location, climate and typography, and can be affected by agricultural practices. Soil erosion accounts for the movement of an estimated total sediment flux of about 35 (±10) Pg yr-1 which equates to 0.5 (±0.15) Pg C yr-1 of agricultural C erosion and an estimate of 0.08 (±0.02) Pg for C removed to river systems by

water erosion (Quinton et al., 2010). In addition, farming practices using heavy machinery can lead to soil compaction making it uninhabitable for worms and mites that are essential in the maintenance of soil structure, and impenetrable for plant roots (Birkás et al., 2004; Rosolem et al., 2002). Ploughing can break up the upper layers of the soil but this can lead to soil erosion and carbon loss, as well as damage to the soil community. Cover crops can reduce soil erosion (Kumar Meena et al., 2019). A leaf canopy can slow the effects of raindrops by breaking their fall and slowing the flow of rainwater, reducing detachment and allowing water to percolate through the soil (Kaspar & Singer, 2011). A plant root system can help to improve the soil structure by penetrating into the soil and increasing infiltration. A field trial in Germany established 7 different cover crops and measured the Root Length Density (RLD) over winter. Of these, crimson clover and winter rye provided a dense root mass within the topsoil; oil radish, winter turnip, rape and phacelia provided a highly branched, dense rooting system; bristle oats provided low-branched primary roots and, blue lupin provided a large diameter root within the topsoil (R. Kemper et al., 2020). Significant improvement ($p < 0.05$) of physical and biological properties of soil were shown where cover crops were used compared to no cover crops. Further more, bulk density decreased by 3.5 % and the C/N ratio decreased with depth (Haruna & Nkongolo, 2015).

1.3.4 Whole farm considerations to growing cover crops

Whilst there are many benefits to be gained from growing cover crops, consideration must be made regarding the extra work and costs associated with planting an additional crop. Cost is often cited by farmers as being the main barrier to their use (Roesch-Mcnally et al., 2021; SARE, 2020; Storr et al., 2019). Additional costs include labour, the seed, fuel, and pest management. Typically, a cover crop is sown following a harvest or undersown between the rows of an established cash crop such as maize. The cover crop is established over the winter months enabling it to provide soil stability, biomass, weed suppression and regulate nitrogen (Adetunji et al., 2020; Blesh, 2018; Florence et al., 2019; García-González et al., 2018). At the end of winter the crop is destroyed and incorporated into the soil releasing nutrients and carbon. The most common ways to remove the crop is to terminate it with the herbicide glyphosate, or use a machine such as a roller, to crush or crimp the plant stems. The latter is usually only effective after a frost (Storr et al., 2020). Termination or management of the cover crop is an important consideration if a cash crop is to successfully follow on without

competition. Many farmers are reliant on glyphosate to clear unwanted plant growth however, in recent years, many countries have banned or restricted the use of glyphosate because of its link with cancer in humans (Myers et al., 2016). Glyphosate is currently approved for use in the EU until 15th December 2022 (European Commission, n.d.). The absence of glyphosate would make it difficult to manage the cover crop at the end of the season potentially leaving rogue plants in the cash crop. However, the effect of glyphosate on the soil microbial community is not well understood and further research into the impacts of glyphosate is needed (Benamú et al., 2010; Hagner et al., 2019; Rosenbom et al., 2014; Silva et al., 2018). With the concern over glyphosate and other intensive farming methods, more attention is being given to alternative, regenerative methods of farming.

Regenerative farming has opened up a growing interest in the use of microbial inoculants (Oviatt & Rillig, 2020). The addition of a mixed microbial inoculant to soil or seed has been found to improve soil and plant health (Alori et al., 2019). Many commercial biological products contain endo mycorrhizal fungi, and beneficial bacteria which are linked to increased plant growth. Whilst such inoculants are not specifically sold to activate the process of aggregate formation, the inclusion of mycorrhizae offers up the possibility of such an eco-service (Gosling et al., 2006; C. Jones, 2009; Wright & Upadhyaya, 1998). The use of inoculants with a cover crops to increase biomass yield may, in turn, increase the soil organic matter (Santos et al., 2019).

In a UK survey carried out in 2017, farmers said they would consider using cover crops if they had more information on the effect of cover crops, the economics, and access to funds for seed purchase (Storr et al., 2019). The focus on mitigating climate change through the creation of a carbon sink held within the soil, is one of several strategies towards the achievement of government targets (DEFRA, 2018). Financial incentives encourage the use of cover crops in the UK through the Arable and horticultural standard (DEFRA, 2021a) , and private sector voluntary soil carbon credit schemes. Understanding how cover crops can be used to specifically increase carbon in soil will provide local financial benefits and make it a more affordable soil management technique. The greater, global benefit is the sequestering of carbon to mitigate climate change, improve soil health and secure long term food supply. Therefore, our understanding of the benefits of the plants together with the mechanism involved in the flow of carbon is crucial. Specifically, there is no research

that investigates the cumulative effects of a mix of common farming practices used to help establish a cover crop including the addition of herbicides (weed clearance and crop desiccation) and application of inoculants.

1.4 Aims and Objectives

The overarching aim of this research is to further understand the impact of cover crops on carbon cycling with particular attention to the effects of cover crops on soil in the context of other commonly used farming practices including the clearance of weeds using glyphosate, and use of a biological inoculant to stimulate growth.

A field trial was designed to best resemble the environmental and agricultural conditions for growing a cover crop and to measure the changes in the soil and microbial community. Chapter 3 describes the design of the trial, methodology, and results for the first year of establishment. Chapter 4 provides the methodology and results of additional analyses carried out in the second year up to the termination of the cover crop and growth of the follow on cash crop. A further objective was to identify if one cover crop species is more attractive than another in building a microbial community conducive to forming carbon aggregates, and compare this in two types of soil, clay and sandy. The methodology and results are described in Chapter 5.

A final objective is to align the experimental work into a practical context, by understanding the use of cover crops by farmers. Chapter 6 describes a survey used to investigate current reasons for uptake of cover crops, and perceived benefits and difficulties associated with their use. Discussion and conclusions are provided in Chapter 7.

2 Literature Review

Cover crops are widely used in farming to reduce soil erosion, water pollution, increase organic matter, improve structure, and regulate nutrients (Paustian et al., 2016; Kessel et al., 2013). Using cover crops to increase soil organic carbon (SOC) stock is a strategy favoured by the UK Government (DEFRA, 2018), however their effectiveness is unclear. As with any crop, the yield can be affected by the management regime – the preparation of the soil, the control of weeds and pests, and application of manure and nutrients and all of these factors can potentially change the stock of carbon. This review describes the soil carbon cycle; the role of cover crops, the effect of adding glyphosate, and the use of inoculants as part of a whole harvest strategy to increase SOC.

2.1 Carbon cycling in soil

Carbon cycling is the movement of carbon from one form to another within and between air, land and water. Carbon is one of the most abundant elements in the Earth's crust. It can form many organic compounds and is found in all forms of life. Soil offers a globally significant capacity for the storage of carbon (Lal, 2015, 2018; Poulton et al., 2018). Soil organic matter (SOM) comprises 50 % to 58 % organic carbon (Pribyl, 2010). The total global SOC in soil to a depth of 1 m is estimated to be 1505 Pg of which 55 % is held in the top 0.3 m (Lal, 2018). It is estimated that between 115-154 Pg of carbon that was once in the soil has been lost to water and air contributing to climate change (Lal, 2018, 2019). Moving carbon from the atmosphere back into soil is one strategy to mitigate the impact of climate change. The stock of SOC can be increased by i) adding organic inputs such as manure or plant biomass, ii) increasing the below ground biomass iii) increasing the symbiotic microbial activity to increase the pull of CO_2 through plants iv) reducing gaseous releases and v) slowing the flow of carbon by stabilizing it (Ahmed et al., 2020). For carbon stocks to increase, inputs of carbon need to exceed outputs.

The movement of carbon within the soil is complex and has, over the years been subject to different theories and models (Lehmann & Kleber, 2015). Humification is based on the decomposition of organic matter and the selective preservation of carbon molecules into stable compounds or mineralized. Other theories consider the

decomposition of organic matter from large to small molecules, predominantly by microbial activity (progressive model) and take this a step further by considering the split between labile and recalcitrant forms of carbon. The current proposed model is the "soil continuum model" which shows a more complex flow of carbon from large to small molecular size and the formation and deformation, and adsorbtion and desorbtion processes occurring throughout. This is stimulated by environmental conditions such as temperature, moisture and soil biota (Lehmann & Kleber, 2015) .

Figure 2.1 shows how soil biota plays a significant role in the cycling of carbon, taking carbon from plants, animals and dead organic residues, and releasing carbon as gases, providing building blocks for new life or stabilizing carbon as aggregate. The composition of the microbial community determines the rate of flow of the carbon into and out of the soil and the microbial community is determined by its environment (Johnson et al., 2015; Malik et al., 2020; Ward et al., 2016) .

Rainfall, temperature, location, and soil type affect the microbial community, together with the preparation of the soil and inputs used during a typical harvest year. The addition of fertiliser for example, can impact the quantity of carbon in soil in grassland. Intensive management with a high input of fertiliser will deliver the lowest SOC, whereas negligible management with no fertiliser gives a greater stock of SOC. However, the highest carbon is found in grassland under intermediate management where some fertiliser is used (Ward et al., 2016).

The relative abundance of bacterial and fungal species alters according to the strategy employed to operate within a given environment (Malik et al., 2020). A nutrient rich, healthy soil that is not subject to climatic or other stress will comprise a different microbial community structure than a soil under stress from drought or intensive farming practices (Fernandez et al., 2020; Jones et al., 2009; Malik et al., 2020). The microbial mix

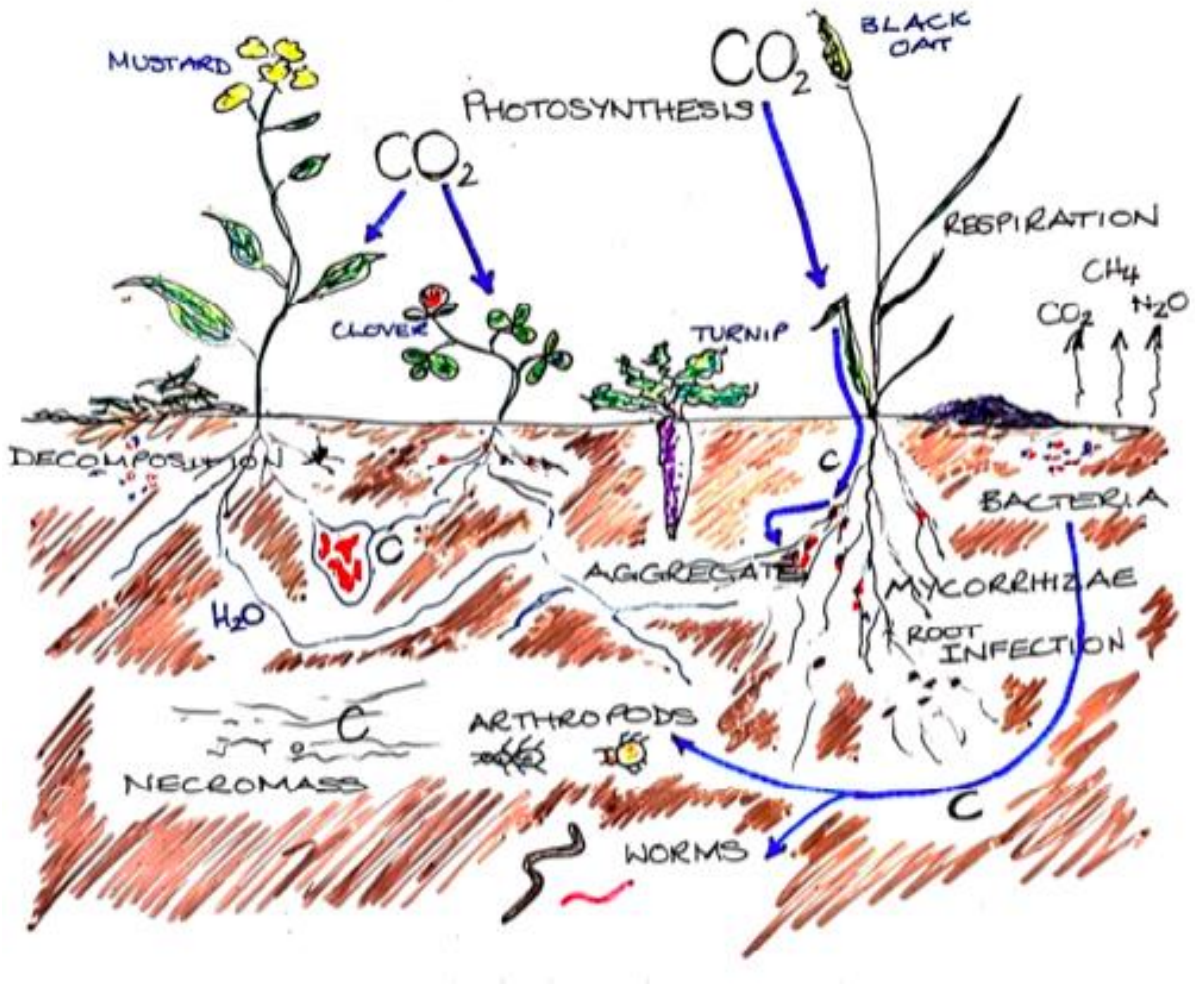


Figure 2.1 The movement of carbon into and out of soil. Carbon enters the soil food web from the decomposition of leaf litter and organic matter by microbes, other earth dwellers, and the infection of roots by mycorrhizae forming stable aggregates. Organic matter, aggregates and necromass all contribute to the soil carbon stock. Carbon leaves soil through respiration and denied by the removal of biomass.

affects the carbon cycle in many ways, for example: the release of enzymes and substrates that invest in biomass formation, or stabilize carbon into aggregates. Three such microbial strategies are outlined below including: High Yield Investment (carbon flows out of the soil into biomass such as a crop), Resource Acquisition (carbon has to be sought from more recalcitrant sources such as wood), and Stress Tolerators (carbon flow slows down and accumulates) (Malik et al., 2020).

2.1.1 High Yield Investment

Microbes in a healthy soil can deliver a "High Yield Investment" strategy. They infect plant roots, draw down sugars and in return gather resources in the form of nutrients and water, generating high yielding crops. In this situation where resources are abundant and there is readily available labile carbon, copiotrophic bacteria are dominant (Fierer et al., 2007). This group of bacteria has a high growth rate and are themselves consumed by other soil dwellers. Dead microbes (necromass) contribute to the carbon pool (Berhongaray et al., 2019; Buckeridge et al., 2020; Chenu et al., 2019; Kallenbach et al., 2016; Lavallee et al., 2020; McDaniel et al., 2014; Poeplau et al., 2018). At the roots, carbon is converted into aggregates, a process linked to the presence of glomalin, a sticky exudate produced by a fungus, Arbuscular Mycorrhizae (AM) (Gillespie et al., 2011; Gosling et al., 2006; Jansa et al., 2013; W. Wang et al., 2017; Wright & Upadhyaya, 1998).

2.1.2 Resource Acquisition

Where soil has a lower carbon stock and fewer nutrients, the microbial community turns to a strategy of "Resource Acquisition". Here microbes seek out nutrients by producing exudates that can decompose tougher, more woody materials, and mine for minerals. In this microbial community there will be a mix of copiotrophic and oligotrophic bacteria, the latter taking the recalcitrant carbon (Dignac et al., 2017; Fierer et al., 2007; Malik et al., 2020). This activity results in the release of carbon as carbon dioxide (CO_2) and methane (CH_4), back into the atmosphere. AM will be active in the formation of aggregates (Wright & Upadhyaya, 1998).

2.1.3 Stress Tolerators

The microbial community in soil under stress is dominated by the oligotrophic bacteria which are "Stress Tolerators". The growth of a microbial community is slow where oligotrophic bacteria are prevalent (Fierer et al., 2007). The Fungal:Bacterial ratio is high under stressful conditions and the mining of resources and cycling of carbon is slow. Carbon is stabilized forming aggregates, adsorbed onto particles and held within fungi (Malik et al., 2020).

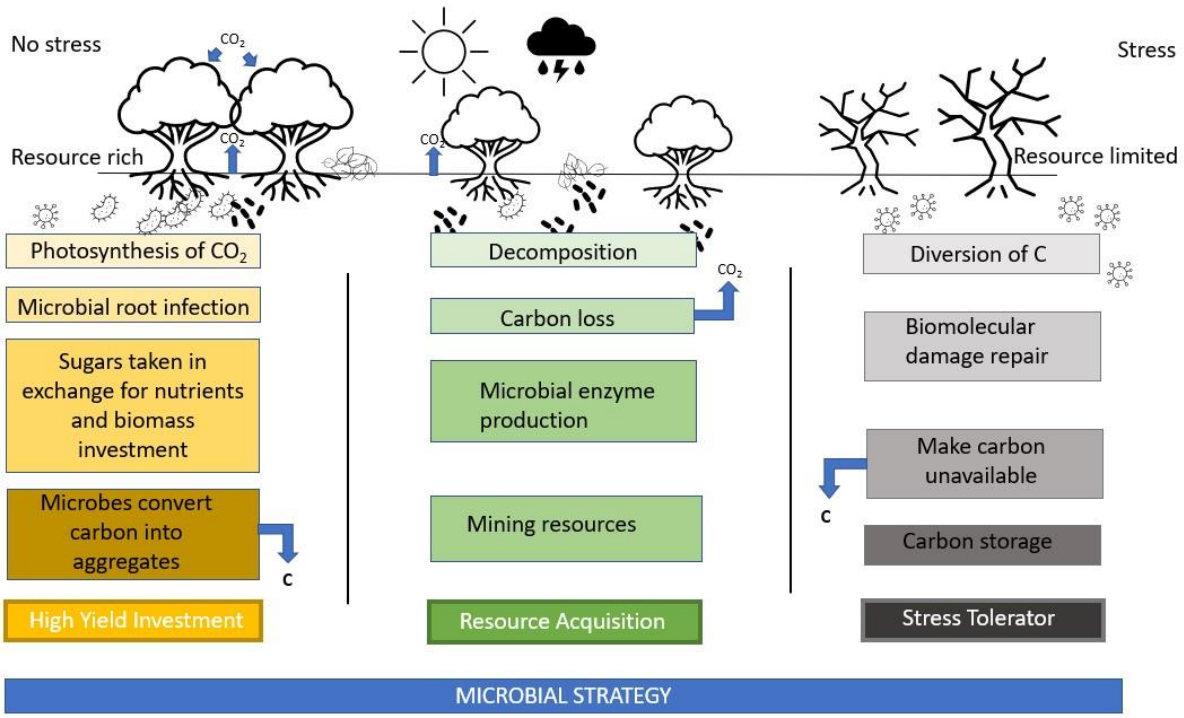


Figure 2.2 The microbial strategy and movement of carbon adopted in soil under a low stress, medium stress and high stress environment.

Recent research has shown that fungal species are more resistant to drought than bacteria (de Vries et al., 2018). Drought causes changes to the microbial community following destabilisation of bacteria which can have repercussions on the future composition of above ground plants and crops (de Vries et al., 2018).

Over the course of an agricultural year, soil is subject to various degrees of mechanical, chemical and biological intervention. Three agricultural interventions are considered below in terms of their impact on the carbon cycle in soil i) the use of cover crops, ii) the use of the herbicide glyphosate and iii) the use of biological inoculants.

2.2 The role of cover crops on SOC

A cover crop, a crop grown between cash crops either seasonally or as a rotation, provides a pathway for the movement of carbon from the atmosphere into the soil. Some farmers use cover crops as a green manure, by ploughing in the biomass or by rolling the crop flat following a hard frost (Couëdel, Alletto, Tribouillois, et al.,

2018; HDRA, n.d.; Rosenfeld & Rayns, n.d.). The biomass decomposes and becomes part of the soil. There are several papers describing field experiments which look at the role of cover crops and the increase of carbon in soil. Table 2.1 provides a synthesis of the most recent papers published which include field plot experiments where a cover crop treatment is established, and results measured over a short or long term. Microbial biomass carbon is the most commonly used measurement with 54 % of 24 studies indicating an increase where cover crops are used.

Several meta-analyses have been completed analysing results from published research papers relating to cover crops. There is agreement that long term use of cover crops increase SOM or at least do not decrease SOM following a cash crop (Jian et al., 2020; Kim et al., 2020; McDaniel et al., 2014; Muhammad et al., 2021; Poeplau & Don, 2015; Shackelford et al., 2019). Meta-analysis completed on 57 papers covering arable farms in the Mediterranean and California concluded that cover crops increased soil organic matter by 9 % and carbon dioxide emissions were 15 % higher than land left fallow (Shackelford et al., 2019). It is recognized that the growth of SOM and changes in the microbial community is influenced by cover crops species, and the length of time a cover crop is used (Jian, Du, et al., 2020; Kim et al., 2020; Lori et al., 2017; Muhammad et al., 2021). The use of cover crops can also result in increased emissions to air in the form of carbon dioxide and can impact water uptake which can have a detrimental affect follow-on crops (Shackelford et al., 2019).

Table 2.1 A synthesis of research outcomes on soil carbon and microbes with the use of cover crops. SOM = soil organic matter, SOC = soil organic carbon, + = an increase, - = no change or a decrease.

Author	SOM	SOC	Microbial Community	Bacteria	Fungal	Details of findings regarding carbon in soil and changes in microbial community
Sainju 2007			+			Increased MBC in non-legume cover crop compared to legume
Brennan 2017			+			Cover crops increase MBC in sandy and loam soils
D Finney 2017			+		+	Arbuscular Mycorrhizae (AM) are abundant under oat and rye and non AM are positively associated with hairy vetch
D Finney 2017			+	+	+	Living cover crops have immediate impacts on MBC structure and function
Mazzola 2015			+			Different plants attract different microbes
Lehman 2015b			+			Living biomass provides resources to support microbial populations
Olson 2014		+				The cover crop treatments had more SOC stock than without cover crops
Strickland 2019			+			In the presence of cover crops, active microbial biomass increased by 64 %, respectively.
E B Moore et al 2014	+					Rye cover crop gave an increase in SOM compared to no cover crop
E B Moore et al 2014	-					Rye cover crop gave no change in SOM when following soybean
Venkateswarlu 2007		+	+			Long term use of legume cover crops increased SOC and MBC
Martinez-Garcia 2018			+			Cover crop species and management affect MBC
Six et al					+	Cover crops increase fungal dominance
Njeru 2014, 2016					+	Improved fungal colonisation with cover crops
Mendes et al 2019			+			Increase MBC where cover crops are used
Salazar 2020		-				After 8 years of winter cover crops, there was no observable changes in SOC
Haruna 2015			+			Cover crop significantly improved soil biological properties
Poeplau 2014		+				Green manure led to a significant increase in SOC. 102/139 observations had an annual change rate of 0-1 mg ha-1 yr-1
Jian 2020		+				Cover crops and cover crops in rotations significantly increased SOC with overall mean change 15%
Shackleton 2019	+	+				In plots with cover crops, there was 13% less water, 9% more organic matter and 41% more microbial biomass in the soil
Kim 2020			+			Cover cropping significantly increased parameters of soil microbial abundance, activity, and diversity by 27%, 22%, and 2.5% respectively, compared to those of bare fallow.
Mohamed 2020			+			Compared to no cover crop, cover crop overall enhanced PLFA, MBC, and MBN by 24, 40, and 51%, respectively
McDaniel 2014		+				Adding one or more crops in rotation to a monoculture increased total soil C by 3.6% and total N by 5.3%, and in rotation, total C increased by 8.5% and total N 12.8%.
Fierer et al 2007				+		Cover crop give rise to increased concentrations in certain microbial groups -Gram-ve leaf litter (labile carbon), Gram + (recalcitrant carbon).

Another factor to consider is the impact of cover crop selection on nitrogen levels because carbon cycling is linked to nitrogen. The ratio of carbon to nitrogen (C:N) determines the rate at which carbon moves out of the system (D. L. Jones et al., 2009; Khan et al., 2007; Lei et al., 2015; van Veen et al., 1985). There is a link between the use of cover crops with a significantly ($p < 0.001$) decreased N leaching, thus retaining N in the soil and significantly ($p < 0.001$) increased SOC sequestration (McDaniel et al., 2014). A C:N ratio greater

than 30 will immobilize carbon whereas a ratio of less than 20 will result in the formation of aggregates and mineralization (Jenkinson et al., 1990). N fixing can be controlled by selecting cover crops with legume species. Cover crops with a low C:N ratio such as legumes, are incorporated into the soil quickly (AHDB, 2018). Commonly used legumes include clovers, vetch, peas and beans and non-legumes include black oats, rye, mustard, radish, and turnips. Genetically, legumes possess an on/off switch that is triggered in response to the availability of nitrogen in the root zone, a process known as quorum sensing. If nitrogen is readily available, the switch activates an inhibitor, creating a barrier to infection by bacteria in the root, however, if nitrogen is limited, the switch is turned off removing the protective barrier, allowing bacteria to infect the plant (Oldroyd et al., 2011). Infection of the root by the bacteria (rhizobia) into the epidermal cell, leads to the release of rhizobia into the primordial nodule cells. Cell division occurs forming nitrogen fixing bacteriods. The bacteria convert atmospheric nitrogen to ammonia via a reaction involving nitrogenase enzymes. The success of this process relies on the presence of the rhizobial bacteria in the soil (Baddeley et al., 2014; Khan et al., 2007).

2.3 The use of glyphosate

In the rotational use of cover crops, glyphosate is commonly used to desiccate the cover crop prior to reseeding with a cash crop. In some cases, glyphosate can be used more than once a year on the same ground. The impact on soil biota following the use of cover crops to build up a microbial community and the subsequent destruction of the cover crop with glyphosate has not been considered together. N-(phosphonometyl) glycine or Glyphosate was invented in 1971 by F.E. Franz and commercially produced by Monsanto Agricultural Products Co. USA in 1974 (Grossbard and Atkinson 1985). Glyphosate is a very successful, broad-spectrum, non-selective herbicide which enters the plant through foliage and translocates throughout the plant. It works by disrupting the shikimic acid pathway by blocking activity of the enzyme 5-enol-pyruvyl-shikimate-3-phosphate synthesase. This prevents the synthesis of amino acids and kills the plant (Kanissery et al., 2019).

Several studies have been carried out to determine the effect of glyphosate on the soil microbial community and the results have been mixed. For example, whilst many early papers concluded that there is no effect on microbial respiration (Hart & Brookes, 1996; Wardle & Parkinson, 1991, 1992) other more recent research

shows that in the short term, 7-38 days, there is a significant increase in microbial respiration (Lane, 2011; Nguyen et al., 2016). Research has also shown changes in the microbial community as a result of being exposed to glyphosate (Hagner et al., 2019; Lane, 2011; Rosenbom et al., 2014; Silva et al., 2018). Early research carried out on pure cultures, showed that many microbial species were inhibited when subjected to glyphosate. However, it was recognised that the dose rate applied under laboratory conditions was considerably higher than would be expected in soil, because ordinarily most glyphosate is taken through the foliage before reaching the soil (Grossbard & Atkinson, 1985).

Glyphosate is exuded through the roots of plants and enters the rhizosphere (Rueppel et al., 1977). The low C:N ratio 3:1 allows glyphosate to be easily mineralized to carbon dioxide by microorganisms (Haney et al., 2009; Nguyen et al., 2016). However, repeated applications can reduce carbon mineralization and increase the glyphosate half-life (Andréa et al. 2003). In a study where glyphosate was repeatedly applied to soil over 180 days, it appeared to stimulate microbial biomass and that repeated application increased microbial populations that degrade glyphosate (Lane 2011). It was found that glyphosate can stimulate the growth of Mycorrhizae fungi in vitro (Laatikainen & Heinonen-Tanski, 2002), yet in another study, AM spore viability was found to be 5.8-7.7 fold higher in soil not treated with glyphosate compared with treated soils (Druille et al., 2013). Thus the increased respiration and decreased spore viability could potentially result in a reduction in SOC.

2.4 The use and impact of inoculants

A bioinoculant or soil inoculant is an agricultural amendment that contains specific beneficial microbes that increase crop yield by creating a symbiotic relationship with plants. The first inoculant was patented in the USA in 1896. Many of the early inoculants contained one species of microorganism chosen for a specific service on a specific crop (Santos et al., 2019). The industry has grown significantly with new micro-organisms being found and multispecies inoculants made on a commercial basis. Some of the main agri-inoculant manufacturers include Symbio, Rizobacter, Bayer, BioConsotia, Groundwork BioAg and AGTIV and a majority on the inoculants are used on legumes such as pulses, pes, lentils, peanuts and beans. Inoculants are generally applied to increase productivity. Table 2.2 provides a list of research carried out on different inoculant

microbial species and the success rate of the crop yield. Every experiment gave an increase in crop yield where an inoculant was used compared to no inoculant. There is a paucity of research on the impact of inoculants on SOC or cycling of carbon. However, ecosystem services provided by microbes can contribute to increased SOC for example, inoculants containing plant growth promoting rhizobacteria (PGPR) increase the yield of crop biomass (Bhattacharyya & Jha, 2012; Glick, 1995; Saad et al., 2020), which in the case of a cover crop would contribute carbon as a green manure (Couëdel et al., 2018; Doltra & Olesen, 2013; Gosling et al., 2006; Rosenfeld & Rayns, n.d.). Inoculants containing AM can stabilise carbon as aggregates and contribute to the fungal necromass (Dignac et al., 2017; Kotsia & Marco, 2017; Liang et al., 2015; Martínez-García et al., 2018; Peng et al., 2013; Wang et al., 2015; W. Wang et al., 2017; Wright & Upadhyaya, 1998). An inoculant that boosts the diversity of a microbial community may reduce the loss of carbon through respiration (Averill & Hawkes, 2016; Johnson et al., 2015; Nazaries et al., 2015).

Table 2.2 Microorganisms, the crop and increase in yield compared to a non-inoculated control (Santos et al., 2019)

Microorganism	Crops	Increased yield with inoculant compared to non-inoculated control (%)
Bradyrhizobium japonicum	Soy Bean	1.6 - 19.0
	Cow pea	38.1
Bradyrhizobium liaoningense	Cow pea	54.8
Bradyrhizobium yuanmingense	Cow pea	38.3
Rhizobium tropici	Common beans	8.3 - 36.0
Rhizobium leguminosarum sv. phaseoli	Common beans	48
Rhizobium leguminosarum sv. *viciae*	Faba beans	5.0 - 81.4
Azospirillum brasilense	Maize	14.3 - 31.0
	Wheat	14.7 - 31.0
	Tomato	11
Pseudomonas fluorescens	Maize	29.0 - 31.0
	Tomato	57
Bacillus polymyxa	Wheat	13.6 - 19.5
Burkholderia vietnamiensis	Rice	12.1 - 22.0
*A. brasilense** and *B. japonicum**	Soy Bean	14.1 - 81.9
*A. brasilense** and *R. tropici*	Common beans	19.6

It is reasonable to consider that selective biological inoculants could enhance the amount of carbon captured by cover crops by increasing biomass, and by boosting the abundance of AM (Wright & Upadhyaya, 1998). The view that inoculants could be used to increase SOC is supported in a review by Ahmed et al (2020). The varying abundance of mycorrhizae and rhizobacteria can have a greater impact on the soil than soil type or climate (Averill & Hawkes, 2016) and fungal activity contributes more towards carbon sequestration than bacteria(Li et al., 2015; Six et al., 2006). AM contributes to the soil carbon stock in several ways. Its symbiotic relationship with plant roots allows it to take plant sugars, formed by photosynthesis, and in return grow a web of mycelium that provide nutrients and water to the plant. AM has been observed to influence the infected plant's ability to photosynthesise depending on its own requirements for carbon (Kotsia & Marco, 2017). Therefore, microbes could possibly be selected specifically for their ability to increase rates of photosynthesis. The biomass of the fungi itself, contributes hundreds of kilograms of stable carbon per hectare within the soil (Olsson et al., 1999). The proportion of fungi making up the microbial necromass accounts for 70.7% compared to 25.9% bacteria (Li et al., 2015). Fungal mycelium has a high nitrogen content that decomposes over a few weeks, however, the cell wall is more resistant and preserved long term (Fernandez & Koide, 2013). AM exudes glomalin, a carbon-based recalcitrant substance that does not degrade easily thus locking in carbon, and it is the glue that binds particles together to form stable aggregates (Peng et al., 2013; Wright & Upadhyaya, 1998). The competition for soil nitrogen by mycorrhizal fungi produces a slowdown of flow of carbon with the formation of aggregates and, up to 65% reduction of carbon respiration from soil (Averill & Hawkes, 2016). Whilst the natural processes and benefits of AM are well documented, the efficacy of AM inoculants is scant and there are concerns over the unintended consequences of adding fungi to soil (Hart et al., 2018).

Other examples of known microbes that provide an eco-service and can be used with cover crops to increase the amount of carbon inputs to soil include *Pseudomonas flourescens* which improves plant growth through control of disease (Ganeshan & Kumar, 2005). Other bacteria such as *Bacillus pumilus* (Komala & Khun, 2014), *Bacillus cereu*s (Han et al., 2013), *Bacillus pasteurii* (Stocks-Fischer et al., 1999), and *Bacillus mucilaginosus* (Z. Zhang et al., 2011), sequester atmospheric carbon and produce anhydrase carbonates in soil. Carbon cycling is affected by the availability of nitrogen in the soil. Nitrogen provides a source of energy to microbes, stimulating growth of the microbial community, which initially cycles the labile carbon out of a system. When

labile sources are short, microbes begin mining recalcitrant carbon and reducing the carbon pool (Khan et al., 2007). It may also be possible to identify specific bacteria that could moderate or inhibit the mining activity of recalcitrant carbon.

Inoculants are important in agriculture, particularly in poor soils (Alori & Babalola, 2018; Babalola, 2010). Research in the use of inoculants for climate change mitigation is receiving some interest, however, there is little research on the effects of inoculant use in a healthy soil, or specifically with a cover crop rather than a cash crop (Ahmed et al., 2020). Also little is understood about the long-term effect of inoculants on the resident microbial community (Mawarda et al., 2020). Identifying changes to the composition of a community that occur under different whole harvest practices is important if carbon cycling needs to be understood and controlled.

2.5 Scientific methods to study soil biota

Quantification of the microbial community in terms of its size and composition is an important part of understanding the impacts of agricultural practices on the flow of carbon. The abundance of bacterial and fungal groups gives a good indication of the direction and speed of carbon flows into or out of a soil ecosystem.

Soil biota research methodologies include measuring i) total organic carbon (TOC) in order to assess microbial biomass carbon (Brennan & Acosta-Martinez, 2017; Frasier et al., 2016; Lei et al., 2015; Martínez-García et al., 2018a; Strickland et al., 2019), and ii) phospholipid derived fatty acids (PLFA) (Buckeridge et al., 2013; Finney et al., 2017; Frasier et al., 2016; Orwin et al., 2010), iii) microbial indicators to assess functionality using DNA in soil (Schmidt et al., 2018; Thakuria et al., 2008; Widmer et al., 2001), iv) soil respiration (Orwin et al., 2010; Sanz-Cobena et al., 2014)

2.5.1 Microbial biomass

The chloroform fumigation extraction method is commonly used to measure TOC and nitrogen bound up in the microbial biomass (Gregorich et al., 1991). The chloroform lyses the cells releasing the carbon and nitrogen

which is then extracted with K_2SO_4. The difference between the TOC in fumigated and non-fumigated sample (control) gives the quantity of chloroform labile carbon; or the microbial biomass. This method is applied to a wide range of situations for example, to highlight changes in the soil under different environmental conditions such as seasonal changes (Buckeridge et al., 2013), or the effects of treatments including different species of cover crops on carbon (Strickland et al., 2019). This method is relatively inexpensive and can quantify the microbial community under different treatments. However, this method does not provide detail on the species of microbes that make up the microbial biomass. More details are provided in Chapter 3.

2.5.2 PLFA

Many papers researching cover crops and the impact on soil biota have used PLFA analysis (Orwin 2010, Frasier et al 2016, Finney 2017, Martinez Garcia 2018, Lehman 2011). The use of PLFA analysis to measure fungal and bacterial biomass in soil was developed by Frostegard and Baath (1984). The method involves the extraction of PLFA from cells within the organism. PLFAs' comprise carbon chains of varying length and structure some of which are identified as key biomarkers, attributed to single species or groups of microbes. Table 2.3 provides a list of fatty acids and the groups they represent. PLFA analysis has the advantage over microscopy because it is not as time consuming. Importantly, and relevant to this research, it can be used to identify different management levels in agricultural soils (Zelles et al., 1995). Not all biomarkers are found to be unique to one species and this can cause problems in the interpretation of results. For example, a biomarker used to identify AM has also been found in some bacteria (Frostegård et al., 2011).

This method shows changes or differences in the soil community structure as a result of different environmental conditions such as pH and organic matter content (Finney et al., 2017), or use of different cover crops, organic or non- organic treatments ((Martínez-García et al., 2018a).

Table 2.3 A list of fatty acid biomarkers and the microorganism or group it identifies

Biomarker Fatty Acid	Microorganism/Group	Reference
i14:0, a15:0, 15:0, 16:1w9, 16:1w7c, 17:1w8, 19:1a	Bacteria	Frostegard & Baath 1992
15:0, 17:0	Bacteria	Tunlid & White 1992
iso and anteiso 15:0	Gram positive	O'Leary & Wilkinson 1988
B10:0, B12:0	Gram negative	Ratlegde & Wilkinson 1989
10Me17:0, 10Me18:0	Actinomycetes	Ferle & Frostegard 1993
Cy17:0, Cy19:0	Anaerobic bacteria	Vestal & White 1989
18:2w6	Fungi	Ferle 1986, Frostegard 1993, Vestal & White 1989

2.5.3 DNA

SOM and the soil genetic pool is dominated by bacteria. The bacteria present in soils can be quantified using deoxyribonucleic acid (DNA). The total nucleic acids (DNA) are extracted from a soil sample and amplifying 16S rRNA genes with primers for all bacteria. The taxa are then identified by sequencing. Using bioinformatics, a species abundance record can be created. Although it does not identify bacterial species but can highlight functional markers such as nitrogenase (nitrogen fixing) and hydrogenase (respiration) for example (Griffiths et al., 2011; Malik et al., 2018, 2020). Several studies use DNA to identify changes in the fungal population for example and how different treatments such as cover crops or climate change can affect it (Benitez et al., 2016; Detheridge et al., 2016; Hanson et al., 2022). Whilst this method is useful, it will not be used in this research due to time and costs constraints.

Methods i) ToC, and ii) PLFA described above will be the core methods employed in this research as they provide the sensitivity to detect small changes in the microbial community over a relatively short time and under different agricultural practices. The equipment requirements are available and the cost per test is within the research budget.

3 The effect of cover crops, herbicide and inoculant on carbon content in field plots- the first year

3.1 Introduction

The degradation of agricultural soils has led to the depletion of organic carbon. Soil organic carbon is vital for the growth of crops and provision of food. In addition, the translocation of carbon from the soil into the atmosphere is contributing to climate change. A solution is required to both remove carbon from the air and then store it in the soil long term (carbon sink). In the select committee report on soil health (Soil Health First Report of Sessions 2016-17, 2017) several experts referred to the use of cover crops as one approach to improving soil health. However, there is more to growing a crop than just sowing the seed.

It is generally accepted by farmers that the success of any crop relies on the preparation of the seed bed, by reducing potential competitive plants and increasing the presence of beneficial microbes. The common use of herbicides, and more recent use of inoculants to enhance crop yield have, under separate studies, shown different outcomes on the microbial community(Alori et al., 2019; Alori & Babalola, 2018; Haney et al., 2009; Lane, 2011; Wardle & Parkinson, 1991). To date, research has not considered the cumulative effects on the soil during a harvest year. Each of these treatments is discussed further below.

The aim of the field trial is to replicate a typical agricultural year and to measure the effect on soil of using a cover crop, glyphosate and an inoculant coated onto the cover crop seed. With respect to the inoculant, this is an unconventional approach as inoculants are expensive and generally only applied to cash crops to increase the yield. This experiment was conducted over three years to allow for the changes to be detected. Each of the treatments is considered below in context of increasing the stock of organic carbon in soil.

3.2 Cover crop

Current evidence is divided about the ability of cover crops alone to provide increased organic matter. Whilst some research shows an increase over time (Aguilera et al., 2013), others show a deficit (Strickland et al., 2019). Table 2.1 in Chapter 2 provides a synthesis of outcomes of organic carbon in a selection of cover crop trials. Cover crops can contribute plant biomass, a source of carbon which is incorporated into soil at the end of the winter season (Couëdel, Alletto, Tribouillois, et al., 2018; HDRA, n.d.; Rosenfeld & Rayns, n.d.). The plants provide a canopy, reducing the impact of raindrops on soil, and holds soil in place with a root system (Kemper & Derpsch, 1980; Michels et al., 1995; Posthumus et al., 2015). A cover crop also provides a connection between air and soil creating a habitat for a microbial community (in the rhizosphere) that can play a role in the sequestering of carbon into aggregates(Gillespie et al., 2011; W. Wang et al., 2017; Wright & Upadhyaya, 1998; J. Zhang et al., 2015). The roles of microbes in the carbon cycle are discussed together with strategies used in times of environmental stress in Chapter 2.

3.3 Use of glyphosate

The condition of the soil biology at the outset is important if a crop is to be established. Glyphosate is commonly used to clear competitive plants before sowing a crop. It works by interrupting the Shikimic acid pathway (Kanissery et al., 2019), a metabolic pathway in plants responsible for the biosynthesis of amino acids. This is not found in animals, however the pathway is present in the belowground organisms so it has the potential to alter the soil's microbial community. Other research has shown the viability of *Arbuscular mycorrhizae* (AM) spores is reduced where glyphosate is used (Druille et al., 2013). This evidence suggests the use of glyphosate has the potential to impact negatively on soil health with regard to the ability to store carbon.

3.4 Addition of microbial inoculants

The abundance and composition of microbes in a community determines the movement and use of carbon. Microbes are important for the delivery of services to increase the overall stock of carbon in soil. In particular,

the presence of AM is important if carbon is to be stabilized (Gillespie et al., 2011; Gosling et al., 2006; Jansa et al., 2013; W. Wang et al., 2017).

Whilst AM occurs naturally, commercial inoculants that contain mycorrhizae and other beneficial bacteria are being used in agriculture to stimulate plant growth, increasing crop yields, such as above ground biomass (Santos et al., 2019). Symbio is one such brand of inoculant, a cream coloured powder that is applied to the seeds(https://www.symbio.co.uk) . A greater the yield of cover crop biomass is likely to result in a greater input of carbon once the crop is incorporated into the soil.

3.5 The hypothesis

The main aims of this field study are:

i. To investigate if three agricultural treatments, cover crops, mycorrhizal inoculant, and glyphosate (alone or in combination) can be used in an agricultural rotation to increase soil organic carbon
ii. To understand the impact of treatments on fungal and bacterial community
iii. To understand what effect treatments have on the flow of carbon

Based on the above evidence, the hypothesis proposed here is, if a cover crop treated with a mycorrhizal inoculant is grown in a soil that has not been pre-treated with glyphosate, it will produce the richest microbial community conducive to increased levels of soil organic carbon.

3.6 Method

3.6.1 Field site

Hilley Farm is a mixed arable and livestock farm located on the flood plain of the River Severn in Shropshire, NGR SJ 37899 16833. It has been continuously farmed by the same family for 3 generations. Herbicide, pesticide, and chemical fertilizers have been regularly used, together with applications of cattle and chicken manures. The field trial was designed to best represent the closest environmental conditions that would occur on a typical farm.

The crop removed prior to the trial was a cover crop of stubble turnips used for grazing sheep over winter. Cover crops are most commonly grown annually, in between cash crops, and removed after the winter period to allow for the cash crop.

In some cases, where soil is particularly impoverished, a cover crop can be used over a year or more as part of a rotation. The aim of the trial is to understand what happens within the soil where cover crops are grown and undergo minimal management. For this experiment it was decided that the cover crop would be left for 3 years and to take measurements annually. Whilst this does not necessarily represent the common use of cover crops, it will provide a record of what changes occur long term, especially with regards to the increase of organic matter. It also makes allowance for analytical techniques which may not be sensitive enough to pick up changes over a short period of time. This Chapter describes the results from the first year which, as far as possible, will reflect the common use of an annual cover crop rotation. It is not a perfect reflection of the use of a cover crop as it was not desiccated and incorporated into the soil. It does however, give a snap shot of the soil biology within the rhizosphere of the standing crop.

A field was identified that represented an intensive cropping system. Table 3.1 lists the crops grown since 2012 and details the quantity of glyphosate administered between cropping.

Table 3.1 Historic crop planting and use of glyphosate from 2012 to 2018 in trial field at Hilley Farm, Shropshire. Three agricultural treatments were considered: a grass-based cover crop, glyphosate and a mycorrhizal inoculant.

Year	Crop	Glyphosate treatment
2017/2018 (Nov-Jan)	Stubble turnips	
2017	Winter Wheat	2litres per hectare
2016	Oil Seed Rape	3litres per hectare
2015	Winter Barley	2litres per hectare
2014	Winter Wheat	2litres per hectare
2013	Winter Wheat	2litres per hectare
2012	Oil Seed Rape	3litres per hectare

The field was prepared for sowing in May 2018 by power harrowing. Six blocks, each with 8 plots of 4 m x 6 m were marked out. Each plot was separated by a 0.5 m gap and at least 1 m between blocks (Figure 3.1 and Figure 3.2).

Figure 3.1 View of the plots at Hilley Farm from the air

Figure 3.2 Viewing plots from the bottom of the Figure to the top -Blocks 1: plot 1-8, block 2: 9-16, block 3: 17-24, block 4: 25-32, block 5: 33-40, block 6: 41-48

A randomized, complete block design replicated 6 times was applied with 8 treatments, 3 factors including a control. The control had no treatments applied and was left fallow (Figure 3.3).

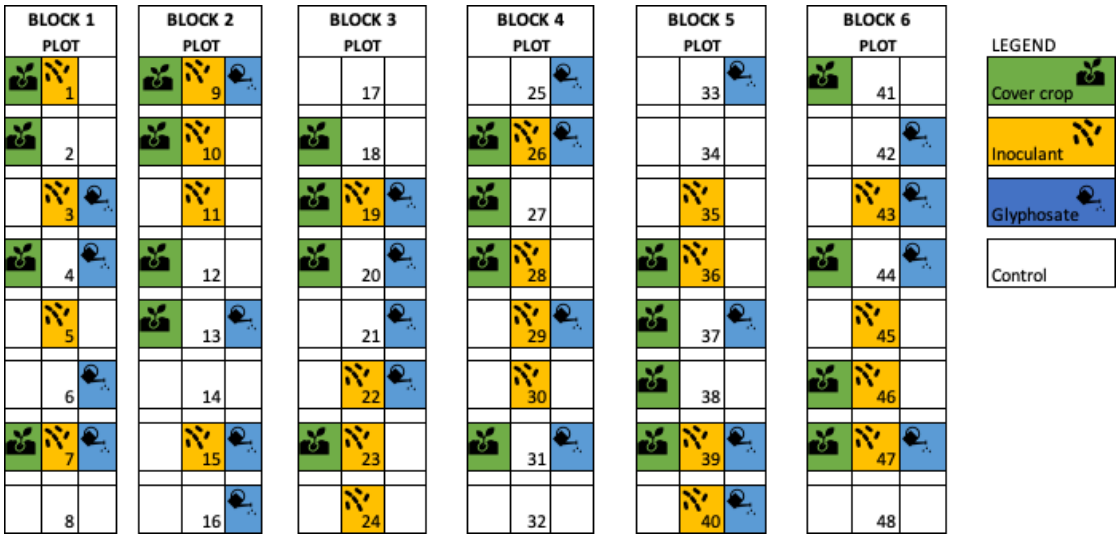


Figure 3.3 Schematic of treatments applied to each plot within 6 blocks

Figure 3.4 Field plots before treatments applied (13 May 2018)

Glyphosate was used to clear weeds before drilling. The commercial brand applied was Garryowen Mapp no. 17508. A solution of 1 litre of glyphosate: 60 litres of water, 1.67 % vol was prepared and applied at the manufacturers maximum recommended rate (6 litres per hectare). A commercial mixed grass cover crop was established in half of the plots and the rest were left fallow (Table 3.2). This was chosen because it was suitable for wetter soil types, it could potentially provide grazing, silage or hay and included a clover mix for nitrogen fixing.

Table 3.2 Details of plant species included in the cover crop

Variety	Ratio (% weight)	Seed rate 100 kg ha^{-1}
AberGreen Int Diploid PRG	11	11
Nifty Int Dipoid PRG	7	7
AberZues Int Diploid PRG	11	11
AstonEnergy Int Tetraploid PRG	14	14
Fintona Int Tetraploid PRG	28	28
AberAvon late Diploid PRG	14	14
Winnetou Timothy	7	7
Rivendel White Clover (small leaf)	2	2
AberHerald White Clover (medium leaf)	4	4
Barblanca White Clover (large leaf)	2	2
Total	100 %	100 kg ha^{-1}

Commercial inoculants are manufactured and sold on the basis of increased yields and improvement of root mass. Symbio Mycoforce Grass Seed Coat is an inoculant specifically for perennial grass species. It claims to contain the following Endo Mycorrhizal species:

- *Glomus claraum*
- *G. aggregatum,*
- *Paraglamus brasilianum,*
- *Gigaspora margarita*
- *G. etunicatum Trichoderma Sp. and Bacillus Sp. Zeolite*
- Trace elements

It is applied by coating the powder onto the seed. On plots that required the addition of the inoculant, the powder was coated on the grass seed at a rate of 0.6 g m^{-2}. Where inoculant was applied on fallow plots, it was coated onto sterile seed that had been heat treated. Seed was heated in an oven at 100 ^{o}C for 1.5 hours (Siddique & Wright, 2003).

The seed was drilled on 3rd June 2018 (Figure **3.5**). This included, untreated cover crop, cover crop with a coating of inoculant and dead seed coated with inoculant into fallow plots.

Figure 3.5 Drilling seed 3 June 2018

3.6.2 Plot management

Between the 16th to the 20th July 2018 the plots were topped. Between 14th and 26th November 2018, sheep were grazed on the plots. No additional fertilisers, manures, herbicides or pesticides were applied during this time.

3.6.3 Soil sampling

Soil samples were taken on 15th and 16th May 2018 before treatments were applied and annually thereafter. Five evenly spaced 1 m cores, with a 2 cm diameter were drilled across the 4 m width of each plot using a rig auger shown in Figure **3.6**. The line across the width was adjusted for each annual sample to avoid previous drill holes. The core was split into three horizons; 0-23 cm, 23-60 cm and 60+ cm and combined for each plot. These were placed in cool boxes to transport to Lancaster University where a subsample was placed in a -80 ^{o}C freezer. The remainder was stored in a <5 ^{o}C cold store.

Figure 3.6 Soil sample rig 15 May 2018

Bulk density samples were taken from 5 pits dug out in the sample field adjacent to the blocks (Figure **3.7**). A total of 9 rings were taken from each pit, 3 from 0-23 cm, 3 from 23-60 cm and 3 from 60+ cm. The rings plus samples were weighed before placing in a drying oven at 105 ^{o}C for 16 hours. The samples were removed and reweighed. The ring was weighed and volume calculated (140.758 cm^3). The bulk density, porosity and air space were calculated.

i) Bulk Density g cm^{-3}= weight of soil g x volume of ring cm^3
ii) Water content g g^{-1} = weight of water g / weight of soil g
iii) Porosity % = 1 - bulk density / Particle density g cm^{-3} where Particle density =2.66 g cm^{-3}

Figure 3.7 Soil section from pit used for bulk density sampling

3.6.4 Soil analysis

SOM was measured using the loss on ignition method. The soil was air dried in a drying cabinet at Rothamsted at ambient temperature for 3 to 4 weeks. 5 g of sieved air-dried soil was heated to 550 ^{o}C for 6 hours to burn off the organic matter. The difference in weight before and after represented the SOM and was presented as a percentage value (% SOM).

The percentage SOC was calculated by multiplying % SOM by 0.58, the ratio of carbon found in SOM (Pribyl, 2010). Using the bulk density and % SOC, the total tonnes of carbon in a hectare was calculated.

The pH was taken for each horizon for all 48 plots. 10 g fresh soil in 25 ml of distilled water was mixed on a horizontal shaker for 30 minutes. Readings were taken using a Mettler Toledo meter with an Inlab Expert Pro ISM probe.

3.6.4.1 Microbial biomass

Aggregation is a biological process that involves fungal and bacterial activity (Wright & Upadhyaya, 1998). Using analytical techniques to measure the changes in the microbial community provides an indication of the soils SOC content and the potential to increase the more stable SOC. Analysis of the microbial carbon and nitrogen biomass (MB_C and MB_N), and phospholipid fatty acids (PLFA) were carried out on the upper horizon of the soil.

Microbial biomass was measured using the K_2SO_4 liquid fumigation method adapted from Fierer 2003 Powlson 1976, Brookes 1982. Fumigation of the cells using liquid chloroform and K_2SO_4, released C and N bound up in the microbial biomass. For each plot two fresh soil samples were taken, one was fumigated and one was not fumigated. Total carbon (TC), total nitrogen (TN), total organic carbon (TOC) and total organic nitrogen (TON) was analysed using a Shimadzu TOC analyser.

The Shimadzu TOC – L series uses the 680 °C combustion catalytic oxidation method. By taking the results for the unfumigated sample from the fumigated sample the microbial biomass carbon and microbial biomass nitrogen were calculated.

3.6.4.2 Phospholipid fatty acids (PLFA)

Phospholipid fatty acids (PLFA) was used to determine the size and composition of the living microbial community associated with each treatment. It is a common analysis used to assess microbial communities in agricultural soil (Buyer 2010, Lehman 2012, Lori M 2017). It is a rapid and sensitive method that recognises single and biomarker groups of fungi, bacteria, Gram-negative and Gram-positive.

Lipids were separated into phospholipids (polar and glycolipids), and neutral lipids (hydrocarbons, sterols and free fatty acids) from a 1 g freeze dried, ground soil sample using a modified version (Lancaster University 2018) of the Bligh and Dyer method (Bligh 1959). The liquid was eluted with chloroform to remove the neutral lipids, and acetone to remove the glycolipids. The phospholids were eluted to a clean tube, and the remaining solvent evaporated off. The further separation of lipids and preparation for GC analysis was carried out using

mild alkaline methanolysis. Standards C13 and C19 were added for reference. The GC used was an Agilent Technologies 6890N Network GC system, in conjunction with Clarity Autosystem software. The volume of sample injected into the GC was 2 µl. The GC initial oven temperature was 60°C, then ran on the following sequence:

Temperature 60°C, Initial T0 : 2 minutes;

Rate 1: 20°C/minute, final T1: 190°C

Rate 2: 3°C/minute, final T2: 250°C

Rate 3: 10oC/minute, final T3: 300°C

Final time: 5 minutes

Table 3.3 List of phospholipid fatty acid biomarkers used to identify the presence of soil microbial functional groups

Biomarker	Identification		Reference
Std C13:0	Methyl tridecanoate	Standard	
14:0	Myristic acid	gram +ve	6
15:0i	iso-pentadecanoic acid	gram +ve	1, 4, 5, 6
15:0a	anteiso-pentadecanoic acid	bacteria	1, 5, 6
15:0	Pentadecanoic acid	gram +ve	6
16:0i		gram -ve	1, 4, 5, 6
16:1 (n-7)	Palmitoleic acid	gram-ve	2, 4, 5
16:1	1-hexadecanol	gram -ve	
16:1 (n-5)	Hexadecenoic acid	gram -ve / AMF*	2, 5, *6
16:0	Palmitic acid -	prokaryotes and Eukaryotes	
17:1 (n-8)	cis-9-heptadecenoic acid	gram +ve	
7Me-17:0			4
br17:0		gram +ve	1, 5
i17:0	15-methylpalmitic acid	gram +ve	1, 4, 5, 6
a17:0	14-methylpalmitic acid	gram -ve	4, 6
7,cy-17:0			4, 5, 6
br18:0		sp fungi	
18:2 (n-6,9)	Linoleic acid (9, 12-octadecadienoic acid)	sp fungi ?	3,4, 5, 6
18:1 (n-9)	methyl oleate (cis-9-octadecenoic acid)	gram -ve	2, 4, 5, 6
18:1 (n-7)	11Z-octadecenoic acid (cis vaccenic acid)		2, 4, 5, 6
18:1 (n-5)	13Z-octadecenoic acid		
18:0	methyl stearate		
19:1	Cis-10-nonadecenoic acid methyl ester	gram -ve	
7,8cy-19:0	Cyclopropane fatty acid	gram -ve	4, 5, 6
Std C19:0		Standard	

References: 1 Gram positive (Lechevalier, 1988; O'Leary & Wilkinson, 1988; Zelles, 1999). 2 Gram positive (O'Leary & Wilkinson, 1988; Zelles, 1999). 3 Fungal biomass (Bååth & Anderson, 2003; Federle et al., 1986). 4 (Rinnan & Bååth, 2009). 5 (Aciego Pietri & Brookes, 2009). 6 (de Deyn et al., 2011)**.**

The following biomarkers were summed to represent the bacterial, fungal, Gram -ve, Gram +ve groups respectively, and to calculate the Fungal:Bacterial ratio and Gram positive:Gram negative ratio.

Table 3.4 The biomarkers identified by functioning group, bacteria, fungi, gram positive and gram negative.

Group	Biomarker
Bacterial	14:0, 15:0i, 15:0a, 15:0, 16:0i, 16:1, 17:1(n-8), br17:0, a17:0, 18:1 (n-9), 19:0
Fungal	18:2 (n-6,9), 18:1 (n-9), 19:1
Gram positive	14.0, 15:0i, 15:0, 17:1 (n-9), br17:0, i17:0
Gram negative	16:0i, 16:1, a17:0, 18:1 (n-9),

3.6.5 Statistical Analysis

Analysis of variance (ANOVA) was completed using GENSTAT (19.1.21390). It was used to determine the effect of different treatments on the size and activity of the biological, chemical and physical attributes of the soil. It was also used to compare differences over one year, pre and post treatment. ANOVA highlighted any variability or trends between treatments and over time. Three treatments were compared singularly and in combinations [TREATMENT 1 x TREATMENT 2 x TREATMENT 3] with plots nested in blocks [BLOCKS/PLOTS]. Data was also analysed across time - [TREATMENT 1 x TREATMENT 2 x TREATMENT 3 x YEAR] and [BLOCKS/PLOTS/SAMPLE]. ANOVA compared each treatment with a control. Excel was used to calculate averages and standard deviation. Bar charts were used to illustrate the data.

3.7 Results

3.7.1 Bulk density

Table 3.5 shows the bulk density, water content and soil porosity of the soil. The bulk density increases down the profile from 1.39 g cm^{-3} at 0-23 cm to 1.62 g cm^{-3} at 60+ cm. Typical bulk density of a clay soil is 1.40 g cm^{-3} (Sumner, 2000) The profile of the soil varies from clay to a courser sandy, silt towards a meter depth. This is recognized in the change of the bulk density (Table 3.5).

Table 3.5 Bulk density, water content and porosity of 3 soil horizons 0-23 cm, 23-60 cm, 60+ cm.

Horizon	Bulk density g cm^{-3}	Water content g g^{-1}	Soil porosity %
0-23cm	1.39	0.30	48%
23-60cm	1.53	0.31	43%
60+cm	1.62	0.24	39%

3.7.2 Effects of cover crops, addition of inoculant, and glyphosate

Soil samples were taken in 2018 before treatments were applied to the plots, and again in the following year after treatment was applied. Data was collected for pH, SOM, for 3 horizons (0-23cm, 23-60cm and 60+), and PLFA for the top horizon only (0-23cm). Microbial biomass was quantified in 2019 in the top horizon (0-23 cm), however data for the baseline year (2018) was corrupted due to instrument failure. Results are presented below for the cover crop treatment, and the cover crop with additional treatment combinations. The effect of glyphosate alone, inoculant alone and these two treatments together without cover crops is presented to observe any effects that they have.

3.7.2.1 Soil Organic Matter

The SOM for all plots decreased in 2019 compared to 2018 with the biggest drop in the top layer (0 -23 cm). The plots treated with glyphosate only, showed a significant effect on SOM (%) in 2019 when compared to plots not treated with glyphosate (p= 011) (

Table 3.6 and Figure 3.8). Using the means generated by running the factorial ANOVA, plots treated with glyphosate compared to those without were 9.24 % and 9.62 %. There were no other significant effects of the treatments on SOM.

Table 3.6 Organic matter mean (%), standard deviation (SD) and P value for 3 soil horizons taken in 2018 before treatments were applied and 2019 a year after treatments were applied. **Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). A P value = <0.05 is in bold text.**

		2018			2019		
Horizon	TREATMENT	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value
0-23 cm	A	10.21	0.77		9.60	0.45	
	CO	10.38	0.58	0.470	9.57	0.43	0.779
	C+G	10.94	2.12	0.655	9.37	0.34	0.322
	C+I	10.38	1.16	0.387	9.56	0.39	0.653
	C+G+I	10.36	0.66	0.573	9.29	0.45	0.334
	GO	10.16	0.81	0.644	**9.39**	0.46	**0.011**
	IO	10.39	0.97	0.919	9.74	0.45	0.438
	G+I	10.44	1.36	0.692	8.90	1.03	0.222
		10.41	**1.09**		**9.43**	**0.55**	
	TREATMENT	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value
23 - 60 cm	A	7.98	4.04		6.61	1.74	
	CO	6.66	1.03	0.824	6.89	1.05	0.859
	C+G	6.91	1.49	0.434	6.03	1.09	0.488
	C+I	6.39	2.32	0.789	5.85	0.48	0.738
	C+G+I	6.79	1.21	0.445	6.78	1.30	0.815
	GO	6.57	1.14	0.634	7.01	1.37	0.633
	IO	6.85	1.06	0.847	6.50	1.25	0.509
	G+I	6.38	1.31	0.879	6.30	1.39	0.737
		6.82	**1.86**		**6.50**	**1.22**	
	TREATMENT	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value
60+ cm	A	4.53	0.91		4.06	0.18	
	CO	4.40	0.14	0.824	3.99	0.62	0.859
	C+G	4.61	1.09	0.434	4.19	0.53	0.488
	C+I	4.38	1.36	0.789	3.94	0.44	0.738
	C+G+I	4.62	0.75	0.445	4.05	0.70	0.815
	GO	4.15	1.04	0.634	4.55	0.94	0.633
	IO	4.38	0.54	0.847	4.07	0.43	0.509
	G+I	4.16	0.55	0.879	4.04	0.62	0.737
		4.40	**0.82**		**4.11**	**0.58**	

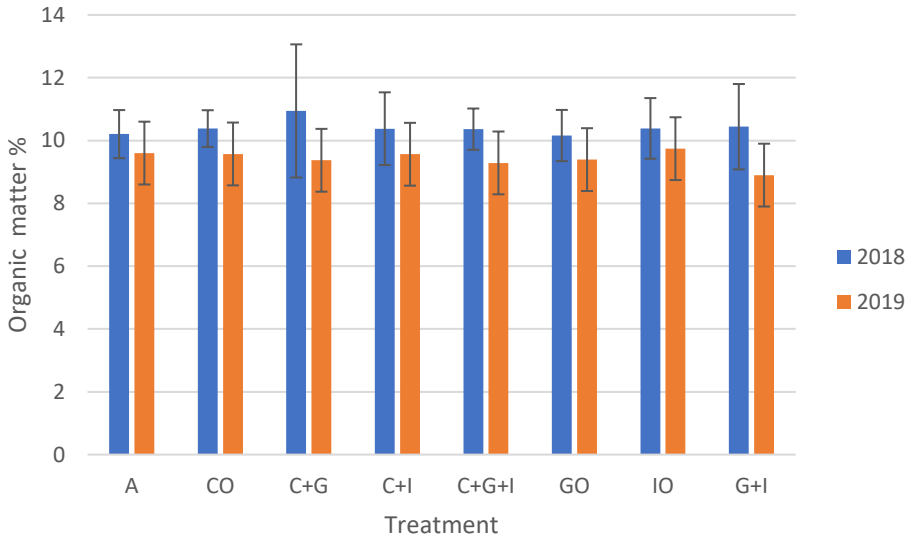


Figure 3.8 Mean SOM (%) and standard deviation taken before (2018) and after (2019) treatments were applied in the upper soil horizon (0 – 23 cm). Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=96).

3.7.2.2 pH

Comparing 2018 and 2019, the pH increased for all treatments in the 0 – 23 cm horizon, decreased in the 23 – 60 cm horizon, and increased in the 60+ cm horizon. There was a small difference between treatments in 2019 with some evidence ($P \leqq 0.5$) that a cover crop only treatment compared with no cover crop, and an inoculant only treatment compared to no inoculant, had an effect on pH in the 0 – 23 cm horizon. The difference in pH showed a mean increase of 0.09 in plots with a cover crop (pH 7.11) compared to those with no cover crop (pH 7.02). The difference in pH showed a mean increase of 0.11 in plots with an inoculant (pH 7.12) compared to those with no inoculant (pH 7.01)

Table 3.7 and Figure 3.9).

It was noted that in 60+ cm horizon in 2018 before treatments were applied, there was a difference in pH in plots assigned for a glyphosate treatment (P=0.024). The mean pH for plots marked but not treated with glyphosate (pH 7.69) compared to those with no glyphosate (pH 7.57).

Table 3.7 pH, standard deviation (SD) and P value for 3 horizons taken in 2018 before treatments were applied and 2019 a year after treatments were applied. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pretreatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pretreatment (C+I), pretreatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pretreatment with glyphosate or cover crop (IO), and a glyphosate pretreatment with inoculant but no cover crop (G+I). P value = <0.05 is in bold text.

		2018			2019		
Horizon	TREATMENT	Mean	SD	P value	Mean	SD	P value
0-23 cm	A	6.53	0.18		7.00	0.16	
	CO	6.41	0.16	0.481	7.05	0.17	**0.057**
	C+G	6.48	0.18	0.195	7.14	0.12	0.119
	C+I	6.47	0.11	0.620	7.10	0.11	0.162
	C+G+I	6.43	0.06	0.576	7.16	0.15	0.455
	GO	6.46	0.13	0.406	6.86	0.33	0.987
	IO	6.51	0.23	0.759	7.11	0.23	**0.040**
	G+I	6.41	0.20	0.395	7.10	0.24	0.629
		6.46	**0.16**		**7.06**	**0.21**	
	TREATMENT	Mean	SD	P value	Mean	SD	P value
23 - 60 cm	A	6.95	0.30		6.95	0.26	
	CO	6.94	0.28	0.300	6.79	0.15	0.380
	C+G	7.03	0.39	0.595	6.92	0.23	0.619
	C+I	7.14	0.17	0.360	6.85	0.41	0.965
	C+G+I	6.92	0.26	0.146	6.88	0.40	0.640
	GO	6.41	1.32	0.331	6.92	0.19	0.626
	IO	6.98	0.27	0.228	6.92	0.30	0.957
	G+I	7.04	0.18	0.636	6.95	0.26	0.949
		6.93	0.53		**6.90**	**0.27**	
	TREATMENT	Mean	SD	P value	Mean	SD	P value
60+ cm	A	7.63	0.20		7.75	0.27	
	CO	7.64	0.09	0.483	7.76	0.22	0.686
	C+G	7.60	0.22	0.693	7.82	0.28	0.904
	C+I	7.49	0.21	0.706	7.74	0.20	0.960
	C+G+I	7.71	0.27	0.432	7.87	0.27	0.557
	GO	7.71	0.15	**0.024**	7.87	0.13	0.130
	IO	7.53	0.33	0.452	7.81	0.26	0.750
	G+I	7.71	0.25	0.706	7.85	0.28	0.960

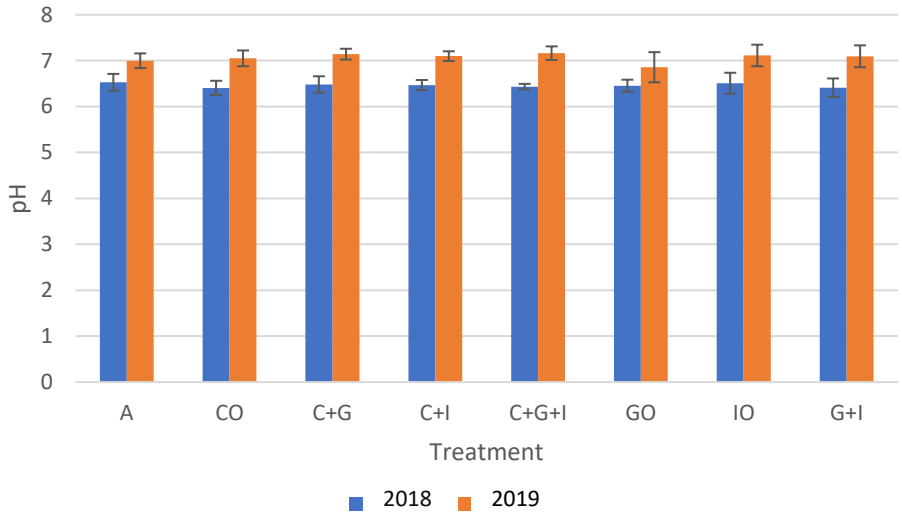


Figure 3.9 Soil pH of each treatment in 2018 and 2019 in the 0 – 23 horizon. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=96).

3.7.2.3 Carbon and nitrogen microbial biomass

In the 0 – 23 cm horizon, the soil MBC and MBN in 2018 or 2019 was not affected by any treatment or combination of treatments (

Table 3.8, Figure 3.10 and Figure 3.11).

Table 3.8 Microbial biomass carbon (MB_C) and microbial biomass nitrogen (MB_N) in 0-23 cm soil horizon in 2019. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I).

	MBc µ g g-1 dry soil			MBN µ g g-1 dry soil		
Treatment	Mean	SD	P value	Mean	SD	P value
A	321.71	96.19		46.92	10.53	
CO	333.34	176.60	0.377	40.97	13.41	0.655
C+G	263.17	103.98	0.749	42.33	6.50	0.749
C+I	336.23	68.38	0.148	42.53	7.44	0.148
C+G+I	354.66	152.90	0.500	44.76	12.86	0.500
GO	398.00	130.68	0.881	51.01	15.81	0.881
IO	317.95	66.30	0.347	42.43	10.22	0.347
G+I	364.39	181.72	0.574	35.72	21.92	0.574

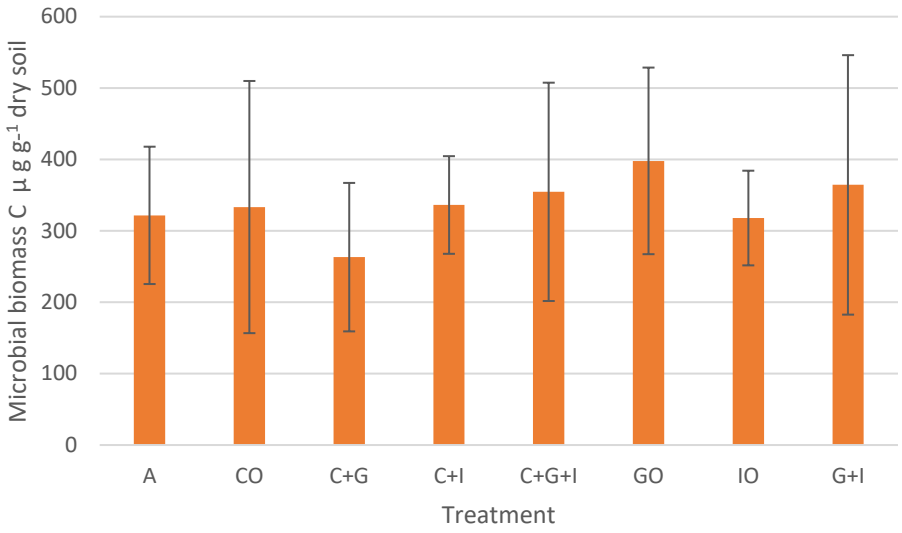


Figure 3.10 The mean and standard deviation of soil microbial biomass carbon after one year of treatment 2019. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=48).

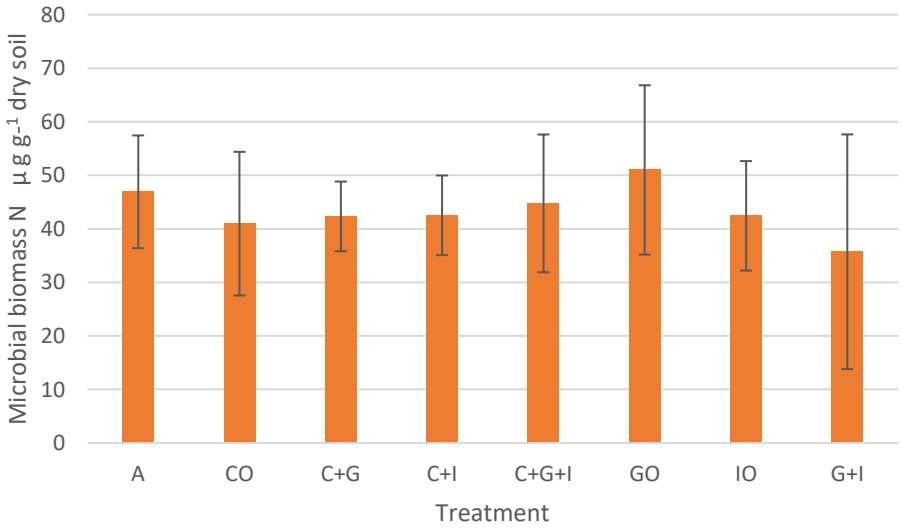


Figure 3.11 The mean and standard deviation of soil microbial biomass nitrogen after one year of treatment 2019. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=48).

3.7.2.4 Phospholipid fatty acids

Results for PLFA in 2018 and 2019 are provided in Appendix 1. The total PLFA for the plots before application of treatment (2018) were consistent across plots. In the first year following the application of treatments, the total PLFA still remained consistent across all treatments. The standard deviation by treatment was lower in the first year compared to the baseline year with the exception of the plots treated with cover crops only (Figure 3.12). There is a visible difference between the overall average PLFA results in 2019 compare to 2018 ($p = < 0.001$).

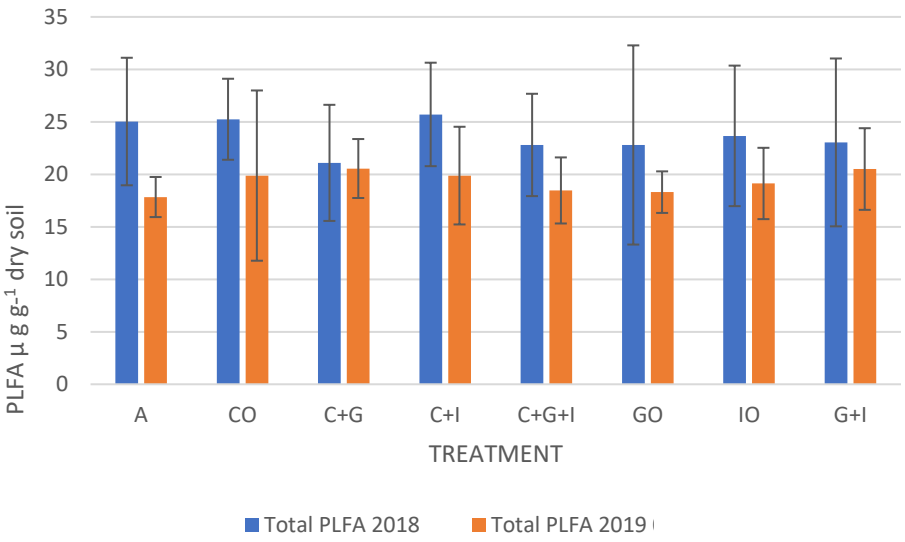


Figure 3.12 The mean PLFA and standard deviation by treatment for samples taken in 2018 and 2019. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=96).

The treatments had no effect on the functional groupings for fungi and bacteria and there was no change by treatment comparing the baseline (2018) with 2019 (Figure 3.13). Of the biomarkers identified, the microbial community comprised around five times more PLFA for bacterial groups compared to the fungi.

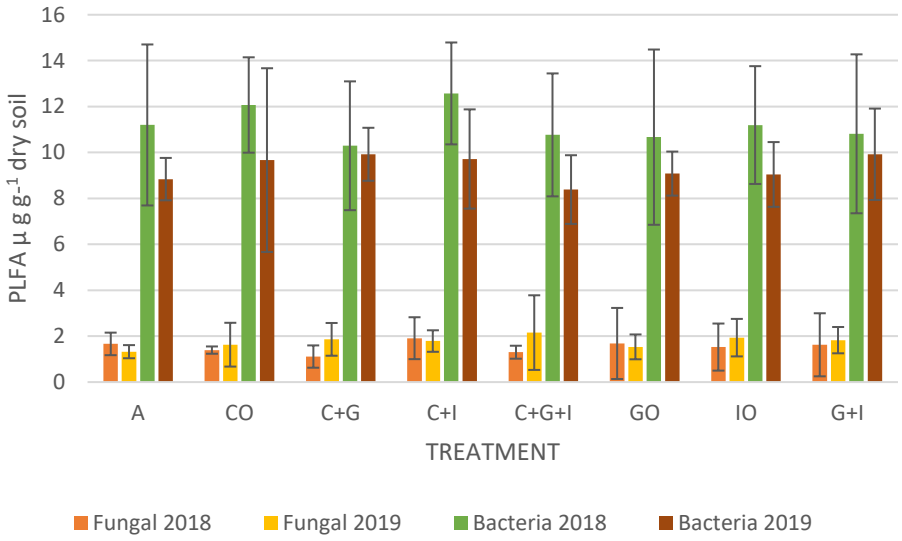


Figure 3.13 The mean and standard deviation of fungal and bacterial groups by treatment for 2018 and 2019 Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=192).

The biomarkers for the gram-negative and gram-positive functioning groups are relatively consistent in 2018 before treatments were applied and in 2019 a year after treatments were applied (Figure 3.14). The standard deviation was noticeably large in the plots under glyphosate only treatment in both 2018 and 2019 compared to the other treatments.

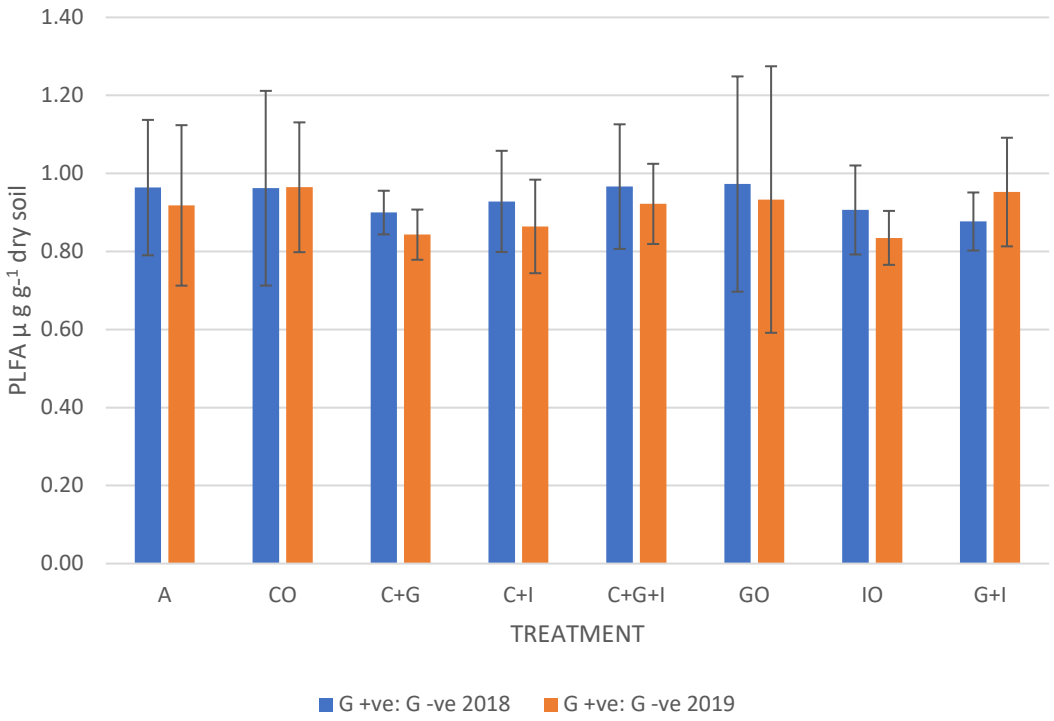


Figure 3.14 The mean ratio and standard deviation of Gram -ve : Gram +ve groups by treatment for 2018 and 2019. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=96).

3.8 Discussion

A hypothesis was proposed that by providing particular conditions within a soil, the amount of SOC would increase. Cover crops would provide the carbon source by removing CO_2 from the air and converting it into sugars used to generate biomass, and supply energy to microbes. Fungal and bacterial communities would take those sugars and transport carbon into the soil. An inoculant was added to boost the microbial community and help accelerate the process. It was also considered that glyphosate could negatively affect the microbial community. Therefore, by not using it prior to drilling seed, the existing microbial community would

not be negatively affected. The plots that had not been treated with glyphosate, sown with a cover crop coated with an inoculant would, hypothetically, offer the greater amount of SOC. Each of these attributes have been discussed in more detail in the literature review in Chapter 2.

The first year's results have shown that there is no increase in soil carbon either as an increase in SOM or PLFA as a result of using the combined treatments, cover crops, inoculant and no glyphosate treatments. The only significant changes occurred in the 0 -23 cm horizon in the soil, with glyphosate only treatment. In this case the application of glyphosate showed a mean decrease in soil organic matter compared to not using glyphosate supporting the theory that glyphosate has a negative impact on SOM. The glyphosate was used to desiccate any plants which would have been incorporated into the soil providing a biomass input. It is therefore surprising that SOM was lower within plots treated with the herbicide.

In the 0 – 23 cm horizon the pH was higher in the cover crop only treatment compared to no cover crop and also in the plots treated with inoculant compared to those without inoculant. Legumes such as clover (included in the cover crop mix) can remove nitrogen from the atmosphere and fix it in soil increasing pH (Ferreira et al., 2016). The results support findings from other studies that have looked at the short-term effects of cover crops (Haruna & Nkongolo, 2015; Strickland et al., 2019). A study of cover crops being used in agricultural rotation on 4 different farms, showed the mean soil pH increase from pH6.56 ± 0.08 to pH6.92 ± 0.08 ($P < 0.001$) (Strickland et al., 2019). Unlike the Hilley Farm results on carbon, research carried out on Freeman farm at Lincoln University, USA showed a 5.6% increase in the carbon to nitrogen ratio (C/N) in the fallow plots compared to the cover crop plots (Haruna & Nkongolo, 2015).

These results are relevant and important to agriculture where farmers are considering using cover crops for the purpose of increasing SOC. Clearly, the short-term use of a cover crop does not always contribute to an increase in carbon any more so than leaving soil fallow. Even where every opportunity is provided to encourage the growth of the microbial community by not treating with the herbicide glyphosate, and adding an inoculant, it still gave no significant beneficial increase in SOC in this case.

There are several limitations of carrying out this study over one year. The cover crop has two opportunities to contribute to the increase of carbon through i) the incorporation of leaf litter and residues into the soil, and ii)

the provision of roots into the rhizosphere. In this experiment the cover crop was not desiccated and incorporated at the end of the year thus reducing the potential feedstock of carbon to soil. The sensitivity of the analysis is also questionable over a short timescale. The increase of SOM is a slow process, and measurable differences using the analytical techniques employed in this study, are possibly too small to measure. SOM was measured using loss on ignition. This technique has been subject to criticism when used on clay soils. Clay has been shown to provide false results because of its ability to bond strongly with water which is not always driven off in the drying process (Hoogsteen et al., 2015). This can lead to an over representation of weight of organic matter. Arguably, in this study the whole site is a clay soil, and comparisons between samples are like for like.

There were significant changes between the samples taken in 2018 and 2019 which were not attributed to any treatments. All plots had plants present after one year and a rhizosphere. The addition of roots from any plant growth is likely to have influenced the bacterial communities which are found a few millimetres from plant roots (Bhattacharyya & Jha, 2012; Bidondo et al., 2011; Hartmann et al., 2008). Weather conditions can also have an effect on soil properties. Research comparing seasonal climate and soil management effects (cover crops, plastic mulch) on the microbial community, found that seasonality had the strongest influence (Maul et al., 2014). Linked with this are the weather conditions such as temperature, rainfall and light hours. In 2018 the weather was unusually hot in spring /summer when the crop was sown and establishing.

3.8.1 Limitations of the experimental set up

In this trial the use of cover crops was part of a long term (2 year) rotation where the focus was on activity occurring in the root zone, requiring roots from a standing crop. The more common approach is to use a cover crop over winter and incorporate the biomass into the soil before drilling the cash crop in the following spring. It is acknowledged that the role of above ground biomass as a carbon source was largely ignored here except for natural seasonal leaf litter and winter die back biodegradation. Most of the research on the use of cover crops is based on the annual incorporation of the biomass and the impact of the biodegradation of plant matter.

Research into the changes in SOC commonly show that growth in carbon stock is a slow process (Chapter 2) and it is possible that the methods used for measuring carbon is not sensitive enough to see small changes. Additional data was included for the second year in order to look for further evidence of the impact of each of the treatments. This included a worm count, used as an indicator of a healthy soil (Stroud, 2019) and quantification of the follow on wheat crop. Chapter 4 describes the results of the trial extended over a second year.

3.9 Conclusions

After one season there is no evidence to support the hypothesis that plots treated with glyphosate, planted with a cover crop and inoculated with beneficial microbes, increased in soil organic carbon. There is some evidence that in the 0 – 23 cm horizon, glyphosate only, showed a significant effect on SOM (%) in 2019 when compared to plots not treated with glyphosate, and cover crop alone, or inoculant alone, increased the soil pH over a year. Extension of the trial for a further year will help to determine if, with more time, the treatments can produce significant changes in SOM, SOC and biology.

4 The effect of cover crops, herbicide, and inoculant on carbon content in field plots- the second year

4.1 Introduction

Climate change has become an increasingly important global issue. The frequency of extreme weather events is increasing, making it more difficult to grow crops and challenging food security (DEFRA, 2020; Philip, 2014). Taking excess carbon from the atmosphere and using soil as a sink is a viable option, and cover crops have been considered a useful solution to aid that process. The flow of carbon, however, is complex and understanding how cover crops, together with other common farm treatments effect carbon sequestration, is

key. Additions of chemical and biological treatments, unless understood may lead to unintentional consequences.

A field trial comprising a randomized 6 block, with 8 plots each, containing 3 treatment factors; cover crops, inoculant and glyphosate was sampled in 2018 (baseline year prior to treatment) and 2019 following treatment (see Chapter 3). Analyses were carried out to measure changes in the quantity of soil organic matter (SOM), and pH in three soil horizons (0 – 23 cm, 23-60 cm, 60+ cm), and microbial biomass and PLFA in the 0 – 23 cm horizon. After one year, the analysis showed SOM in 0-23 cm horizon was decreased by a treatment of glyphosate and pH was affected by cover crop only and inoculant only. However, SOM changes slowly and it was unlikely we would see changes over the relatively short space of time (Bhogal et al., 2009; Kaspar & Singer, 2011).

A meta-analysis of cover crop use showed that, used over several years, they can lead to an increase in SOM (Aguilera et al., 2013; Jian, Du, et al., 2020; Kim et al., 2020; Olson et al., 2014; Poeplau & Don, 2015). But what happens if a cover crop is left in situ over a longer period allowing foliage to drop and decompose, and roots to grow? How does the microbial community change and will it result in change of SOM? It is anticipated that the microbial community will increase in quantity and biodiversity with a dominance towards a greater fungal composition.

For this study, it was decided that the cover crop would remain for 2 years to see how the soil would change. As well as the original aims set out in Chapter 2, in this second phase of the study, the specific aims were:

i. To see if standing cover crops could significantly increase SOM during this extended growth period
ii. To measure changes in the composition of the microbial community
iii. To assess the long-term effect of the inoculant and glyphosate on the soil
iv. To assess the soil quality on yield of a subsequent wheat crop.

Additional analyses were completed including: worm count, above ground biomass and yield of a follow on cash crop of wheat cash.

4.2 Methodology

The details of establishment of the plots with different treatments is described in Chapter 2.

4.2.1 Plot maintenance for year 2

In July 2019, the plots were cut for hay, and sheep were grazed for a week in November. Deer were observed grazing on the field from time to time. This is considered typical activity during an agricultural year. The original plots were used and defined by re-marking and mowing paths between them in September (

Figure 4.1).

In February 2020 the field was partially flooded, and water covered part of Block 1, plots 1-8. It was difficult to judge the extent of the flood as all fields surrounding it were also severely flooded denying access for several weeks. In October 2020, the plots were ploughed incorporating cover crop and fallow weeds into the plots. The ground was rolled then disc drilled with winter wheat (a variety called Graham) and rolled again. Post emergence fungicide and herbicide was applied at a rate of 150 l ha^{-1} on 31st March 2021. This included growth regulator ("Canopy"), (0.503 l ha^{-1}), a fungicide (0.75 l ha^{-1}), manganese sulphate 32% (0.28 kg ha^{-1}), Nutriphos (MBS979) (Phos 8.75% w/w, N 5% w/w).

Figure 4.1 The paths between plots were flailed once per year

Another flood event occurred in January 2021 affecting Block 1 plots 1-8 and part of Block 2 plots 9-12. The water drained away within two weeks. Visible differences were noticeable in the wheat crop in Blocks 1 and 2 immediately after the flood receded but appeared to recover well by summertime.

4.2.2 Sampling and analysis

A soil sample was taken from each of the plots on 14th - 16th June 2020. The methodology for sampling is explained in Chapter 3. Analysis for pH, loss on ignition (SOM), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), and phospholipid fatty acid (PLFA) were carried out using the methodology detailed in Chapter 3. Additional analyses were completed during the final 18 months.

4.2.2.1 Worm count

Earth worms are a good indicator of soil health (Stroud, 2019). Worms were sampled on two occasions, 10th-12th Sept 2018 and 18th-23rd August 2019. The method used was based on (Stroud, 2019). 1 pit (20 cm x 20 cm x 20 cm) was dug for each plot and the soil put to one side to check for worms. A mustard solution (1 tbsp mustard powder per 2 litres water) was tipped into the pit to encourage deep burrowing worms to the surface. Total worms were counted and recorded. Adult worms were separated and categorized into Epigeic (surface worms), Endogeic (topsoil worms) and Anecic (deep burrowers) (Figure 4.2).

Figure 4.2 Anecic worms taken from a soil sample in 2019

4.2.2.2 Above ground Biomass

In May 2020 the total amount of above ground biomass growing in each plot was quantified. For each plot the above ground vegetation was cut within 4 quadrats (50 cm x 50 cm), bulked up and weighed to give total grammes in a square meter (g m^{-2}).

4.2.2.3 Crop yield

In August 2021, a day before harvesting, a sample of the crop was taken from each plot. The ear was taken from 3 sets of rows, 1 m long 7-10 cm wide (Figure 4.3). The width was measured for each sample taken. The wheat samples were bulked for each plot. The samples were dried overnight in an oven at 105 °C. The dried ears were put through a standing thresher and the total corn weighed and counted.

Figure 4.3 Winter wheat crop before harvesting

4.2.3 Statistical analysis

Data from 2020 was analysed together with the data for the baseline year 2018 and the first year 2019. ANOVA was completed on all data using GENSTAT (19.1.21390). Comparisons were made between treatments and across years as outlined in Chapter 3. The data set was analysed for each individual treatment, and combinations of 2 and 3 treatments over 3 years. The differences that may have been presented due to the field's topography, soil variation or other environmental aspects were addressed through the randomized blocking design. The nested plots in blocks were included in ANOVA and all treatments were checked alongside each other. Microsoft excel was used to calculate averages and standard deviation which were used to generate bar charts.

4.3 Results

4.3.1 The effect of treatments on above ground biomass

Observation showed a clear difference between those plots that had a cover crop and those that were fallow. The fallow plots were dominated with thistles, docs and nettles with, at times, areas of bare soil underneath. The cover crops comprised a mix of tall and low-lying grasses with good ground coverage. Photographs of each of the plots taken on 14th September 2019 are provided in Appendix 2. The results of the yield of above ground biomass per square meter produced by each treatment are provided in Table 4.1. Biomass was

greatest in the plots treated with inoculant only. Those plots treated with cover crop only, or cover crop with other treatments, generated the lowest amount of biomass. Treatment with cover crop only, compared to no cover crop had a significant effect on the amount of biomass produced (P = 0.002).

Table 4.1 The mean above ground biomass (g/m^2), standard deviation and P value for each treatment

Treatment	Mean (g/m^2)	Standard deviation	P value
Control	712.67	169.69	
Cover crop only	614.33	260.92	**0.002**
Cover crop and glyphosate	616.67	106.29	0.321
Cover crop and inoculant	615.33	206.47	0.064
Glyphosate only	645.00	133.35	0.174
Inoculant only	904.33	211.72	0.142
Glyphosate and inoculant	758.33	170.34	0.516
Cover crop, glyphosate and inoculant	579.00	93.54	0.825

The inoculant applied (refer to Chapter 3) was a commercial brand marketed to improve the yield of a grass type sward. Whilst not significant (P = 0.064), there was a trend showing a treatment of a cover crop with inoculant produced a smaller above ground biomass compared with a fallow plot with inoculant.

4.3.2 The effect of treatments on soil organic matter

The SOM was not affected by any individual or combination of treatments in 2020 or across the 3 year trial period (

Table 4.2, Figure 4.4, Figure 4.5 and Figure 4.6). In 2019 the SOM decreased compared to the baseline 2018 (prior to the addition of treatment), then increased the following year in 2020. The mean SOM of all plots for each year showed a significant change in the 0-23 cm horizon (p = <0.001), the 23-60 cm horizon (P = 0.032), and the 60+ cm (P = 0.012).

Table 4.2 Percentage mean soil organic matter for each treatment in 3 horizons (0-23 cm, 23-60 cm, 60 +cm) in 2018, 2019, 2020. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I).

		2018			2019			2020		
Horizon	TREATMENT	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value	Mean (%	SD (%)	P value
0-23 cm	A	10.21	0.77		9.60	0.45		9.99	0.56	
	CO	10.38	0.58	0.470	9.57	0.43	0.779	10.08	0.39	0.431
	C+G	10.94	2.12	0.655	9.37	0.34	0.322	9.76	0.46	0.680
	C+I	10.38	1.16	0.387	9.56	0.39	0.653	9.90	0.83	0.688
	C+G+I	10.36	0.66	0.573	9.29	0.45	0.334	10.05	0.52	0.548
	GO	10.16	0.81	0.644	**9.39**	0.46	**0.011**	9.73	0.76	0.318
	IO	10.39	0.97	0.919	9.74	0.45	0.438	9.87	0.71	0.993
	G+I	10.44	1.36	0.692	8.90	1.03	0.222	9.73	0.80	0.318
		10.41	**1.09**		**9.43**	**0.55**		**9.89**	**0.61**	
	TREATMENT	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value	Mean (%	SD (%)	P value
23 - 60 cm	A	7.98	4.04		6.61	1.74		7.06	1.45	
	CO	6.66	1.03	0.824	6.89	1.05	0.859	7.51	1.24	0.388
	C+G	6.91	1.49	0.434	6.03	1.09	0.488	6.88	0.60	0.807
	C+I	6.39	2.32	0.789	5.85	0.48	0.738	6.25	0.50	0.646
	C+G+I	6.79	1.21	0.445	6.78	1.30	0.815	6.96	1.52	0.326
	GO	6.57	1.14	0.634	7.01	1.37	0.633	8.35	2.06	0.924
	IO	6.85	1.06	0.847	6.50	1.25	0.509	6.91	1.35	0.191
	G+I	6.38	1.31	0.879	6.30	1.39	0.737	7.00	1.36	0.569
		6.82	**1.86**		**6.50**	**1.22**		**7.12**	**1.37**	
	TREATMENT	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value	Mean (%)	SD (%)	P value
60+ cm	A	4.53	0.91		4.06	0.18		4.02	0.58	
	CO	4.40	0.14	0.824	3.99	0.62	0.859	3.97	0.61	1.000
	C+G	4.61	1.09	0.434	4.19	0.53	0.488	3.88	0.55	0.700
	C+I	4.38	1.36	0.789	3.94	0.44	0.738	3.78	0.37	0.501

C+G+I	4.62	0.75	0.445	4.05	0.70	0.815	4.36	0.81	0.692
GO	4.15	1.04	0.634	4.55	0.94	0.633	4.21	0.65	0.732
IO	4.38	0.54	0.847	4.07	0.43	0.509	4.15	1.14	0.906
G+I	4.16	0.55	0.879	4.04	0.62	0.737	3.73	0.65	0.855
	4.40	**0.82**		**4.11**	**0.58**		**4.02**	**0.66**	

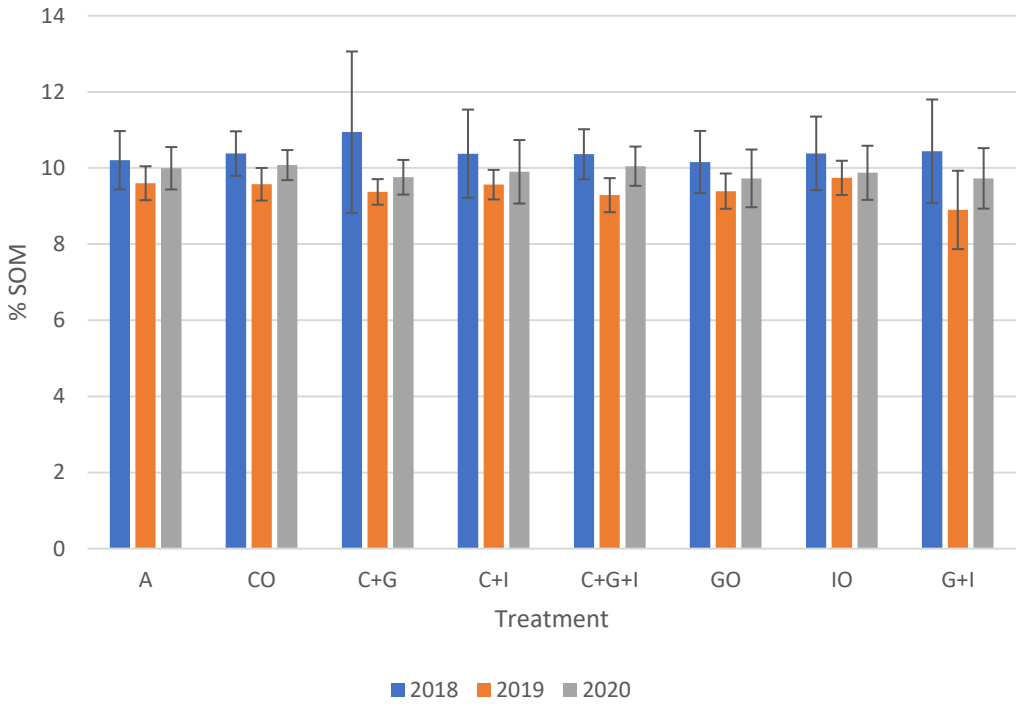


Figure 4.4 Horizon 0 – 23cm. Mean SOM (%) taken before (2018) and after (2019 and 2020) treatments were applied. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

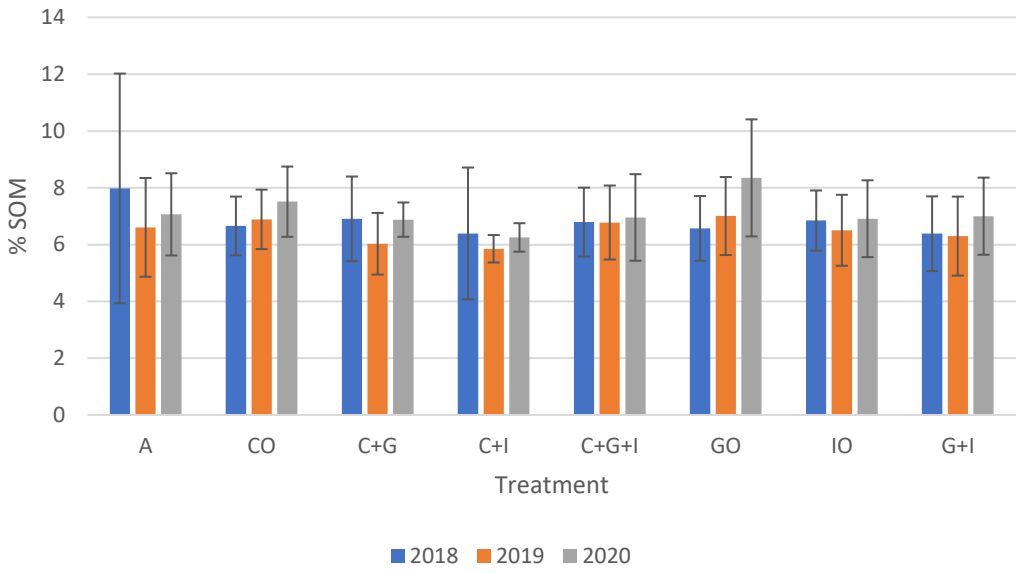


Figure 4.5 Horizon 23 – 60cm. Mean SOM (%) taken before (2018) and after (2019 and 2020) treatments were applied. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=192).

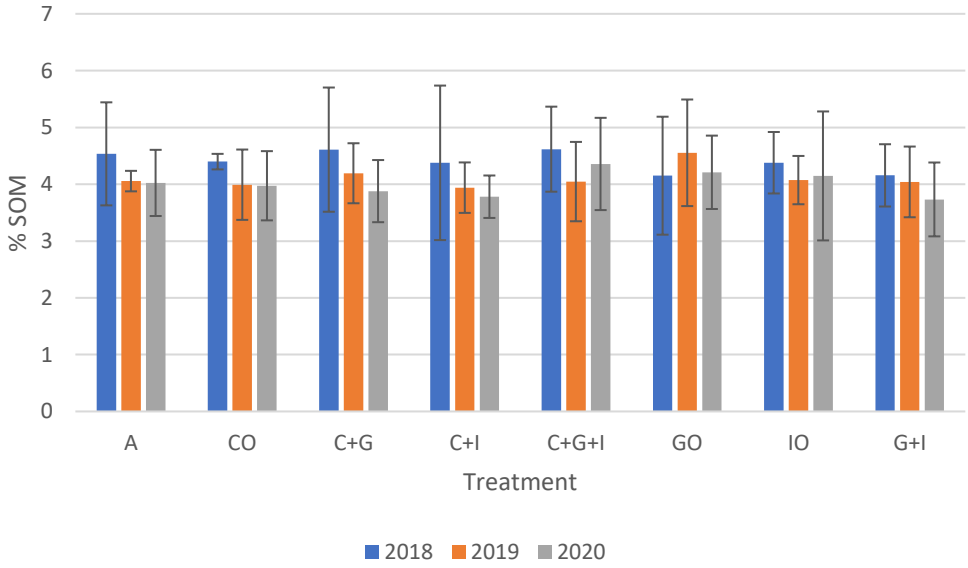


Figure 4.6 Horizon 60 + cm. Mean SOM (%) taken before (2018) and after (2019 and 2020) treatments were applied. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

4.3.3 Effects of treatments on pH

The pH went up and down across all the treatments between 2018 and 2020 but it was only in 2020 where treatments demonstrated significant (<0.05) effects in the upper horizon (0 – 23 cm) including cover crop only, cover crop and glyphosate, cover crop with glyphosate and inoculant, and inoculant only (Table 4.3). The pH in the lowest horizon (60+ cm) showed very little change across time or between treatments.

Table 4.3 The mean pH for each treatment, in 3 horizons (0-23 cm, 23-60 cm, 60+ cm), in 2018 , 2019 and 2020. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I).

		2018			2019			2020		
Horizon	TREATMENT	Mean	Std Dev	P Value	Mean	Std Dev	P Value	Mean	Std Dev	P Value
0 - 23cm	A	6.53	0.18		7.00	0.16		6.49	0.11	
	CO	6.41	0.16	0.48	7.05	0.17	0.57	6.28	0.17	**0.02**
	C+G	6.48	0.18	0.19	7.14	0.12	0.12	6.45	0.10	**0.02**
	C+I	6.47	0.11	0.62	7.10	0.11	0.16	7.07	0.06	0.29
	C+G+I	6.43	0.06	0.58	7.16	0.15	0.46	6.54	0.05	**0.03**
	GO	6.46	0.13	0.41	6.86	0.33	0.99	6.38	0.10	0.81
	IO	6.51	0.23	0.76	7.11	0.23	0.04	6.53	0.11	**0.05**
	G+I	6.41	0.20	0.40	7.10	0.24	0.46	6.54	0.12	0.42
	TREATMENT	Mean	Std Dev	P Value	Mean	Std Dev	P Value	Mean	Std Dev	P Value
23 -	A	6.95	0.30		6.95	0.26		7.16	0.33	

	CO	6.94	0.28	0.30	6.79	0.15	0.38	7.06	0.16	0.26
	C+G	7.03	0.39	0.56	6.92	0.23	0.62	6.98	0.22	0.93
	C+I	7.14	0.17	0.36	6.85	0.41	0.97	7.18	0.11	0.35
	C+G+I	6.92	0.26	0.15	6.88	0.40	0.64	7.15	0.33	0.27
	GO	6.41	1.32	0.33	6.92	0.19	0.63	7.54	1.23	0.34
	IO	6.98	0.27	0.23	6.92	0.30	0.96	7.03	0.08	0.45
	G+I	7.04	0.18	0.66	6.95	0.26	0.95	7.08	0.28	0.54
	TREATMENT	Mean	Std Dev	P Value	Mean	Std Dev	P Value	Mean	Std Dev	P Value
	A	7.63	0.20		7.75	0.27		7.46	0.37	
	CO	7.64	0.09	0.48	7.76	0.22	0.69	7.45	0.28	1.00
	C+G	7.60	0.22	0.69	7.82	0.28	0.90	7.51	0.16	0.70
60 + cm	C+I	7.49	0.21	0.71	7.74	0.20	0.96	7.50	0.28	0.50
	C+G+I	7.71	0.27	0.43	7.87	0.27	0.56	7.49	0.33	0.69
	GO	7.71	0.15	**0.02**	7.87	0.13	0.13	7.51	0.28	0.73
	IO	7.53	0.33	0.45	7.81	0.26	0.75	7.49	0.11	0.91
	G+I	7.71	0.25	0.07	7.85	0.28	0.97	7.39	0.30	0.86

4.3.4 Effects of treatments on microbial biomass

None of the treatments showed a significant effect on the microbial biomass carbon (MBC) in 2020. The range of data points was high and considered to be higher than expected for this soil type. (

Table 4.4).

Table 4.4 Microbial biomass carbon mean, standard deviation(sd), and p value for 2020. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I).

Microbial Biomass Carbon µ g^{-1}soil

Treatment	2020 mean	2020 sd	p value
A	1460.79	1407.36	
CO	931.25	624.81	0.477
C+G	1063.09	683.79	0.790
C+I	836.68	534.98	0.998
C+G+I	1028.75	612.27	0.189

GO	860.15	287.26	0.583
IO	757.14	580.78	0.659
G+I	1234.51	612.27	0.150

There was a general overall change in MBN in soil between 2019 and 2020 (P= 0.016) but no significant differences between treatments (Table 4.5). In 2020 the cover crops only treatment showed a trend towards an increase in MBN compared to no crops (P = 0.064) and comparing 2019 with 2020 (P = 0.07).

Table 4.5 Microbial biomass nitrogen mean, standard deviation (sd), and p value for each year and over time. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I).

Mean Microbial Biomass Nitrogen µ g-^{1}soil

Treatment	2020 mean	2020 sd	p value
A	42.40	24.67	
CO	63.56	21.05	0.064
C+G	70.03	22.25	0.870
C+I	52.98	29.93	0.459
C+G+I	59.03	33.76	0.738
GO	51.45	27.54	0.505
IO	42.43	10.22	0.347
G+I	35.72	21.92	0.574

4.3.5 The effect of treatments on Phospholipid Fatty Aids

The results from the three years of PLFA are provided in Appendix 2, including mean, standard deviation and p value. Comparison of data across the baseline year (2018), 2019 and 2020, showed a significant effect on total PLFA with the cover crop only treatment compared to no cover crop (P = 0.037) (Figure 4.7). However, the standard deviation reflected the wide range of data points amongst these plots, similarly with the plots treated with glyphosate and cover crops.

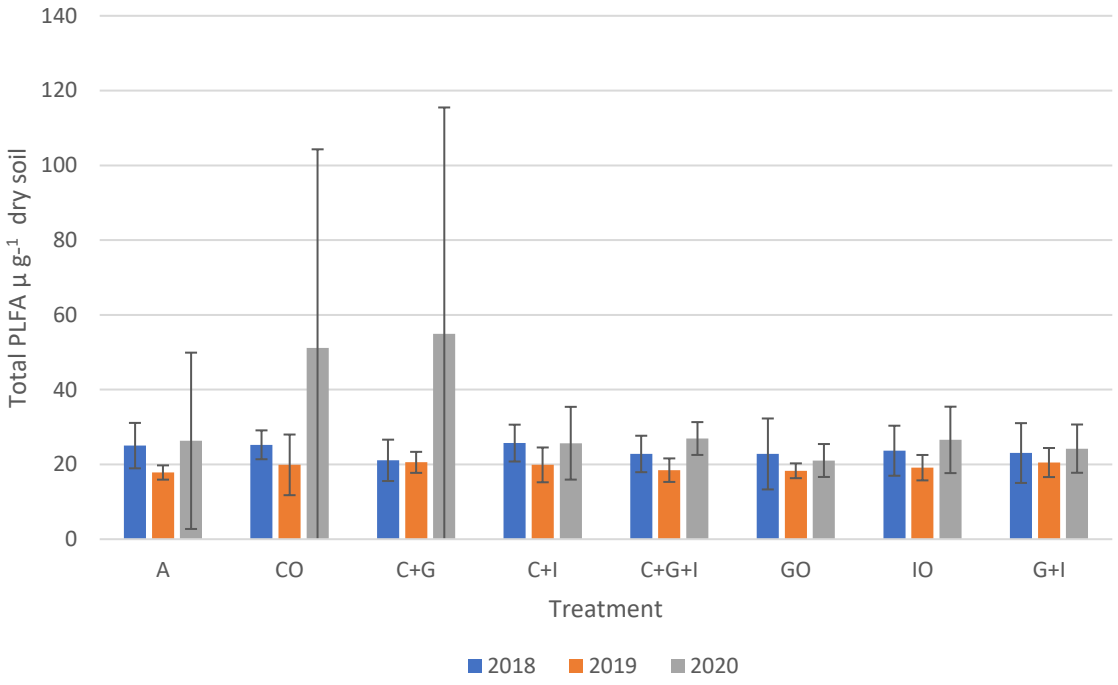


Figure 4.7 Total PLFA for each treatment over three years (2018 – 2020). Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

The PLFA fungal biomarker group showed an increase compared to the base year in the control plot, the plots treated with glyphosate and cover crops, and cover crop only. The range of data points was large, indicated by

the large standard deviation (Figure 4.8). In 2020 ANOVA showed that inoculant only had a significant effect on the fungal group compared to no inoculant (P = 0.04). This treatment across 2018, 2019 and 2020 also showed a significant effect on the fungal group (P = 0.01) shown in Figure 4.9. The fungal group in 2018, before treatment, was fairly even. Following treatment in 2019, it increased in the plots that were inoculated. In the second year the plots not treated with inoculant showed an increase of more than three times that in the inoculated plots (Figure 4.9).

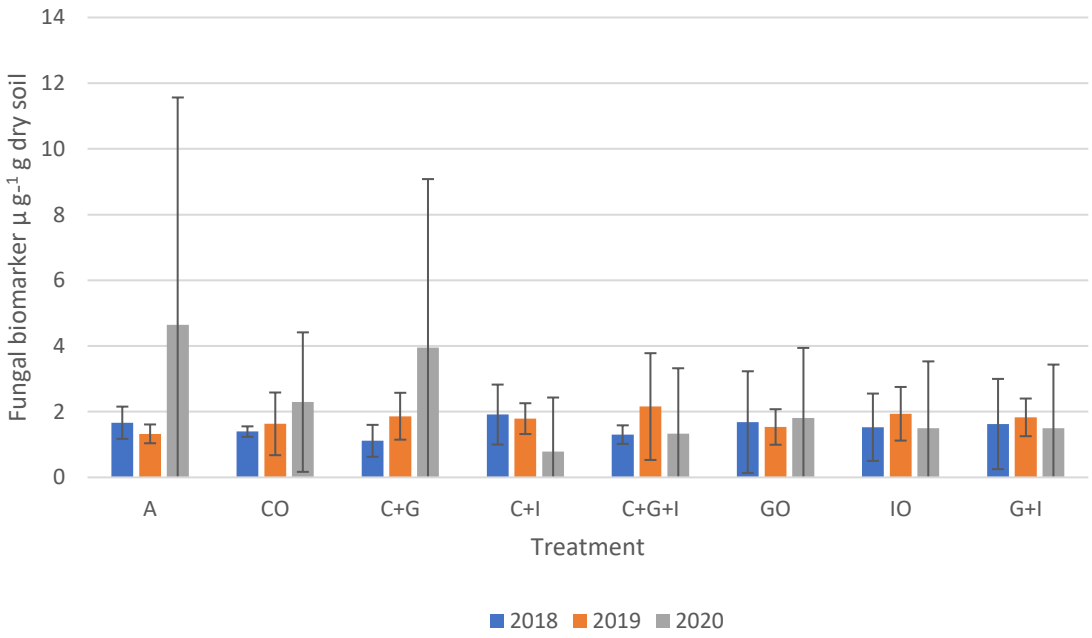


Figure 4.8 The fungal biomarker group of PLFA for each treatment for years 2018, 2019 and 2020
Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

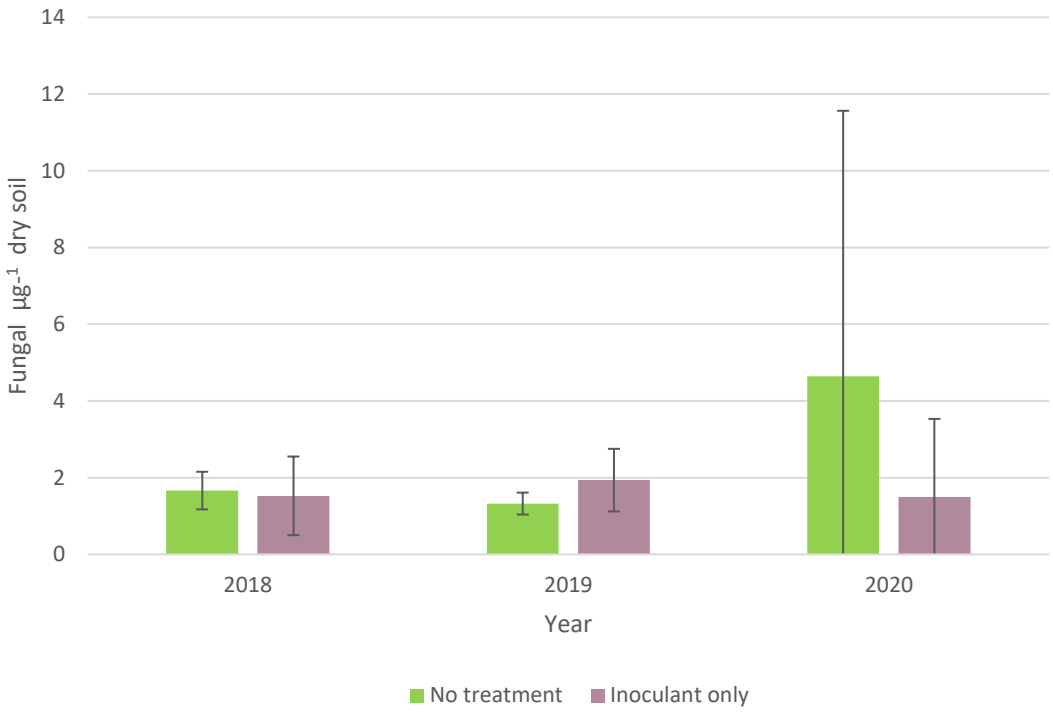


Figure 4.9 Bar chart showing the change in mean and standard deviation of fungal biomarker group in plots with inoculant compared to plots with no inoculant in 2018, 2019 and 2020.(n=144)

The bacteria in plots treated with cover crops only increased from 11.43 µg g^{-1} in 2018 (soil before treatments added) to 17.05 µg g^{-1} in 2020, compared to the fallow which was 10.96 µg g^{-1} in 2018 and 10.72 in 2020 (P = 0.045) (Figure 4.10). The plots treated with the cover crop and inoculant showed a trend towards a lower bacterial presence compared to the cover crop with no inoculant (P = 0.065) (Figure 4.11).

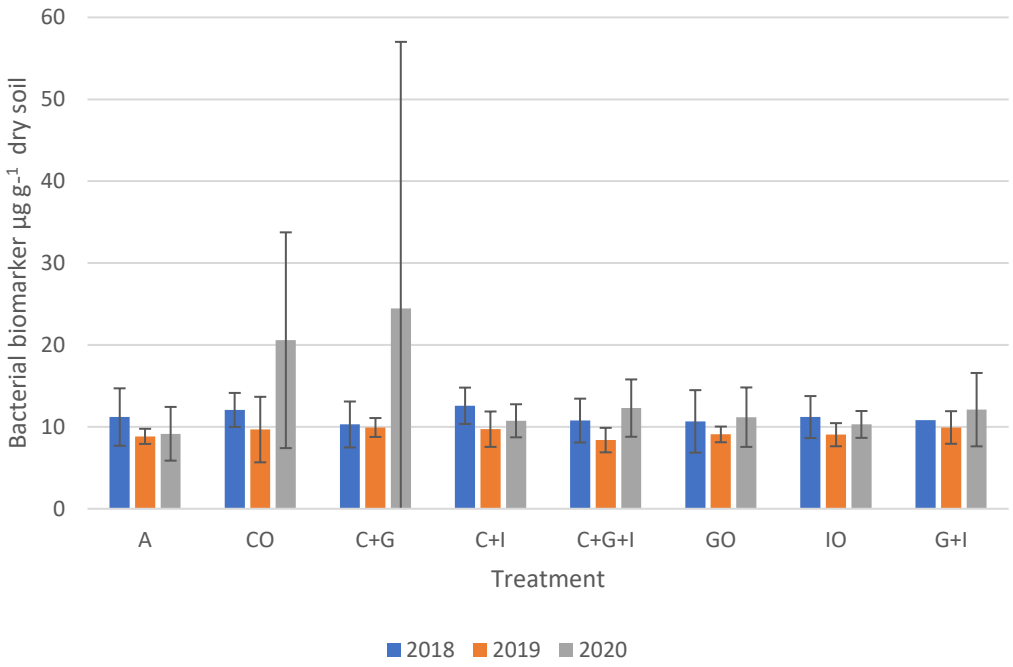


Figure 4.10 The bacterial biomarker group of PLFA for each treatment in 2018, 2019 and 2020.
Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I).

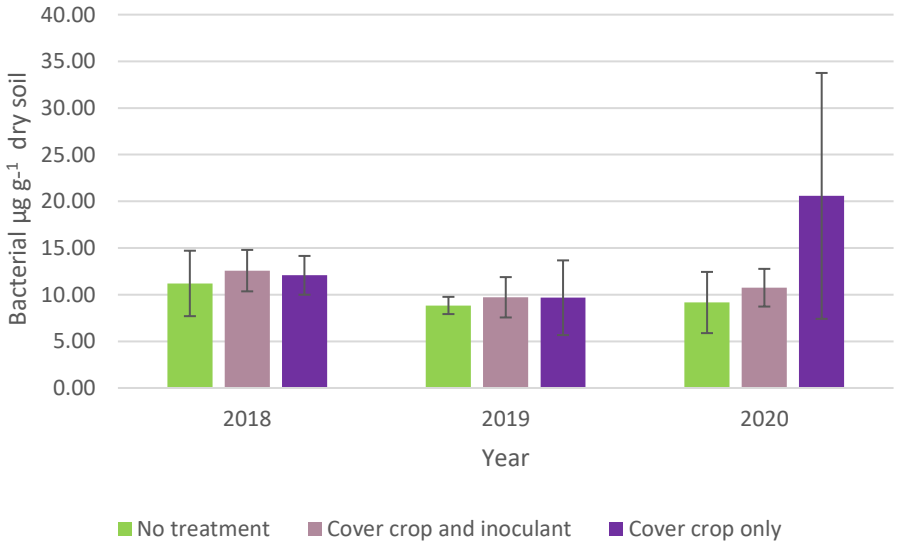


Figure 4.11 The change in bacterial biomarker in plots with cover crop only (P = 0.045) compared to plots with no cover crop, and plots with a cover crop plus inoculant (P = 0.065) in 2018, 2019 and 2020.

The fungal to bacteria ratio (F:B) is an important indicator that shows the likely flow of carbon within the soil. A high ratio is linked to carbon stabilization as aggregate, low is linked to the movement of carbon out of soil into plant roots, shoots and fruit. In 2020, treatments showed no differences in the fungal : bacterial ratio. However, over the trial period between 2018 and 2020, the plots treated with an inoculant (IO) compared to no inoculant (A) effected the fungal:bacterial ratio (Figure 4.12).

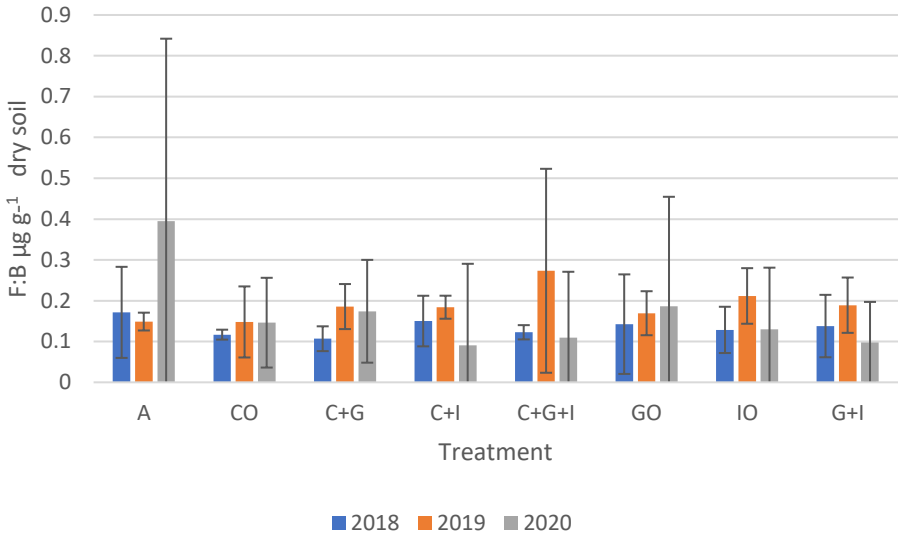


Figure 4.12 The fungal : bacterial ratio for all treatments in 2018, 2019, and 2020. The inoculant only (IO) gave a P value of 0.020. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144)

Gram-positive functionality group are linked to a presence of more difficult (recalcitrant) carbon sources. In this trial the gram-positive biomarker group was affected by the inoculant only treatment compared to no inoculant in 2020 (P = 0.037) and between the years 2018 to 2020) (P = 0.013) (Figure 4.13 and Figure 4.14. The plots with the inoculant only, showed a year-on-year decrease of gram-positive biomarkers.

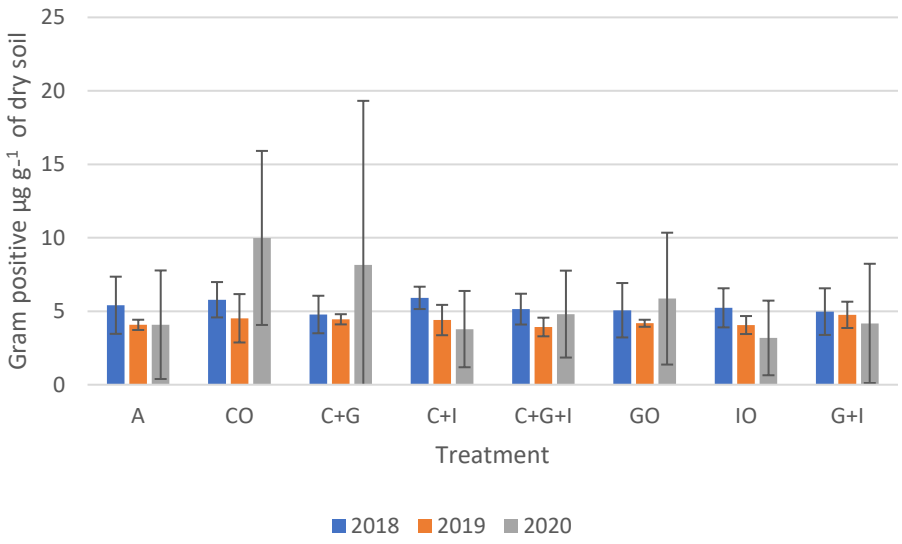


Figure 4.13 Mean gram-positive biomarker group of PLFA for each treatment in 2018, 2019, and 2020
Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

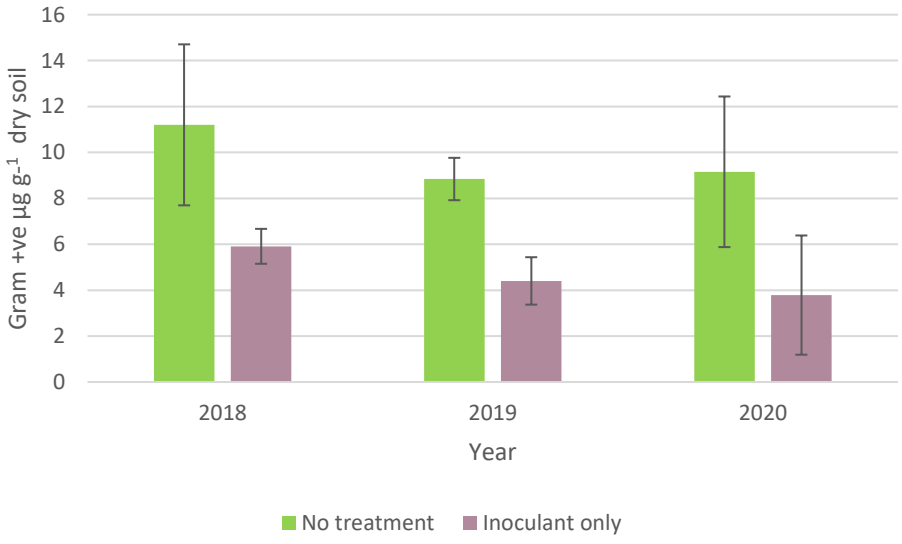


Figure 4.14 The effect of inoculant only on the gram-positive biomarker across years 2018 to 2020.

Gram–negative microbes are associated with more readily available carbon (labile). Cover crops plus the inoculant showed a depressed effect on the gram negative biomarker group over 2018, 2019 and 2020 (P =

0.037) and a trend in 2020 when compared with cover crop and no inoculant (P = 0.66) (Figure 4.15 and Figure 4.16).

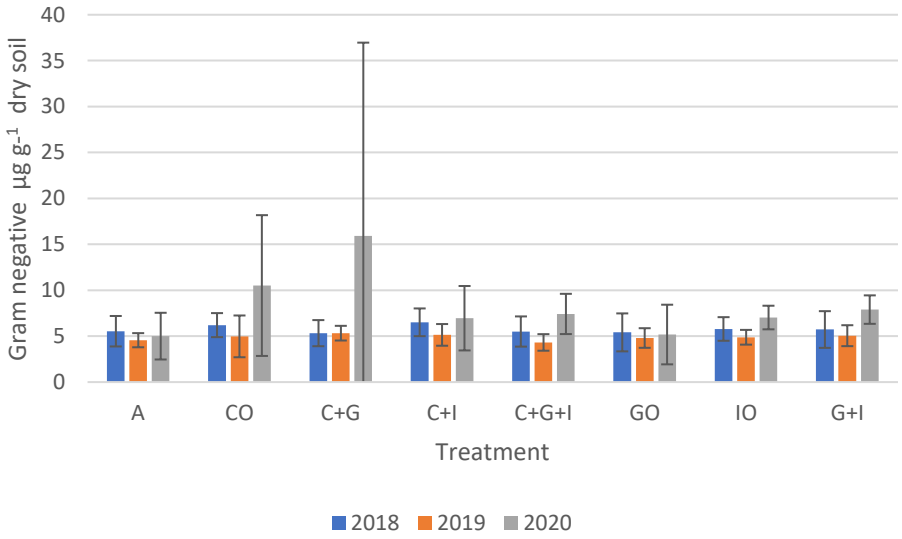


Figure 4.15 Mean gram negative biomarker group of PLFA for each treatment in 2018, 2019, and 2020
Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

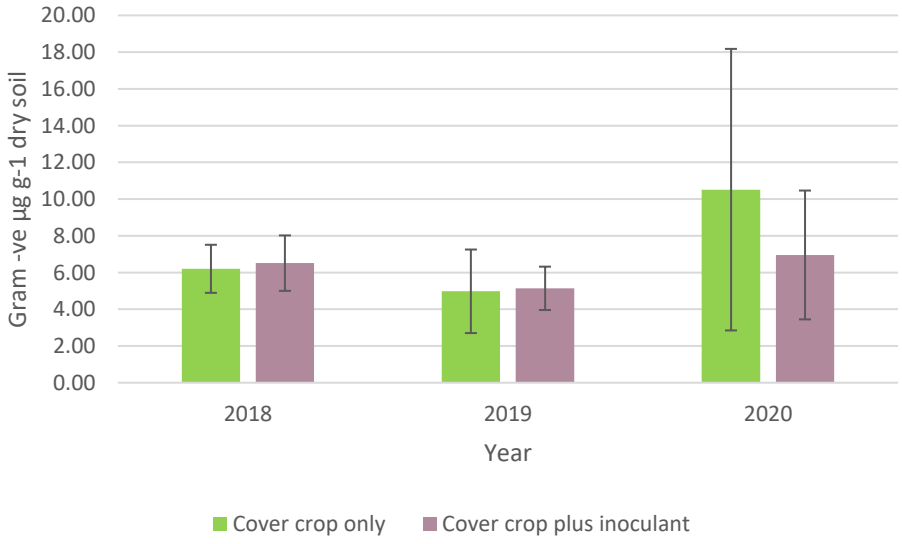


Figure 4.16 The effect of cover crop plus inoculant compared with cover crop and no inoculant on the gram-negative biomarker across years 2018 to 2020.

No one treatment effected the change in the gram-positive: gram- negative ratio in any one year or over three years. The range of data points were widespread in 2020 in the control, cover crop plus inoculant, and glyphosate only (Figure 4.17).

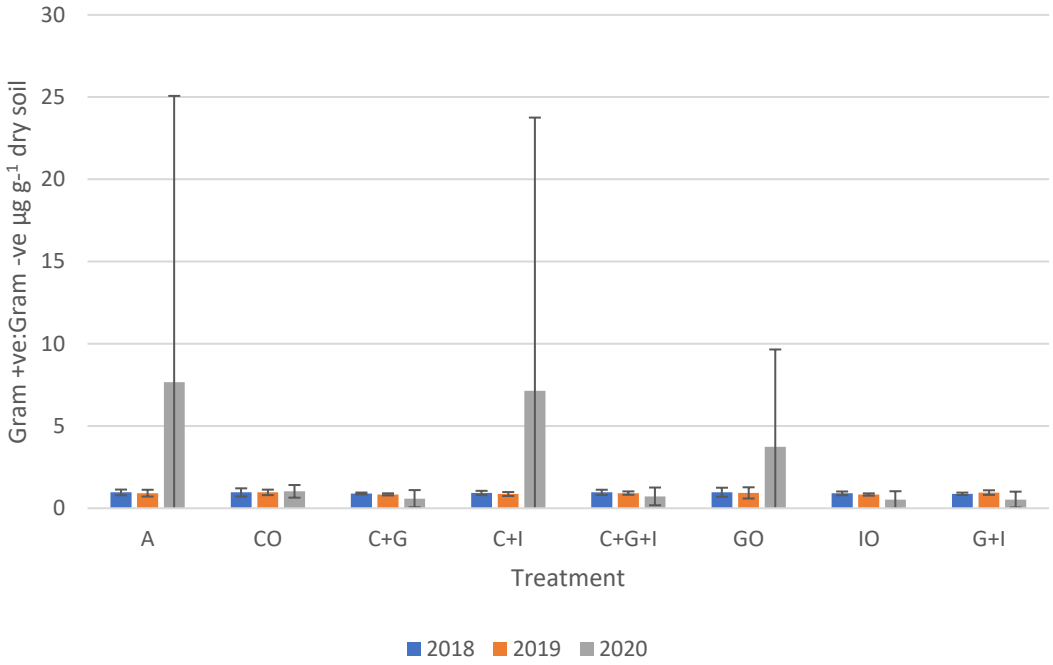


Figure 4.17 Ratio of gram-positive : gram-negative group for each treatment in 2018, 2019, and 2020
Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=144).

4.3.6 The effect of treatments on worm count

The worm sampling occurred after treatments had been applied. There was a significant increase in worms between 2018 and 2019 (P = <0.001). The mean total worm count and adult anecic worm count were effected where glyphosate was used with cover crops (mean 2.25, 0.83) compared with cover crops with no glyphosate (1.25, 0,25) in 2018 (P = 0.007, 0.016). In 2019 the cover crop and inoculant, showed a significant effect on anecic worm numbers (P = 0.34) with a mean of 1.33 with no treatment, and 8.96 with inoculant. In 2018 the epigenic worms count was affected by cover crops (0.167) compared to no cover crops (0.583).

The biggest annual increase of total worms occurred in the plots treated with cover crop only which increased from an average of 1.2 in 2018 to 11.25 in 2019. The cover crop plots pre-treated with glyphosate gave the lowest annual increase between 2018 and 2019 (Figure 4.18).

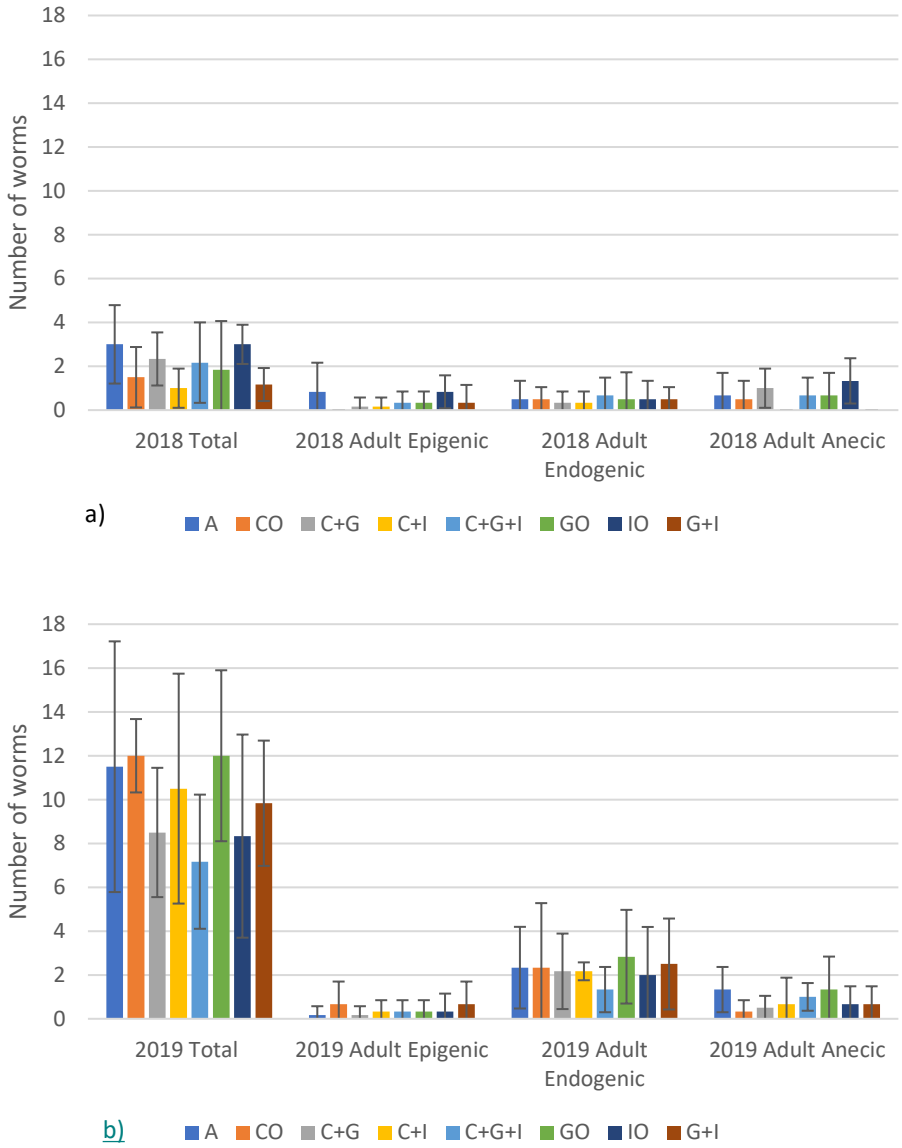


Figure 4.18 Average worm count by type for each treatment in a) 2018 and b) 2019. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). .

4.3.6.1 Yield of follow-on cash crop

The yield of the follow-on cash crop of winter wheat (g m^{-2}) showed no significant difference across the treatments.

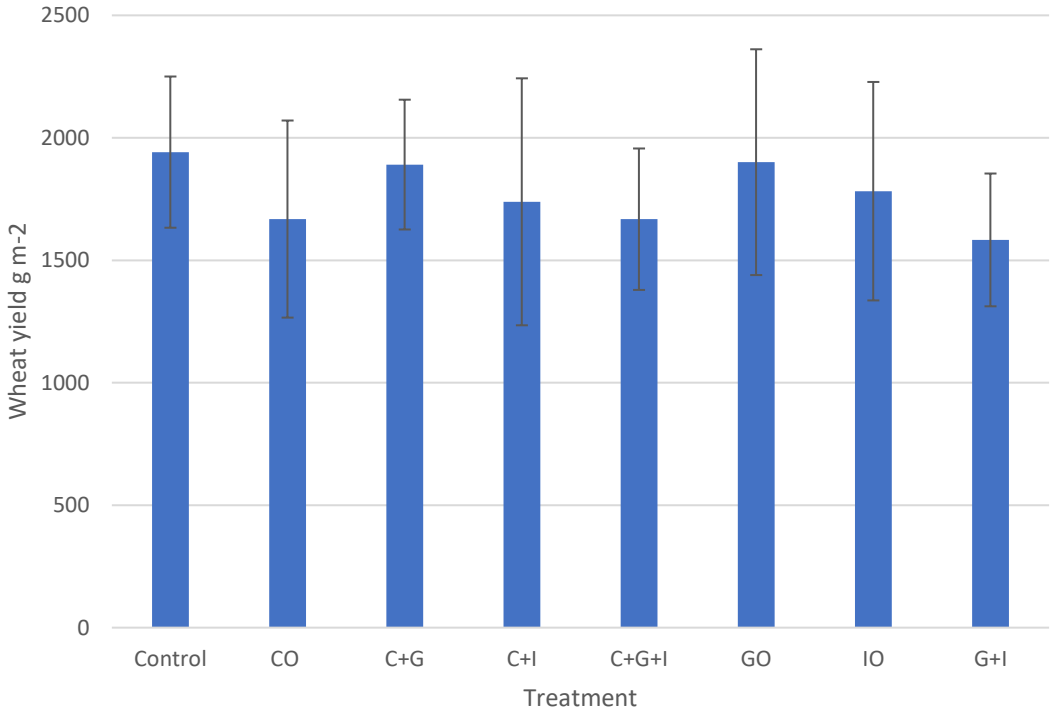


Figure 4.19 The yield of wheat grain harvested from plots previously treated with cover crop, glyphosate and inoculant combinations. Treatments include: the control with no treatments (A), cover crops only (CO), cover crop and pre-treatment with glyphosate (C+G), cover crop coated with inoculant without a glyphosate pre-treatment (C+I), pre-treatment with glyphosate and cover crop coated with inoculant (C+G+I), glyphosate treatment only (GO), Inoculant only with no pre-treatment with glyphosate or cover crop (IO), and a glyphosate pre-treatment with inoculant but no cover crop (G+I). (n=48).

4.4 Discussion

The whole harvest approach examined the effect on soil of not only a cover crop but included the use of glyphosate, a commonly used herbicide, and the application of a beneficial inoculant. This research was focussing on the effects of a standing crop where roots remained intact for two years. The treatments are discussed in response to their effect on increasing the carbon content of soil and the impact on the flow of carbon.

4.4.1 The effect of treatments on the quantity of carbon in soil

After two years of standing crop, SOM was not affected by any of the treatments in any combination, in any of the three soil horizons. Whilst the total SOC (equating to 50 % to 58 % of SOM) varied significantly between the years, this could not be attributed to any specific treatments that were applied. This result is similar to that found in other research. Tauges et al. (2019) found in a long-term experiment measuring SOC to a depth of 2 m showed no change where winter cover crops had been used. However, what was recognised was where cover crops were not used, the SOC decreased by 5.6 %. Other studies also found it difficult to prove that SOC increased by using cover crops in the short term concluding that it can take decades to show significant improvements in SOC. Evidence on long-term use of cover crops compared with fallow showed an increase in SOC (Moore et al., 2014; Venkateswarlu et al., 2017). Meta-analysis of 269 studies where cover crops were regularly used over up to 12 years, showed an increase in SOC (Jian, Lester, et al., 2020; Olson et al., 2001). Olson showed that there was a significant increase in SOC in the root zone where cover crops had been used for 12 years and most SOC was maintained where cover crops had been used with no till over 8 years.

Compared to the research described above, in this trial the crop was not desiccated each year, turned into the soil, and resown the following year which potentially limited the availability of plant carbon and SOM. The above ground biomass was significantly lower in plots with a cover crop compared to fallow. Research carried out by Berhongaray et al (2018) showed above ground biomass had a very low SOC formation efficiency of 9% whereas below ground biomass had a high conversion efficiency to SOC of 76% (Berhongaray et al., 2019). Although a large above ground biomass can improve a soil eco system, it is the functional traits of a plant that have a greater influence on microbial activity than biomass (Finney et al., 2016; Florence et al., 2019; Lal et al., 2007). This appeared to support findings in the second year of this trial in the PLFA results. The PLFA analysis showed a general drop between 2018 (before treatments were applied) and 2019, and a significant increase in 2020 in the plots treated with cover crops ($P = 0.037$), and in plots pre-treated with glyphosate plus cover crops (Figure 4.7).

The microbial biomass carbon increased up to 4 times in all plots but was not significantly affected by any one treatment. Allowing any roots to establish and remain without interruption appeared to encourage a rich microbial presence.

4.4.2 Impact of treatment on flow of carbon

Fungal biomarkers increased in the control plot, plots treated with cover crops only and plots pre-treated with glyphosate and a cover crop. However, the plot treated with inoculant only compared to no inoculant gave a significantly lower result in 2020. The impact of inoculants is discussed further, below. Where fungal groups are dominant the carbon flows slowly. Olsson (1999) identified the biomarker 16:1 (n-5) as a dominant PLFA present in Arbuscular Mycorrhizae (AM), and it is often used as a biomarker to identify AM. AM are key in the formation of aggregates and the stabilizing of SOC (Jones & Carbon, n.d.; Kotsia & Marco, 2017; Q. Wang et al., 2015; W. Wang et al., 2017; Wright & Upadhyaya, 1998). Finney et al (2017) used this biomarker to identify the presence of AM and similarly found that AM (16:1 n-5) was higher in standing cover crops compared to the fallow control (Finney et al., 2017).

There is controversy over the use of the 16:1 (n-5) biomarker to identify AM as it has been found not to be unique to AM. 16:1 (n-5) was found to be present in gram-negative bacteria, and although in relatively small amounts, it could present false readings (Ngosong et al., 2012). Similarly, 18:2 (n-6) a biomarker for saprophytic fungi could also be identified where there was bacteria although again, this is in small amounts (Frostegard et al., 1996).

The plots treated with cover crops only showed a strong trend towards increased MBN ($P = 0.064$). The ratio of C:N has an impact on the flow of carbon. N speeds the flow of C out of the soil. The differences in available C and N in cover crop plots and fallow could have been affected by plant species, via plant traits or photosynthesis (Oertel et al., 2016). The process of decomposition supplies the microbial community with energy and releases carbon.

4.4.3 The impact of inoculant

Inoculants are sold commercially to provide ecosystem services to promote healthy plant growth and increase yield. The inoculant used (Symbio Mycoforce Grass Seed Coat) contains AM and plant growth promoting bacterial species and is specifically for perennial grass species. Inoculants work by providing a plant with microorganisms that stimulate activity to perform specific functions such as mobility of nutrients (Suyal et al.,

2016), defence against pests and pathogens (Babalola, 2010) and plant growth (Beneduzi et al., 2012; Glick, 1995; Kundan & Pant, 2015) thus improving plant health and increasing crop yield. However, in this experiment there were several negative effects noticed where inoculant was used. The PLFA biomarkers were significantly lower in the fungal biomarker group in plots that had inoculant only, compared to no inoculant (Figure 4.9) and in bacteria in plots with cover crop treated with inoculant compared to cover crop on its own (Figure 4.11). The bacteria and gram-negative groups more than doubled in quantity in the second year. 7,cy-17:0 (gram-negative bacteria) was greater in plots with cover crop only compared to cover crop treated with the inoculant. Cover crop and fallow with inoculant had similar levels of the bacteria. One biomarker, 18:0 (bacteria) was significantly greater in the cover crop plot with the inoculant compared to fallow with and without inoculant.

Hertzberger et al (2015) found a similar result using inoculant to increase growth of prairie grasses. In this case the grasses initially did well but over time their performance declined. The performance of an inoculant is often trialled and measured in poor soils that are degraded and thus tend to give a positive outcome (Hart et al., 2018). In healthy, well-established soils, the performance of an inoculant is limited and potentially damaging (Hart & Brookes, 1996; Oehl et al., 2017). The baseline SOM at Hilley Farm was between 9-10% which does not represent poor soil therefore this could explain the unexpected results where the inoculant was added.

Two biomarkers changed significantly in plots with inoculant compared to plots with no inoculant – 7me 17:0 (gram-positive) and 16:0 (bacteria). The bacteria 16:0 increased in the plots with no inoculant whereas, the gram-positive bacteria (7me 17:0) showed no change over the three years in the plots with no inoculant, but dropped significantly in the third year (2020) in the inoculated plots. The inoculant is developed to increase yield and provides a dose of gram-negative bacteria conducive to plant growth (Butterbach-bahl et al., 2013). The abundance of gram-negative may have resulted in competing with the gram-positive bacteria.

4.4.4 Effect of glyphosate

The worm population was greatest (p.0.007) in plots not pre-treated with glyphosate and planted with a cover crop. The annual increase was significantly lower in total worm population where glyphosate has been used.

This outcome is supported by other research. In greenhouse experiments where glyphosate was applied to soil, worm casts decreased (Gaupp-Berghausen et al., 2015) and in another, the worms were heavier but fewer (Zaller et al., 2014).

Two biomarkers 7,8cy 19:0 and 15:0 showed significant changes over time. 7,8cy 19:0 (gram-negative bacteria) increased in year 2 where there was glyphosate. Other examples have shown an increase of bacteria when glyphosate is applied suggesting that it provides a source of carbon for some bacteria (Lane, 2011). 15:0 (bacteria) decreased significantly in the plots with no glyphosate. Glyphosate works by disrupting the shikimate pathway (a metabolic pathway) used by plants to synthesise aromatic amino acids. This pathway is also present in some fungi and bacteria (Hagner et al., 2019) which could be a reason for the decrease in this biomarker (Arango et al., 2014; Cherni et al., 2015).

4.4.5 The combined effect of glyphosate and inoculant

The quantity of total PLFA, fungi and bacteria in plots treated with combinations of an inoculant and glyphosate showed no differences between treatments in any year (Figure 4.7, Figure 4.8, Figure 4.9, Figure 4.10).

The p values for PLFA, bacteria, and fungi functional groups across treatments in each of the 3 years, 2018, 2019 and 2020, were 0.978, 0.962 and 0.874 respectively. This may be explained by the microbial community displaying resistance (the degree to which microbial composition remains unchanged when disturbed), and resilience to change when the soil is subjected to the addition of man-made chemicals and biological inoculants (Allison et al, 2009). Although the addition of glyphosate alone increased bacterial species, and inoculant alone decreased bacterial species (Figure 4.10) in combination there was consistently no effect. The microbial community was expected to react to the glyphosate with a microbial flush as observed in research carried out by (Lane, 2011). It was also expected that the inoculant would boost the microbial community with beneficial microbes (Alori & Babalola, 2018; Babalola, 2010; Beneduzi et al., 2012; Saad et al., 2020). The reason for this lack of response when in combination is not clear although it could suggest some kind of antagonistic interaction. Similar results were found where two different growth promoting bacteria were isolated and treated with glyphosate. The root and shoot of maize was shorter when treated with the

inoculant alone, the glyphosate alone, and both inoculant and glyphosate compared to neither of the treatments(Shahid & Saghir Khan, 2017).

The p value for PLFA, bacteria, and fungi functional groups across treatments in each of the 3 years, 2018, 2019 and 2010, was p. 0.978, p. 0.962 and p. 0.874 respectively. This steady state may be explained by the microbial community displaying resistance (the degree to which microbial composition remains unchanged when disturbed), and resilience to change when the soil is subjected to the addition of man-made chemicals and biological inoculants (Allison & Martiny, 2009). This outcome demonstrates that disturbance to the microbial community, either by the addition of glyphosate and the inoculant did not appear to change it. The microbial community was expected to react to the glyphosate with a microbial flush as observed in research carried out by (Lane, 2011). It was also expected that the inoculant would boost the microbial community with beneficial microbes (Alori & Babalola, 2018; Babalola, 2010; Beneduzi et al., 2012; Saad et al., 2020). The competing microbes may have kept the community in check making the community more resilient.

4.5 Conclusions

After 2 years there was no evidence that cover crops produced more SOC than fallow weeds. There is evidence to show that cover crops can increase the soil microbial community particularly the gram-negative group, and potentially AM indicated by the biomarker 16:1 (n-5). It was hypothesised that, if a cover crop treated with a mycorrhizal inoculant is used as a feedstock for carbon and grown in a soil that has not been pretreated with glyphosate, it will produce the richest microbial community conducive to increased levels of soil carbon. The evidence suggests that this is not true. Inoculants do not necessarily provide the outcome expected and the existing soil health is an important consideration before deciding on its use. Addition of new bacteria can create competition and change the well-established microbial community leading to negative or unintentional outcomes.

Glyphosate had minimal effect on the microbial community but did negatively affect worms which was supported by other research. There are interesting interactions occurring within the microbial community where glyphosate and inoculants are used together. There is little research on this but as these chemicals are

used together in agriculture for separate and different purposes, further research is needed to understand how they interact and what the consequences are for soil health and fertility.

5 The effect of plant materials on microbial community in clay and sandy soils

5.1 Foreword

The method was developed and executed during the COVID pandemic, the experiment was severely impacted by restrictions imposed during the pandemic. Access to the campus, laboratory equipment and accommodation was restricted for an extended period. The trial was set up and managed at my home in Shropshire using the limited facilities available, the conditions were not ideal but allowed for an experiment to be completed. The soil laboratory at Lancaster University had restricted access and limited staff support which led to logistical problems including limited supply of chemicals and repeated instrument failure. This caused significant delays in analytical work, some of which could not be completed within the limits samples could be stored for.

5.2 Introduction

If cover crops become more widely adopted as part of an arable rotation, as encouraged under DEFRA's new agricultural Standards for grass and arable land (DEFRA, 2022b) then there is a need for a better understanding of the microbial communities associated with different plant species. Is it possible, for example, to plant selectively to achieve a microbially rich soil that offers greater fertility, or increases resilience and resistance to future environmental stresses? Carbon sequestration is a specific service that is required to reduce the impact of climate change. This chapter is focused on whether it is possible to use crops in a more precise and considered way to add carbon as well as attract specific microbes that slow the flow of carbon, such as fungal communities. In the field trials (Chapters 3 and 4), the focus of the research was on what happened in the soil of standing, living crops. However, it is useful to know what the plant materials offer when incorporated into the soil, and whether some cover crop species are more attractive to a microbial community than others.

5.2.1 The effect of plant material on microbes

Research carried out by Strickland et al (2019) found that cover crops increased the active microbial biomass and bioavailable carbon (C) by 64% and 37% respectively, thus improving soil health and indicating increased C sequestration. The cover crops used were all mixes with 4 variations: i) triticale, vetch, crimson clover, radish; ii) barley, crimson clover, radish; iii) rye, crimson clover, radish; iv) triticale and vetch. Apart from adding biomass, the clover can fix nitrogen, radish help break up compacted soil and trap nutrients, vetch has an extensive root system and recycle nutrients, triticale and rye sweep up nutrients and nitrogen. It concluded that species made a difference and there was a positive correlation between cover crop biomass and microbial biomass C and N (Strickland et al., 2019). The greater the biomass the lower the C:N ratio. In other research, monocultures of different grass species resulted in different and predictable soil functionality. This was linked to plant traits and their survival strategies (Orwin et al., 2010). Plant species with a high relative growth rate (RGR) were associated with plant material that had a low toughness, high nitrogen concentrations, an elevated biomass of bacteria to fungi ratio in soil, high rates of soil nitrogen mineralization and concentrations of extractable inorganic nitrogen, and to some extent, higher available phosphorus pools (Orwin et al., 2010).

Research has identified plant species that exhibit preferential colonizing of fungi for example oats have been found to enhance the presence of saprotrophic fungi which are linked to decomposition, and improve the quantity, quality and accumulation rate of the SOM. Vetch and clover attract arbuscular mycorrhizae, and yet red clover, white clover and chicory attract significantly divergent fungal communities which appear to be linked to soil nitrogen (Benitez et al. 2016; Detheridge et al., 2016; Martínez-García et al., 2018b).

5.2.2 The effect of soil on microbes

Land use intensity and soil type strongly affected the presence and quantity of microbial community composition. A model developed by Prout et al (2020) to estimate the condition of soil in terms of its Soil Organic Carbon (SOC) content, determined that 21 % of the SOC variance could be explained by land use, soil

type, annual precipitation and soil pH (Prout et al., 2020). An estimated 53% of the 61 observed AM fungal species were exclusively found in specific soil types (Oehl et al., 2010).

Soil texture has been shown to have a varied impact on microbes and may modify the response of the microbial community to vegetation. For example, fine pore structure can protect the microbes from grazers reducing the loss of the microbial pool, however the clay minerals can adsorb SOC onto its surface thereby protecting SOC from microbes, denying access to a labile carbon source (Liddle et al., 2020). Access to carbon and nitrogen within the soil will attract different microbes for example Gram- positive bacteria are found where carbon sources are more difficult to mine, and Gram-negative bacteria will dominate where carbon is easily available.

Compared to clay soil, sandy soils experience a higher rate of mineralization because of the courser structure and lack of protection (Verberne et al., 1990). Sandy soils have a pore size ranging from 6–30 µm compared to less than 0.2 µm in clay and therefore more vulnerable to erosion from exposure to water for example. A change from dry to wet conditions causes the sudden death of microbes resulting in a reduction in the carbon source for other microbes. This can result in a shift in the microbial community. The soil type together with moisture content has demonstrated its role in terms of the potential effect on microbes and the flow carbon. This combined with particular cover crop materials may add further complexity to the growth and content of a microbial pool.

5.2.3 Aims

The aim of this study was to determine the effect on the soil microbial community when two soil types, clay and sandy, were dosed with a solution of plant matter (SPM) from 7 commonly used species of cover crop. The species and key traits of each plant, and known benefits are listed in Table 5.1.

Table 5.1 List of traits and benefits associated with 7 cover crop species used in this experiment

Cover crop species	Key traits	Benefits
Berseem clover (*Trifolium alexandrinum*)	Legume Low C:N	Reduced soil erosion Add nitrogen to soil Quick breakdown Attractive to pollinators
Black oats (*Avena strigose*)	Long roots Low C:N Nutrient capture	Improve soil structure Quick breakdown Retain nutrients from erosion
Mustard (*Brassica rapa*)	Brassica Medium C:N Allelopathic potential	Biomass Biofumigation
Phacelia (*Phacelia tanacetifolia*)	High root length density Low C:N Non-legume	Improve soil structure Quick breakdown Weed suppression Attractive to pollinators
Radish *(Raphanus sativus*)	Brassica High root length density High C:N	Winter fodder Improve soil structure Slow breakdown
Turnip (*Brassica rapa olieifera)*	Brassica High root length density Improve soil structure High C:N	Winter fodder Improve soil structure Slow breakdown Biofumigation
Vetch (*Vicia villosa*)	Legume Low C:N	Nitrogen fixer Reduce soil erosion Add biomass

5.3 Method

Seven different commonly used cover crops *Trifolium alexandrinum, Avena strigose, Brassica rapa Phacelia tanacetifolia, Raphanus sativu, Brassica rapa olieifera, Vicia villosa,*) were collected from 3 farms: Sefter Farm, Bognor Regis; Rothamsted Research Farm, Woburn; and Hilley Farm, Shropshire (Figure 5.1). Due to time limitations and in order to avoid the long wait for plant material to biodegrade naturally into the soil, a solution of plant material was made with each plant. 200 g of each plant, including the leaves, stem, roots and flowers, was weighed out and chopped. The chopped plant material was mixed with with 2 litres of Milliq water in a 5 litre plastic container and shaken on a horizontal shaker at 200 rpm for 24 hours. The solution of plant material (SPM) was filtered through a soil sieve (1 x 1 mm), bottled and stored at -20^{o}C (Figure 5.2).

a)Black oats (*Avena strigose*)

b)Berseem clover (*Trifolium alexandrinum*)

c)Mustard (*Brassica rapa*)

d)Phacelia (*Phacelia tanacetifolia*)

e)Turnip (*Brassica rapa olieifera)*

f)Radish (*Raphanus sativus*)

g)Vetch (*Vicia villosa*)

Figure 5.1 Images of each of the cover crops species samples taken from a), b) Sefter Farm, Bognor Regis (17/1/2019), c), d), e) Hilley Farm, Shropshire (4/1/2019), f), g) Rothamsted Farm, Harpenden (8/1/2019).

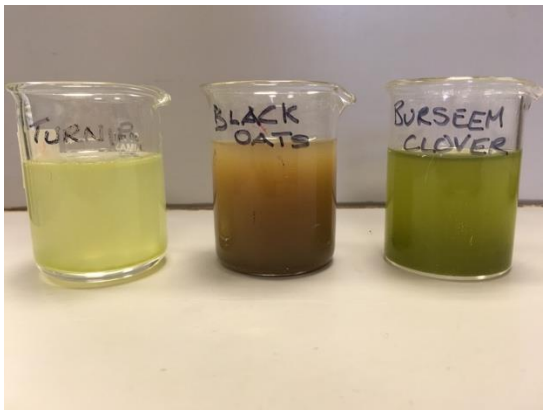


Figure 5.2 Solution of plant material (SPM) from turnips, black oats and berseem clover showing the colour variation produced by the plants.

Two soils of contrasting texture were collected - a sandy soil from Rothamsted Research institute's farm in Woburn, and a clay soil taken from Hilley Farm, Shropshire. 300 g of soil was measured into clean, dry plastic plant pots. A total of 144 pots were filled, 72 with sandy soil and 72 with clay soil.

The pots were divided into 16 trays of 9 pots each. Separately for each plant, 10 ml of SPM was dosed into 9 pots of sandy soil and 9 pots of clay soil. The control was dosed with 10 ml Milliq water. This was repeated twice over 3 weeks providing a total of 30 ml per pot. A litre of water was added to each tray at weekly intervals. The trays were placed on a bench in a shipping container (Figure 5.3). This sheltered the pots from weather, but kept them at ambient temperature. The trays were covered to exclude light preventing plant growth and to reduce the effect of airborne microbes.

Figure 5.3 Samples set out in an open fronted container. Each tray holds 9 pots with one soil type dosed with SPM from one cover crop species.

5.3.1 Microbial biomass carbon and PLFA

At 3 (July), 6 (October), and 9 (January) months, the soil from 3 pots for each cover crop species plus the control were bagged separately and stored in a -5^{o}C freezer. A subsample from each bag was taken and placed in -80^{o}C freezer in preparation for PLFA analysis.

The microbial biomass carbon was measured using fresh soil samples using the K_2SO_4 liquid fumigation method (Fierer 2003, Powlson 1976, Brookes 1982), and analysed on a Shimadzu analyser. The results from the 6 month cull gave extraordinarily high readings and for this reason the data was not used.

Phospholipid fatty acid (PLFA) was used to determine the abundance of groups of fungi and bacteria. The methodology used is described in Chapter 3. Unfortunately, because of access restrictions imposed due to COVID, and subsequent technical problems that could not be fixed in a timely manner, the 9 month PLFA samples could not be analysed.

5.3.2 Statistical analysis

ANOVA was completed on all PLFA data using GENSTAT (19.2) and GENSTAT (22.10) for the microbial biomass. There were a number of missing data for the microbial biomass carbon samples which skewed the results. Consequently, these were log10 transformed before carrying out ANOVA. Comparisons were made between plant species and soil treatments across culls for PLFA results.

5.4 Results

5.4.1 Effect of plant solutions and soil type on PLFA

The total PLFA was significantly greater in the clay soil compared to the sandy soil across all species in the 3 month sample (p. <0.001) and 6 month sample (p. <0.001), and the total PLFA μg g^{-1} were highest in the 3 month sample in both soil types compared to the 6 month samples (p. <0.001). SPM had a significant impact on total PLFA across both soil types at 3 months (p. <0.05) and 6 months (p. < 0.05) (Figure 5.4). Total PLFA showed a significant difference different in the 2 soil types, treated with the different species between the 3 and 6 month samples (p. 0.002). Where SPM was applied, the total PLFA in soil sampled in month 3, was greater in clay soils for all species except phacelia, and lower in sandy soils for all cover crop SPM compared to the control. Compared to the 3 month sample, the 6 month sample, showed a decrease of total PLFA in each of the cover crops species in the clay soil. The total PLFA in the sandy soil increased in the 6 month sample for the radish, turnip and vetch and decreased in all of the other cover crop SPM.

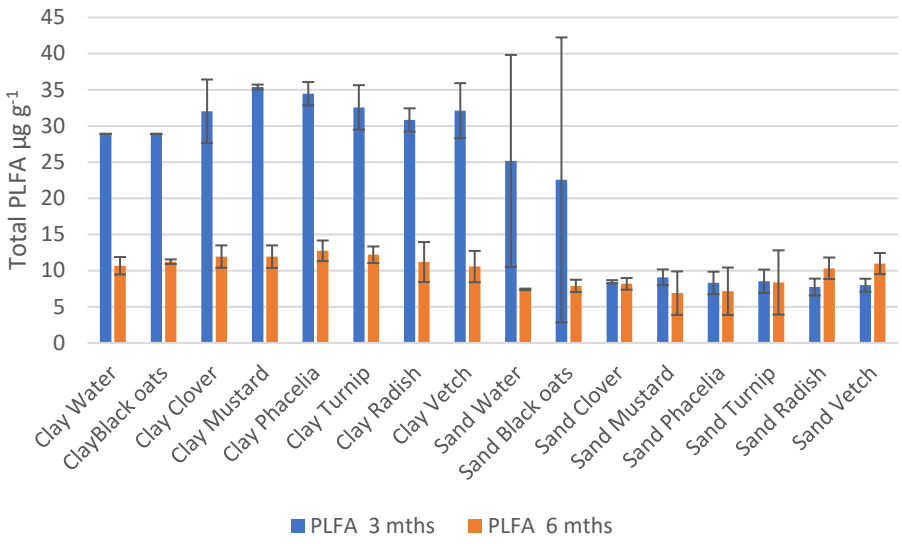


Figure 5.4 Total PLFA in clay and sandy soil following an application of SPM from 7 different species of cover crop. Clay compared to sandy soil (p. <0.001), cover crop species (p. 0.048) and a significant difference in PLFA between the soils dosed with different cover crop species in 3 and 6 months (p 0.002). (n=48).

The fungal group was significantly different between the soil types with more than double the concentration in the clay soil compared to the sandy soil in both the 3 month and 6 month samples (each p. <0.001), and 3 times more in both soil type in the 3 month sample taken in July compared to the 6 month sample taken in October (p. <0.001). The fungal group was significantly different between the cover crop species SPM in the 3 month sample only (p. 0.017), but no significant effects were observed as a result of the soil type and SPM combined. In moth 3, the mean fungal group was greatest for turnip 3.15 µg g^{-1} dry soil (4.14 µg g^{-1} dry soil in clay and 2.17 µg g^{-1} dry soil in sand), with black oats being the lowest at 1.39 µg g^{-1} dry soil (2.77 µg g^{-1} dry soil in clay and 0.0 µg g^{-1} dry soil in sand). It was noted however, that the pots dosed with water (control) gave a mean of 2.89 µg g^{-1} dry soil (3.94 µg g^{-1} dry soil in clay and 1.85 µg g^{-1} dry soil in sand). There was no significant difference in fungal group between SPM in the 6 month cull (Figure 5.5).

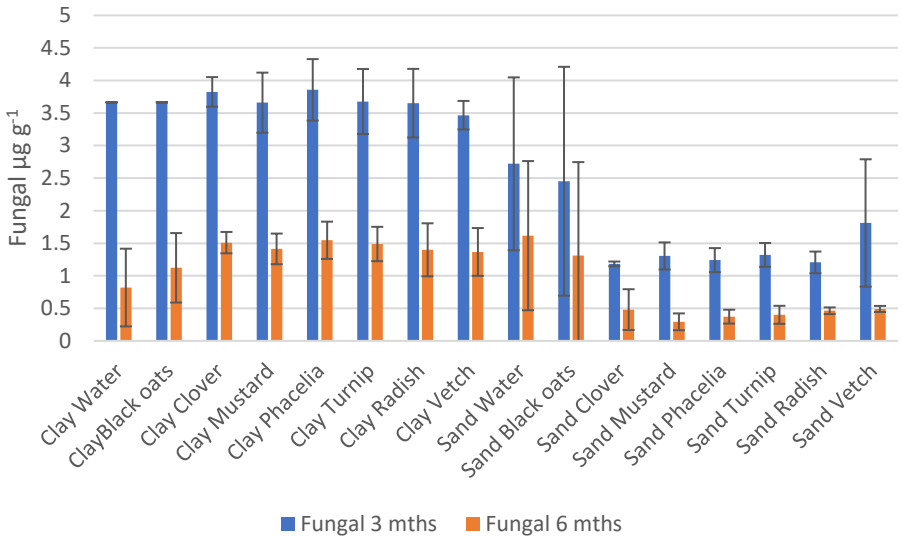


Figure 5.5 PLFA fungal group in 2 soil types treated with SPM from 7 cover crop species at 3 and 6 months. Clay compared to sandy soil p. <0.001.

The bacterial group was significantly different between the 2 soil types in both the 3 and 6 month samples (p.< 0.001). The greater amount was found in the clay soils in both the 3 month (14.29 µg g^{-1} dry soil) and 6 month (5.31 µg g^{-1} dry soil) culls but were relatively consistent in 3 and 6 month culls in the sandy soils (3.85 µg g^{-1} dry soil and 3.72 µg g^{-1} dry soil respectively, (Figure 5.6). The bacterial group was significantly different over time in the two soil types for the different cover crop species (p. 0.003). The 6 month sample showed a significant difference in the mean bacterial group between the cover crop species SPM (p. 0.019) across all soil types, and significantly different between sandy and clay soil types and cover crop species (p. 0.003). Bacterial group from the turnip SPM across both soil types in the 6 month cull averaged 5.72 µg g^{-1} dry soil, the lowest was clover at 3.93 µg g^{-1} dry soil. Across all samples at 6 months, the control dosed with water was 3.80 µg g^{-1} dry soil (Figure 5.6).

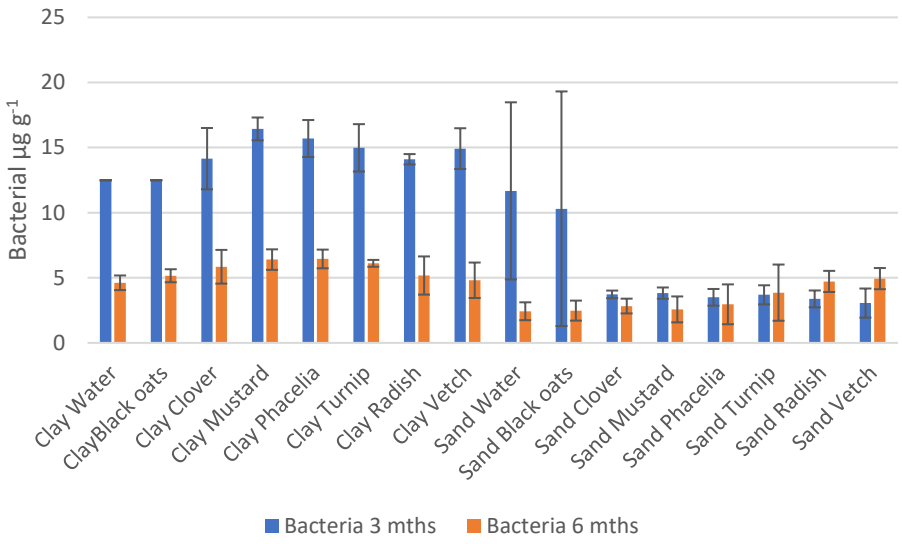


Figure 5.6 PLFA, bacterial group in 2 soil types treated with SPM from 7 cover crop species at 3 and 6 months (p. 0.003). Bacterial group was significantly different across species in the 6 month cull (p. 0.019) and across the different species in different soils (p. 0.003). (n=96).

The mean gram-negative bacterial group was significantly different between soil types, and between the 3 and 6 month sample (p. <0.001, and p. <0.001 respectively) (Figure 5.7). The different crop species SPM, in different soils across 3 and 6 months showed significantly different gram-negative values (p. 0.007). There was a significant difference in gram negative bacteria in the 6 month sample between the SPM for all soil types (p. 0.015) and between all cover crop SPM in each soil types (p.0.002). Averaged between both soil types the turnip provided the greatest overall mean gram negative bacteria at 2.655 µg g^{-1} dry soil and clover was the lowest 1.774 µg g^{-1} dry soil and water was 2.043 µg g^{-1} dry soil. For the different soil types, the highest and lowest in clay was mustard (2.727 µg g^{-1} dry soil) and black oats (2.194 µg g^{-1} dry soil), and in sand, turnip was highest (2.938 µg g^{-1} dry soil) and lowest was clover (1.354 µg g^{-1} dry soil) (Figure 5.7).

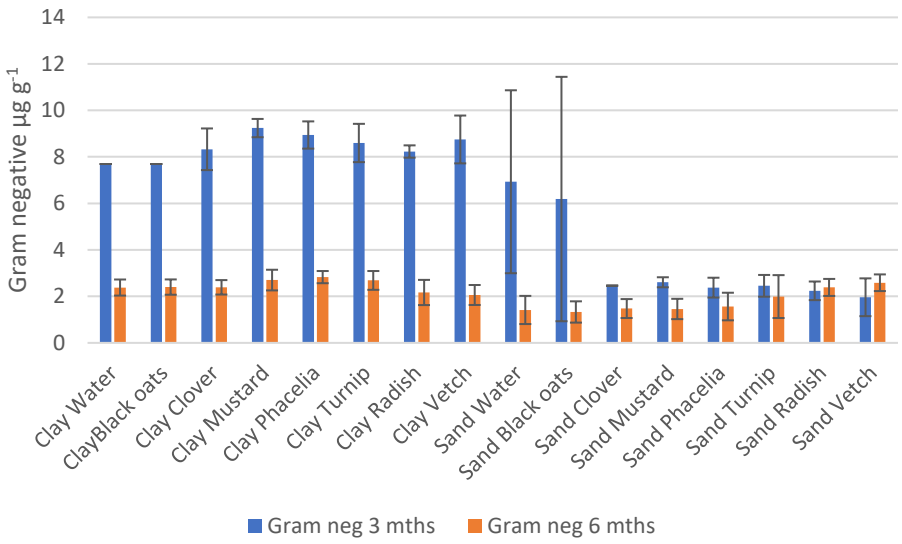


Figure 5.7 The gram-negative bacteria in 2 soil types treated with SPM from 7 cover crop species at 3 and 6 months showed a significant difference (p. 0.007). Gram-negative were significantly different across species in the 6 month cull (p. 0.015) and across the different species in different soils (p. 0.002). (n=96).

The mean gram-positive bacterial group was significantly different between soil types, and in the 3 and 6 month sample (p. <0.001, and p. <0.001 respectively) (Figure 5.8). There was a significant difference in gram positive bacteria in the 3 month and 6 month sample between the cover crop species SPM and soil types (p. 0.051 and p. 0.018 respectively). The mustard provided the greatest mean gram positive bacteria in clay in month 3 at 6.530 µg g^{-1} dry soil, and phacelia was the lowest 1.774 µg g^{-1} dry soil in clay. In sand, the greatest mean gram-positive bacteria in month 3 was black oats at 2.034 µg g^{-1} dry soil and the lowest was vetch at 0.923 µg g^{-1} dry soil. The control was 5.533 µg g^{-1} dry soil in clay and 1.460 µg g^{-1} dry soil in sand. In the 6 month sample, the turnip provided the greatest mean gram positive bacteria in clay at 3.430 µg g^{-1} dry soil and vetch was the lowest 2.172 µg g^{-1} dry soil in clay. In sand the greatest mean gram-positive bacteria in sand in month 6 was vetch at 2.451 µg g^{-1} dry soil and the lowest was mustard at 1.156 µg g^{-1} dry soil. The control was 2.553 µg g^{-1} dry soil in clay and 0.909 µg g^{-1} dry soil in sand.

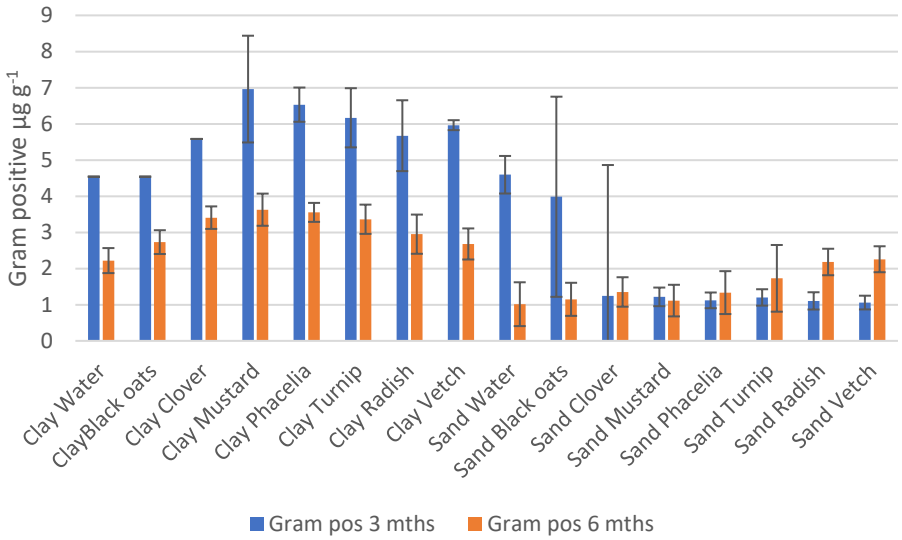


Figure 5.8 The gram positive bacterial group in 2 soil types treated with SPM from 7 cover crop species at 3 and 6 months showed a significant difference (p. 0.003). Gram positive bacterial group was significantly different across species in the 3 and 6 month cull across the different species in different soils (p. 0.051, se = 0.361 and p. 0.018 , se = 0.323 respectively). (n=96).

5.4.2 Effect of soil and SPM on microbial biomass carbon

Clay soil had a significantly higher microbial biomass carbon than sand (p. 0.046). The microbial biomass was not significantly affected by SPM from any species, and there was no significant interaction between species and soil type (Figure 5.9).

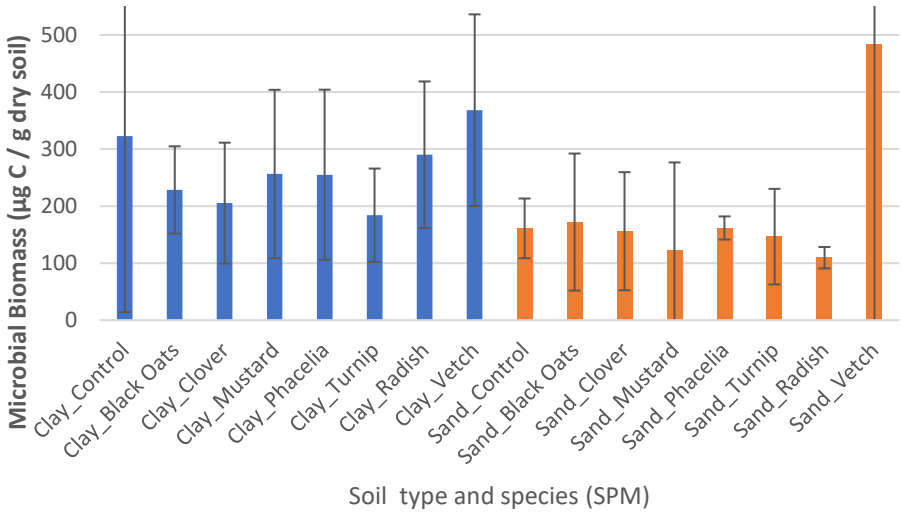


Figure 5.9 Average microbial biomass carbon in 2 types of soil, clay and sand, treated with seven different species of plant derived SPM at 3 months (p. = 0.046). (n=48).

At 9 months, there was significantly more microbial biomass carbon present in the clay soil compared to the sandy soil (p = <0.001). The species alone had no significant effect on the microbial biomass carbon however, the combination of soil type and the SPM showed a significant difference (p = 0.044). In clay, the mustard SPM gave the greatest microbial biomass carbon, and radish gave highest result in sandy soil (Figure **5.10**).

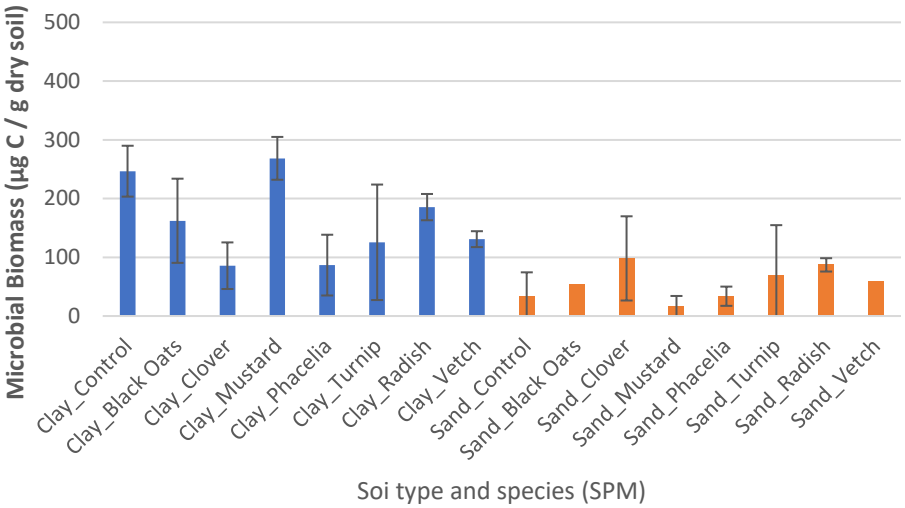


Figure 5.10 Average microbial biomass carbon in 2 types of soil, clay and sand, treated with seven different species of SPM at 9 months (p. = <0.001 between soil types, p.= 0.044 between soil and species). (n=48).

5.5 Discussion

The type of soil, and the period of time the SPM was left in the soil presented the greatest effect on the microbial community rather than the species of cover crop. Across all samples, clay had more than double the amount of PLFA than the sandy soil. This supports findings that clay soil has a greater capacity to preserve biomass and hold a greater proportion of microbial decay products (Berg, 2009; Marschner et al., 2001.; van Veen et al., 1985a). As discussed in Chapter 2, soil organic carbon, is strongly and positively linked to the clay content (Prout et al., 2020; Lal, Kimble, Follett, 1996). Availability of labile C is positively associated with soil clay content (Setia et al., 2012). Some plant tissues contain more labile C than others and this is linked to the soil C:N ratio. When SPM is added to an existing pool of micro-organisms they rapidly exploit any labile C offered. Once consumed, the recalcitrant C is mined which is associated with gram-positive bacteria. This is reflected in the PLFA results which show a peak of activity occurring in the 3 month sample in clay and for the

same species (e.g.turnip and radish), there was a peak in 6 months in sandy soil. More frequent sampling between the point of dosing through to 9 months would have offered an opportunity to present a distribution curve of bacterial groups over time by species for each soil type. Results indicate that each plant is associated with a specific microbial community (Berg, 2009; Marschner et al., 2001), and that plant traits such as extensive root mass (e.g.vetch), large biomass (e.g. Phacelia), woody stems (e.g. Mustard) (Orwin et al., 2010), in different types of soil, have an effect on the microbial community.

The species of plant had an impact on microbial community composition and there was an interaction with soil type. For example, SPM from turnips gave the greatest fungal group of PLFA biomarkers. Cover crop species can offer different ratios of C:N and will attract different types of bacteria(Khan et al., 2007; Orwin et al., 2010). Microbial and fungal biomass are positively linked to low leaf and litter quality (e.g. high toughness) and strongly and negatively related to nitrogen in leaf litter (Orwin et al., 2010). Turnips (and radish) capture residual nitrogen and have a high C:N ratio, therefore carbon rich, and release nutrients slowly, which is attractive to microbes (Mäkelä et al., 2011).

Total bacteria and gram-negative bacteria groups showed significant differences between crop species in the 6 month cull. Turnip gave highest presence of the gram-negative group, and clover the lowest averaged across all soil types. In clay soil gram-negative bacteria were highest with SMP of mustard, and lowest with radish SPM, and in sandy soil the highest was turnip SPM and lowest with clover SPM. Gram-negative bacteria are more abundant where leaf litter is high and a carbon source is readily available (Finney et al., 2017; Maul et al., 2014). Clover and vetch will fix nitrogen where soil is lacking nitrogen (Oldroyd, 2013; Oldroyd et al., 2011). It would have been expected therefore, that these legume species, that have a low C:N, would have broken down most readily and attracted a greater abundance of gram-negative bacteria earlier on than the turnips, radish and mustard (all from the mustard family). The results seem to indicate that the crop with the high C:N provided for the Gram- negative bacterial group for longer such as the turnip in sandy soil, in comparison to the low C:N of clover in sandy soil which would have been consumed more readily. However, in the microbially rich clay soils, the radish, which has a high C:N ratio, showed a comparatively low presence of the Gram-negative group. Other lower C:N species had more of the Gram-negative group in clay. It appears that

there is a shift in the microbial community over time and that time period is linked to soil type and availability of carbon and nitrogen.

Gram-positive bacteria averaged across all soil types were significantly different between species. It was most abundant with an application of mustard SPM (3.901 µg g^{-1} dry soil), and lowest with the phacelia SPM (2.921 µg g^{-1} dry soil). This result was different however if the soil type was considered. In clay the most abundant gram-positive bacteria was found in mustard SPM and the lowest in phacelia whereas in sandy soil, the gram-positive group was most abundant in black oats and lowest in vetch. Gram-positive bacteria are more abundant where nutrients are low and carbon is not so readily available (Gurevitch et al., n.d.; Maul et al., 2014). Mustard attracts Gram- positive bacterial group as it comprises and has a low nutrient value, (Gurevitch et al., n.d.; Orwin et al., 2010). Oats have been found to enhance saprophytic fungi (Benitez et al., 2016), although this was not evident in this experiment.

Not all SPM from cover crop species provided a more abundant microbial community than in the control (soil dosed with water). Phacelia in clay gave a lower total PLFA than the control in month 3. Within the total PLFA of the Phacelia SPM, the fungal, bacterial and gram-negative functional groups were all comparatively lower than the water control, however the gram-positive group was higher. The phacelia had been subjected to frost before it was collected from a field, and this may have contributed to the low results. Frost can cause the plant cell membrane to burst and lose nutrients (Inouye, 2000). The tissues may have partially degraded before being made into a SPM and dosed into the soil, and therefore been quickly exploited by microbes in the clay.

5.6 Limitations

The use of plant material solutions in a pot experiment is unlikely to provide a realistic picture of what would have been observed in the field. If time allowed it would have been more realistic to have grown the cover crops in pots under constant conditions and then allowed them to naturally decompose. Not only would this have given decomposition under realistic conditions and include all of the components of the plant material, but by completing the experiment with a living plant, the benefits associated with the presence of

decomposing roots in the rhizosphere could be observed. The microbial community may have been different to that observed with the plant material in a solution.

The soils were sourced from different farms that were subjected to a history of different agricultural management techniques which can affect the soil structure and organic matter (Poulton et al., 2018; Powlson et al., 2014). However, unfortunately a full history was not available for all of the sites. Further measurement of the chemical and physical properties of the soil may have helped to understand the baseline soil conditions. There will also have been impacts due to abiotic conditions such as temperature and humidity, with conditions in the pots differing from those in the field. Furthermore, although the pots were covered, there was likely to have been some exposure to airbourne microbes over the 12 months.

5.7 Conclusions

Plant species of cover crop is likely to influence structure and functionality of microbial communities. These impacts will be modified by soil type with clay soil retaining a larger microbial community than sandy soil. While there were many limitations to this experiment the results indicate the importance of the cover crop plant species for determining impacts on the microbial community and indicate the need for further research in this area. Further work needs to be completed to understand the changes in the microbial community during the decomposition stage as part of an agricultural rotation.

6 Farmers survey on the use of cover crops

Agriculture is undergoing a second green revolution as described in Chapters 1 and 2. Priorities are shifting away from greater yields at any cost, towards a more compassionate system that invests in the natural environment and provision of nutritious food (A Green Future: Our 25 Year Plan to Improve the Environment Annex 3, 2018). Methods that enrich agricultural land, including the use of cover crops are being taken more seriously but it is questionable whether the roll-out will be fast enough to make the serious changes needed to achieve those goals(HM Government, 2021).

The use of cover crops in the UK has not been recorded on a regular basis since 2015. A total of 2395 farm holdings responded to the 2014/15 DEFRA survey on the land managed voluntarily under the campaign for the farmed environment (DEFRA, 2015). In keeping with this research, cover crops include winter cover crops, brassica fodder crop and over winter stubble. The last recorded data in 2014/15 showed a total of 99,305 ha under cover crops however, this was a drop from 158,426 ha the year before. The most recent UK survey regarding cover crop use was carried out in 2017. Of 117 respondents 66 % used cover crops in 2016(Storr et al., 2019).

Table 6.1 Area of land in England under cover crops 2013 to 2015

			Total ha	
Voluntary Measure	**2013**	**2014**	**2015**	**2014/15 change**
Winter cover crop	22,543	28,472	26,454	-2,018
Brassica fodder crop	15,045	21,171	14,781	-6,390
Over winter stubbles	265,697	108,783	58,070	-50,713
Total	303,285	158,426	99,305	-59,121

If this is compared to the experience in the US, the Conservation Information Technology Centre (CITE) completed 6 surveys on cover crop use since 2012 on behalf of Sustainable Agriculture Research and Education (SARE) program (Table 6.2). The number of respondents increased by 26 % since 2013, with the average number of acres of cover crops grown per respondent increasing by 53 % (Wallander et al., 2021).

Table 6.2 The average area planted with cover crops per respondent from 2013 to 2019 in the US (http://CITE.org)

Year	2013	2015	2016	2017	2018	2019	Change since 2013
Respondents	750	837	856	914	936	950	+200
Average acres planted	303	337	359	390	422	465	+162

Results from the 2019/20 survey included respondents that did not use cover crops. 70 % of non-users considered using cover crops. Barriers included not owning the land and a reluctance to invest, or a belief that the owner would not give permission. Some early adopters of cover crops became disillusioned following negative results or a difficult experience and gave up (Dunn et al., 2016) .

Dunn et al. (2016) suggest that farmers that take up cover crops or any new idea (early adopters) have similar personality characteristics. They are motivated and have the ability to process information and data (Blackstock et al., 2010), tend to rely on self-learning, and are able to deal with a high level of uncertainty (Dunn et al., 2016). The behaviour of farmers can be influenced by using legal or policy instruments, financial incentives, advice and voluntary collective action (Blackstock et al. 2010), but successful long-term behaviour has been linked to the uptake of new ideas on a voluntary basis (Ayer et al 2018).

With the introduction of the Environmental Land Management Scheme (ELMS) to the UK, there will be policies and financial incentives that look to encourage the use of cover crops. There will be a need for advisors who can help break down the barriers to implementation and provide guidance on new and developing management practices to get the most from cover crops.

A key element of ELMS is the provision of advice and technical guidance. Current thinking on how advice is to be given includes:

- Provision of 1:1 advice and support direct to land managers
- Group advice and training
- Telephone and online support

- Facilitation of peer-to-peer learning

The aim of this survey is to get an up to date understanding from the farmers' perspective, of the benefits and pit falls of using cover crops, and why some farmers choose not to use them. It is also important to understand how scientific research on the use of cover crops can be communicated to farmers and growers at a practical level. Science can help to assure farmers in the decision-making process, filling gaps where there are misunderstandings or potential barriers, however the information needs to be effectively communicated.

6.1 Method

The survey was aimed at UK farmers that predominantly grow crops. It was compiled and disseminated in 2020. The survey was designed to be accessible to as wide an audience as possible, notwithstanding restrictions and limitations of the Covid 19 pandemic. Google forms, a digital platform, was used to create the questionnaire which was accessible to anyone with a computer or digital device. This method was inexpensive and one that was familiar to many farmers, particularly this year when most communications and transactions are commonly completed online. Links to the form were provided for dissemination to agronomists, farming groups, the NFU and via communication links at Rothamsted Research and Lancaster University.

The questionnaire was scrutinized and approved by the Faculty of Science and Technology Research Ethics Committee at Lancaster University. Information was provided to potential respondents at the outset about the survey, reasons for doing it and about the author. It was made clear that all data would be made secure, and no personal details would be kept. An email address was given by those that chose to take up the offer to receive a copy of the summary of the survey on completion, with the assurance that those details would be deleted once the report was sent. All respondents had to give consent to continuing with the questionnaire. All respondents were expected to complete the first section of the survey which was to identify the farm type and location. They were also asked if they had ever used cover crops on their farm. This determined the direction of the questionnaire which split into either a section "About your cover crops" or "Barriers to the use of cover crops". Finally, all respondents were directed to the last section which was to find out how and where they source scientific and technical information (Figure 6.1).

USE OF COVER CROPS

SECTION 1: About your farm

Where do you farm – county?

Type of farm?

Have you ever used cover crops on your farm?

YES

SECTION 2: ANSWER YES

How long have you used cover crops?

What cover crops are you using (tick all that apply)?

Why do you use cover crops?

How do you prepare for the next cash crop

How did you first find out about cover crops

Is there a downside to using cover crops and if so what?

NO

SECTION 2: ANSWER NO

Do you feel you know plenty about the use of cover crops?

If you had more evidence about the benefits and use of cover crops, how likely are you to give them a go?

If you were financially rewarded to increase the carbon in your soil would you use cover crops as a first choice solution?

If there's anything further you would like to say about why you don't use cover crops or have specific things you would like to know, please write below.

SECTION 3: Sources of information - science and technology in agriculture ?

Which of the following do you regularly use to find out latest farming ideas? Tick any that are relevant.

Are you aware of the target set in the Paris Agreement to increase the soil organic matter by 0.4% per year?

On a scale of 1 to 10 how likely are you to implement new agricultural techniques to increase the organic matter content in your soil?

Figure 6.1 Summary version of the questions included in the survey.

The data was collated and exported into excel. For display purposes, the counties were grouped into countries - England, Scotland, Wales and Northern Ireland. The species of cover crops were combined according to the number of times it was mentioned either as a tick box, or part of a description of a mix. The survey was carried out over a 3 month period from October to December 2020.

6.2 Results

A total of 53 completed responses were received. Of those, 52 farmed in England, predominantly the Midlands and 1 from Scotland. Figure 6.2 shows the breakdown by farm type and the regional location of the farm represented. Only 3 respondents said that they had never used cover crop. (Figure 6.3).

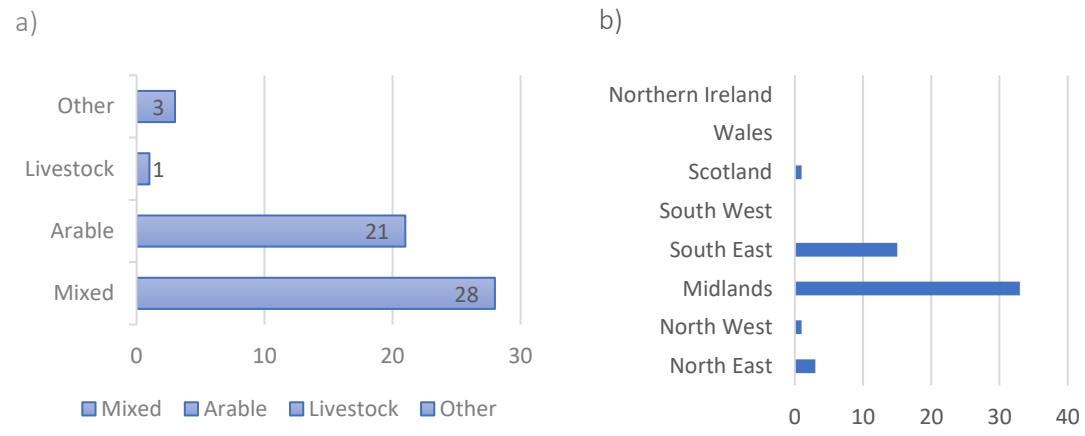


Figure 6.2 The division of respondents by a) type of farm and b) geographical location, bars show the total number of respondents for each category (n=53).

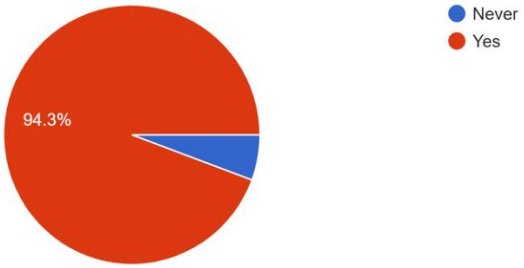


Figure 6.3 The proportion of respondents that have used or never used cover crops (n=53).

Of the 50 that had used cover crops, 78 % of them had used cover crops for more than 3 years (Figure 6.4). Several reasons were given for using cover crops but 90 % said it was winter cover, and 88 % used them to increase soil organic matter and improve soil structure. Only 1 respondent said the reason for using them was to increase the yield of the follow-on crop (Figure 6.5).

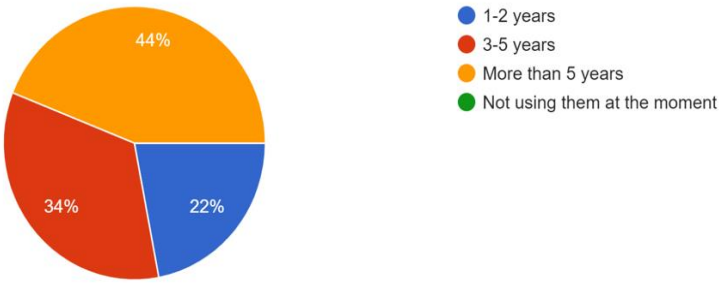


Figure 6.4 The proportion of farmers that have been using cover crops for 1-2 years, 3-5 years, more than 5 years or not currently using them (n=50).

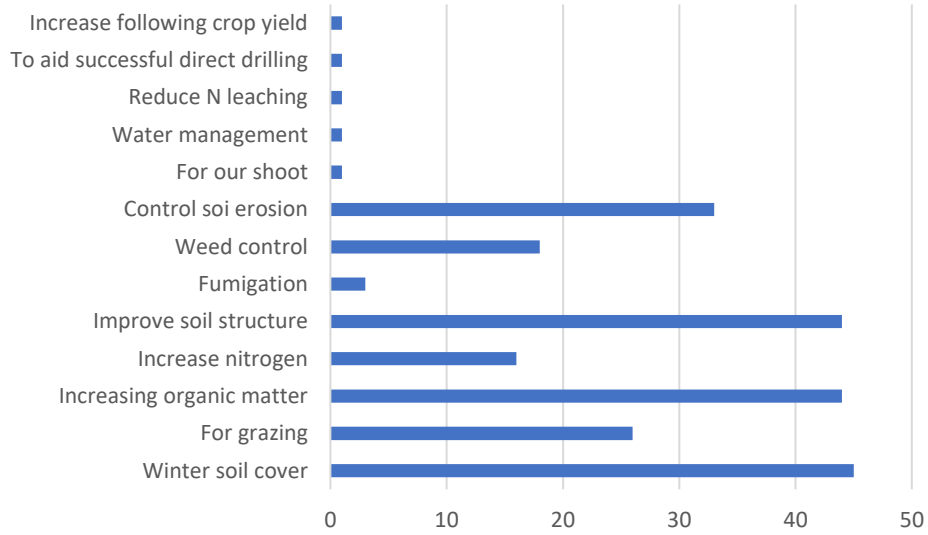


Figure 6.5 Reasons given for the use of cover crops, bars show the total number of respondents selecting each option. More than one reason could be chosen (n=50).

The number of cover crop species being used is limited (Figure 6.6). Many (40 %), use a mix suggested by their agronomist. Phacelia has been used by 78 % of respondents. One or two respondents have used more unusual species such as a mix for bird seeds, sunflowers, quinoa, lupins, and camelina.

The destruction of the cover crop is approached in various ways, but the majority (66 %) use the herbicide, glyphosate to desiccate the crop (Figure 6.7). This is then followed by either direct drilling of the cash crop, ploughing in or minimum till. The alternative methods of destruction are to roll or graze the crop. Some respondents would use different methods depending on the circumstances. If frost had caused senescence for example, rolling would be used in place of spraying with herbicide.

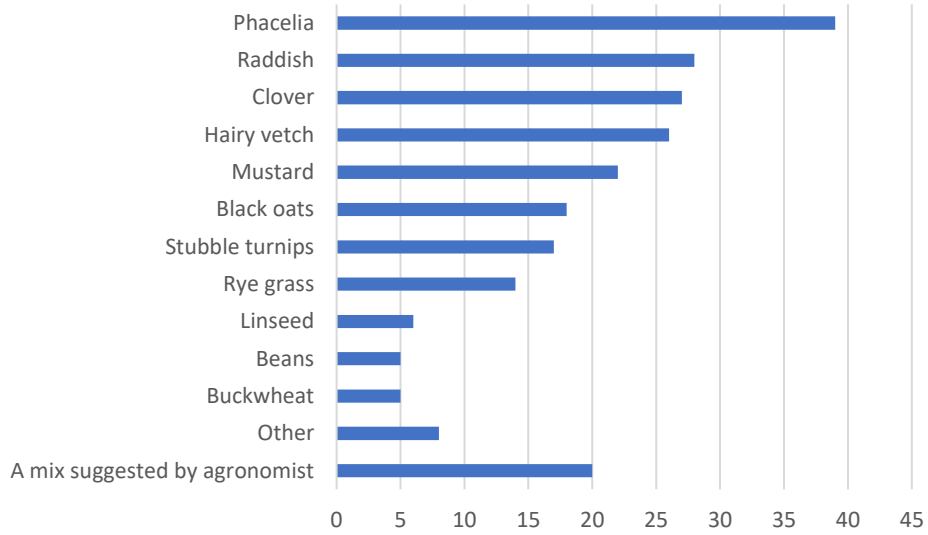


Figure 6.6 The species of cover crops used by respondents. Bars show the total number of respondents selecting this option. More than one could be identified. (n=50).

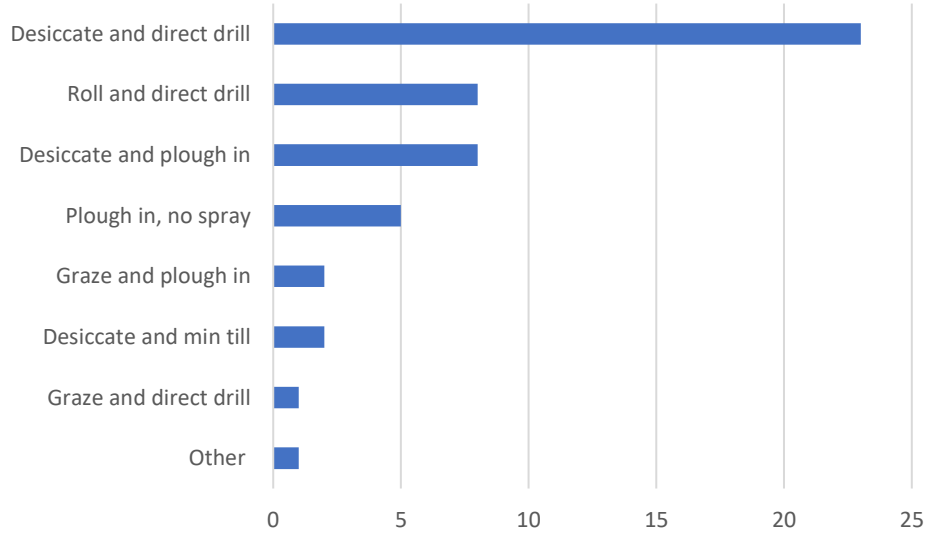


Figure 6.7 Methods used by respondents for destroying the cover crop (n=50).

When asked if there was a downside to using cover crops, 43 of the 50 cover crop users responded and of those, 4 said there were no problems using cover crops (Figure 6.8). The concerns they listed were placed into categories. The biggest downside was the cost with 16 (41 %), out of the 39 respondents considered it a downside. Eleven (28 %) respondents mentioned the problem of slugs and one mentioned disease in the following crop. The issue of ground not drying out in spring was mentioned several times and this was in connection with either the weather being wet, cover crop density protecting the ground from wind or sun, or late timing on desiccation.

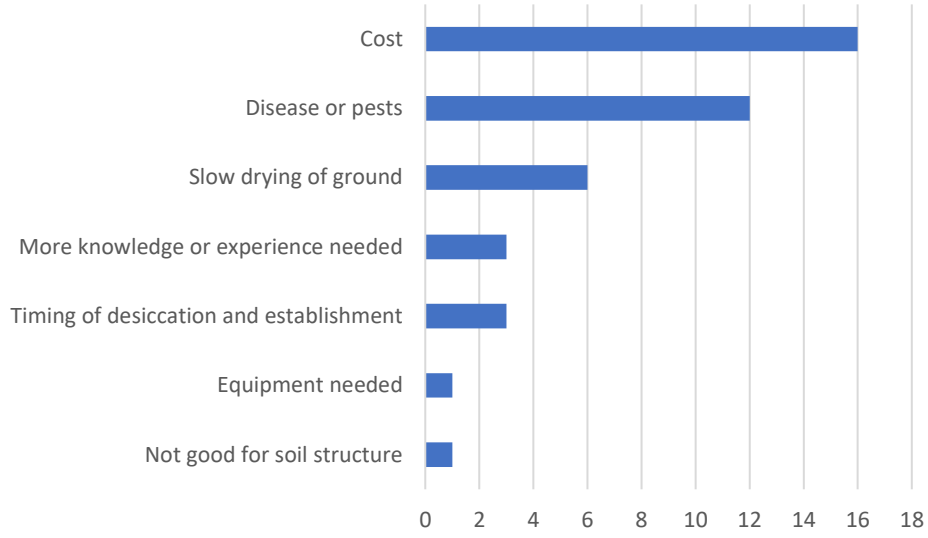


Figure 6.8 The downside of using cover crops identified by respondents (n=39). More than one reason could be selected. Bars show the total number of respondents for each category

Three of the respondents had never used cover crops. Two out of the 3 said that they do not know which cover crops to use, that they heard that they create a problem to future cropping and it did not fit in with their rotations.

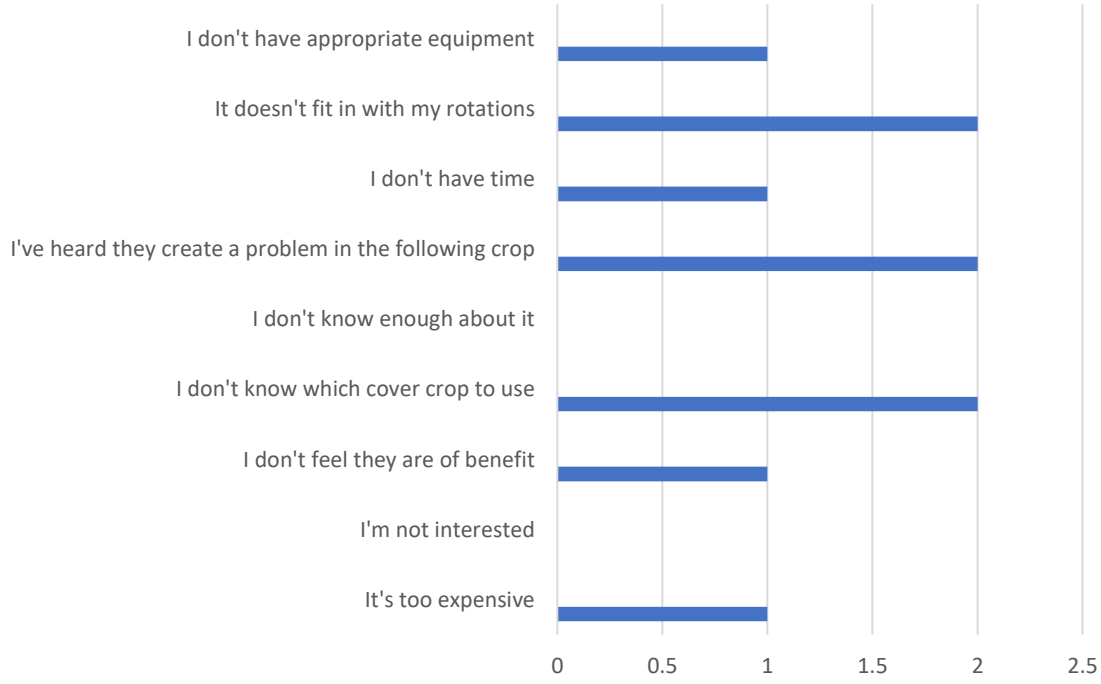


Figure 6.9 Reasons selected for not using cover crops. Bars show the total number of respondents for each category. All that applied could be selected (n=3)

Expense, lack of equipment, time and unsure about the benefits were other reasons suggested for not using a cover crop. None of the respondents said that they were not interested in cover crops or did not know enough about it. When asked if they would consider using cover crops if offered a financial incentive, all of them said “maybe”.

When asked about how they found out about cover crops, the majority (50 %) found out via, agricultural events they attended (28 %), word of mouth from family, friends or neighbours (22 %). The rest found out through farming magazines (18 %) or their agronomist (14 %), and the remaining responses were spread across media such podcasts, you tube, TV, and part of training or education. 6 % could not remember (Figure 6.10).

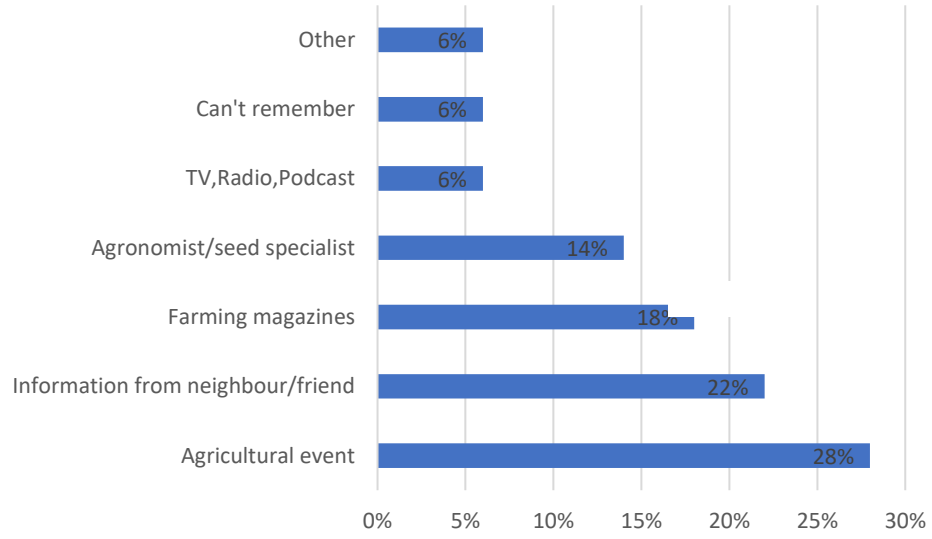


Figure 6.10 A ranked bar chart to show the main source of information used to find out about cover crops. 50 respondents selected the key sources used. (n=50).

All 53 respondents shared the range of media that they refer to for updates on new agricultural techniques and technology. 70 % used advice provided by their agronomist, agricultural events or from specialist invitation events. Farming magazines, the internet, farming groups, family and friends are also considered highly where information is passed on (Figure 6.11).

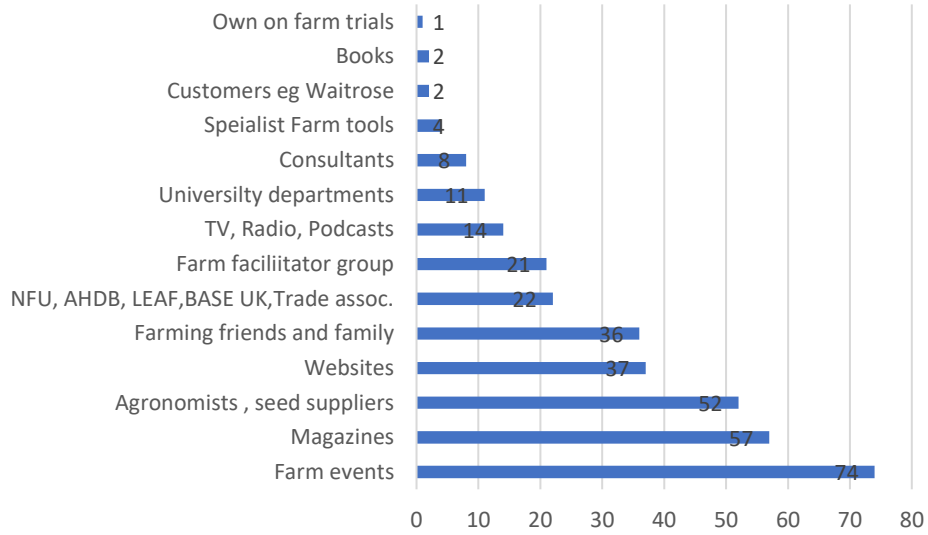


Figure 6.11 Common routes to finding new agricultural techniques and technology. Bars show the total number of respondents for each category, more than one could be selected (n=50)

6.3 Discussion

The majority of respondents were adopters of cover crops. It cannot be assumed that this is a proportional representation of cover crop use in the UK agricultural sector. It is more likely a reflection of the design of the survey. The title of the survey "The use of cover crops" possibly led to a bias in the type of farmers that responded - farmers that used cover crops. It would have been useful to have engaged with more farmers that do not use cover crops to get a better understanding of the barriers.

The number of responses was lower than expected. This could have been a result of poor timing. It was carried out at the end of 2020, during the pandemic so the dissemination via electronic media may not have achieved the reach it may have done if not constrained by lock down rules. Also, farmers were subject to several online surveys from researchers, and it is suspected that survey fatigue had set in.

In 2017 a survey was carried out on the use of cover crops (Storr et al., 2019). At that time 75 % of respondents had 5 years or less experience of cover crops. This was considered to support the general trend for increased interest in the use of cover crops. In this survey 94 % had used cover crops of which 44% for more than 5 years (Figure 6.4). The trend for using and continuing to use them appears to be growing. Comparatively, the use of cover crops in France and the United States (US) is more advanced than in the UK. In

the US, uptake has been further encouraged by the setting up of the Sustainable Agriculture and Research Education (SARE) program in the 1990s (Groff, 2015b).

In the survey used for this research, the range of crop species used was limited to around 11, with Phacelia (*Phacelia tanacetifolia*) being most commonly used (Figure 6.6). If this is compared to practices in the US where the use of cover crops was adopted many years before the UK started, 63 % use a cover crop mix and the popular species include, in order of popularity, radish(*Raphanus sativus*), cereal rye (*Secale cereale*), oats (*Avena sativa*) as one mix, and crimson clover (*Trofolium incanartum*) and turnip (Brassica rapa) in another mix (SARE, 2020).

It is noticeable that the crops species used by farmers in this (UK) survey are generally those that have been offered by agronomists or meet the criteria of greening under the basic payment scheme. In a brochure provided by Agrovista (Cover crops, key mixtures and straights), the crops offered included mustard (*Brassica rapa*), red clover (*Trifolium pratense*), rye (*Sacale cereale*), rye grass (*Lolium multiflorum*), tillage radish, black oat (*Avena strogosa*), vetch (*Vicia villosa*) and berseem clover (*Trifolium alexandrinum*) (Agrovista, 2019). These were chosen based on research carried out by Agrovista at their Lamport field trial site. In 2019, the Agrovista brochure had little information regarding set mixes of species however, the 2021 brochure identifies 7 mixes alongside their main ecosystem services. The mixes are predominantly focused on the control of grassweeds. Whilst this is an improvement, there is still a long way to go in understanding what services can be offered by individual species and species working together as a mix. Similarly, AHDB (a commonly used source of agricultural information) provided a report in 2016 listing similar species to Agrovista and information on the benefits they provided (White, Holmes, and Morris 2016).

All respondents had more than one good reason for using cover crops. The top reasons cited included over winter cover, reduced soil erosion, increased organic matter and improved soil structure. These examples align with the results found in the 2017 survey (Storr et al., 2019) and the annual SARE report (SARE, 2020). These benefits however, do not appear to be enough to persuade others to take up and continue to use cover crops (Table 6.1). The biggest barrier for not using cover crops is cost and this was also cited as one of the draw backs of using them. The financial benefits have been presented by farmer influencers such as Gabe

Brown from the USA, in terms of the increase in yield of the follow on crop and reduced cost of chemical inputs such as fertilizer, pest and weed control. Research evidence however is limited, possibly due to time it takes to measure a change. It is indeed considered a financial risk to buy seed, establish it and later destroy it without an immediate financial reward. Seed alone costs between £15-60/ha, with drilling between £15-40/ha (White, Holmes, & Morris, 2016), €144/ha (£124/ha) in total in Europe (Commission & Environment, 2019) . Across Europe, adopters of cover crops estimated fertilizer costs were reduced by 6.6% for the following crop. Despite quantitative proof of benefits, the financial returns are more difficult to evaluate for the reduced erosion, improved organic matter and increased yield (White, Holmes, & Morris, 2016).
A number of respondents expressed difficulties with cover crops including increased slugs and wet ground in spring. Many farmers are still learning how to manage cover crops. When asked for further comment on managing cover crops, one farmer added that cover crops should be managed in the same way as cash crops, meaning the same care and attentions was required as that given to a cash crop.

Of some concern is the reliance of most farmers to spray off the cover crop with glyphosate. There will be cases where, in the same year, cereals are treated with glyphosate and then again to desiccate the cover crop. Glyphosate is a herbicide used all over the world. Concerns were raised over its links to human cancer following a report published by the International Agency for Research on Cancer (World Health Organisation, 2015). It has also been linked to the decline of insect populations and causing damage to ecosystems (Benamú et al., 2010; Druille et al., 2013; Hagner et al., 2019). The increased use of the herbicide has been blamed in part, on the resistance developed by some plants to the active ingredient (Myers et al., 2016). This is compounded further by the double dose in some rotations where a cover crop is used (Chapter 2).

In the context of climate change, agricultural practices have been shown to be a significant source of emissions (A Green Future: Our 25 Year Plan to Improve the Environment Annex 3, 2018; Hallsworth & Thomson, 2010; Office for Science, 2011), in particular the application chemical fertilizers (Abdalla et al., 2019). One of the main benefits of cover crops is the use of certain species to fix nitrogen or catch soil nutrients preventing or reducing leaching into water courses (Couëdel, Alletto, Tribouillois, et al., 2018; Johnson et al., 2015; Kaye & Quemada, 2017; White, Holmes, Morris, et al., 2016). It was surprising therefore, that the reference to this as a benefit was not as important as other benefits. Adjusting the nitrogen levels in soil through strategic

planting of nitrogen fixing plants does not appear to be considered as a key service. In addition, the inclusion of nitrogen fixing species in a field that have high nitrogen levels is potentially unproductive and a lost opportunity for alternative planting.

6.3.1 Knowledge transfer

The Campaign for the farmed environment – survey of land managed voluntarily in the 2014/15 farming year (DEFRA, 2020) showed a drop in land coverage of cover crops. Considering that the majority of respondents of this survey found out about cover crops from farm events or their agronomist, suggests that the suppliers of seed and equipment are amongst the best placed to transfer knowledge. In the US, SARE plays an important role in gathering and disseminating information. The latest survey included data from non-users of cover crops. It highlighted that farmers who gave up using cover crops was down to a negative experience. Assistance and transfer of knowledge at the outset may help to improve a long term or permanent change of behaviour (Blackstock et al., 2010; Dunn et al., 2016). A survey carried out in Iowa, USA looked at the barriers to cover crop use by setting up four focus groups of farmers who discussed the challenges and barriers. The focus group has since enabled many of the participants to find solutions through the group discussions and net working with farmer groups (Roesch-Mcnally et al., 2021). There are also several high-profile farmer influencers that speak at conferences and provide on-line information through videos such as Gabe Brown from the US, and in the UK, John and Paul Cherry who started the farming event “Groundswell” five years ago. In a survey carried out across Europe (Spain, France, Netherlands and Romania) on the technology and management options for the use of cover and catch crops, it stated that most adopters of cover crops did so on the basis that there are policies that made it mandatory (EU Commission Adoption of cover crops for climate change) (Smit 2019).

6.4 Conclusions

Farmers that are growing cover crops have embraced it year on year with many having used them for 3 or more years. They also have a good understanding of the benefits of the cover crops they use. Respondents showed determination and long-term dedication to improve soil health and, by doing so, positively contributing to Government’s net zero carbon targets.

The survey identified some key problems in the use, application, and management of cover crops. It is still a relatively new practice in the UK and educational and technological gaps need to be addressed to encourage continued use. The financial risk of taking up this practice is a concern to both users and those not using cover crops. Further proof of the financial benefits would go a long way to overcome current barriers.

There is room for improved communication on the use of cover crops and a need for a deeper understanding of the biological benefits. The UK can develop policies and incentivize cover crop use through the recently passed Agricultural Act 2020 (Coe & Finlay, 2020). In June 2021, Defra published standards for arable land which incentivizes the use of cover crops and is paid by area of coverage and time left down. There is an urgent need for the transfer of some of the scientific information about the benefits of cover crop species and the best way to manage them to get the best results.

7 Discussion

Farmers are under increasing pressure to embrace sustainable farming for the future and improve the organic richness of agricultural soils (Defra 2018; 2022). Policy driven incentives are being offered to use cover crops (Defra, 2021) however, as this is an added cost to the farmer, the benefits need to be realized, and information on how best to use the cover crop is vital if changes are to be made (Storr, et al 2019). This research set out to understand the contribution of cover crops on carbon cycling, and the effects on soil when used alongside other commonly used farming practices including weed clearance using glyphosate, and biological inoculants to stimulate growth. A cover crop can be planted in between cash crops, covering the land over the winter months, or as part of a rotation for up to a year. The effect of cover crops on carbon cycling, was considered from i) a whole standing crop, and ii) with respect to the cover crop species and legacy of different plant material in the soils. The standing cover crop was trialed in-field with the additional treatments of glyphosate and an inoculant (Symbio Mycoforce). To investigate whether plant species is important, plant materials were tested in pots of clay and sandy soils.

7.1 Contribution of cover crops to soil health

Cover crops have an important role to play in agriculture. Adding carbon back into soil has become increasingly important to reduce the impact of global warming and secure a global food supply. There is plenty of evidence demonstrating the multiple benefits of cover crops including the reduction of soil erosion(García-González et al., 2018), weed suppression (Kunz et al. 2016; Florence et al. 2019; Brennan et al. 2020) the capture of nutrients and nitrogen, and the supply of biomass (Couëdel et al. 2018; Kaye and Quemada 2017; Finney et al 2016). In this context, sequestering carbon from the atmosphere and converting it into sugars for further use by animals and soil biota, is a valuable ecosystem service that fits in with current agricultural policy.

For this research, the flow of carbon was measured in terms of the changes made in soil organic matter (SOM) and the microbial composition of the soil. Such changes gave an indication of the direction of flow of carbon (addition or depletion) to the soil. This research showed that by establishing a cover crop over a 3 year period,

there were no significant differences in the amount of SOM, of which 50 % to 58 % is soil organic carbon (SOC) (Pribyl, 2010). However, there was a significant and positive difference in the growth of a microbial community in a cover crop plot compared to fallow plots, measured by the total PLFA. The total PLFA was more than double in plots treated with just a cover crop, and where the plot had been treated with cover crop plus glyphosate. Cover crops provide a rich root system vital for many bacteria and fungi that rely on plants for their source of energy in the form of sugars. Compared to leaving soil fallow, cover crops provided a significantly greater abundance of total PLFA (24.6 μg g^{-1} dry soil, 39.8 μg g^{-1} dry soil, respectively), particularly the bacterial functional group (10.72 μg g^{-1} dry soil, 17.05 μg g^{-1} dry soil, respectively). However, the difference was not found to be significant until two years on from establishment. In her research, Finney (2017) found, significant results in a standing cover crop within months of establishment in Central Pennsylvania, US.

The stabilization of carbon in aggregates is associated with i) the presence of arbuscular mycorrhizae (AM) (W. Wang et al., 2017; Wright & Upadhyaya, 1998), ii) a high fungal: bacteria ratio, and iii) a high C:N ratio (Jenkinson et al., 1990). The PLFA biomarker, 16:1 (n-5), is commonly found in AM and whilst not unique to AM, it is a key indicator used in similar research (Finney et al., 2017; Ngosong et al., 2012) . In this experiment, 16:1 (n-5) was significantly more abundant in the cover crop plots compared to fallow after 2 years of establishment. This supports the findings of other research exploring the use of cover crops to increase carbon directly from the root/soil interaction and as a contributor to biomass. Other research has shown that it can take many years to build up SOM and depends on the balance of flow of carbon and nitrogen into and out of the system (Khan et al., 2007). The microbial biomass carbon and nitrogen showed no significant changes as a result of cover crops or combinations of treatments.

Other measurements were taken that are used to indicate the health of soil including soil pH and yield of a follow-on cash crop. In the second year of treatments, the pH was significantly affected in the top horizon only (0-23cm) by treatments that included a cover crop. The pH however, was lower for these treatments compared to fallow. Following desiccation and incorporation of the cover crop, a cash crop of wheat was planted, and the yield measured. There was no significant difference resulting from any of the treatments.

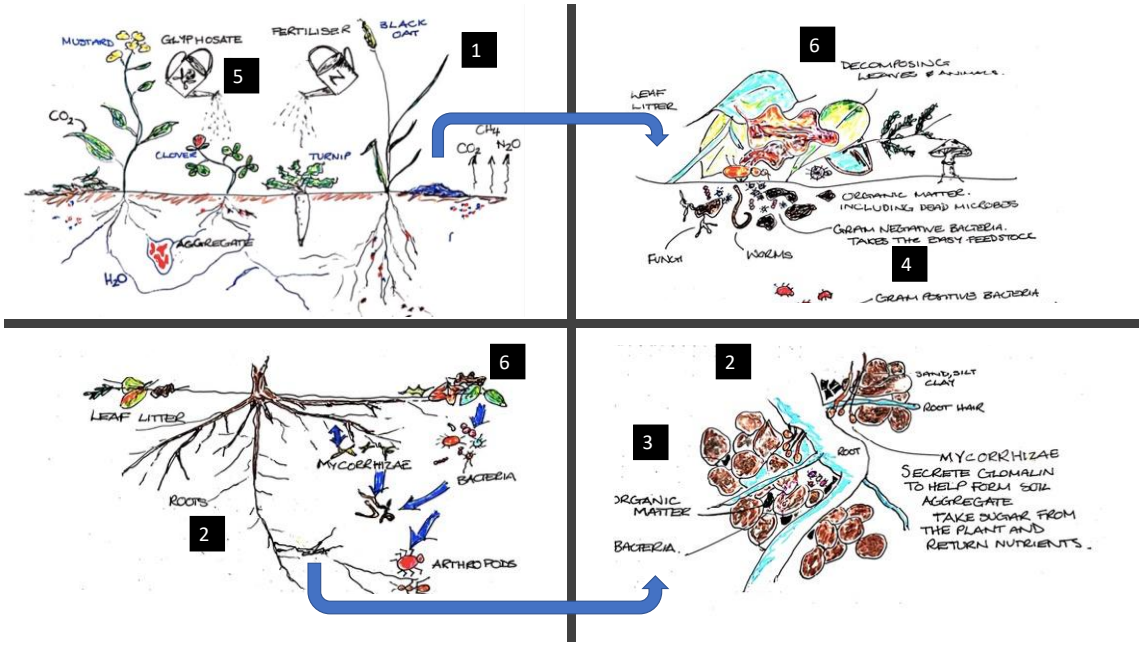
Measurement of soil health can be complex and no one measurement can be taken in isolation as an indicator of soil health.

7.1.1 The effect of cover crop species

A further objective of this research was to identify if one cover crop species provided plant material that was better at building a microbial community conducive to stabilizing carbon into aggregates. The popular use of a cover crop is to grow it over winter then destroy it in early spring, incorporating the plant biomass into the soil. In Chapter 5, evidence showed that when soil was provided with a source of organic material, in this case a solution of plant material (SPM) from different species of cover crop, it had an impact on the microbial community. For example, SPM from turnips gave the greatest fungal group of PLFA biomarkers and the highest presence of the gram-negative group, and clover the lowest averaged across all soil types. Gram-positive group averaged across all soil types were significantly different between species. The gram-positive group was most abundant with an application of mustard SPM (3.901 μg g^{-1} dry soil), and lowest with the phacelia SPM (2.921 μg g^{-1} dry soil). In other research, plants with different traits have different ratios of C:N attracting different types of bacteria (Khan et al., 2007; Orwin et al., 2010). Microbial and fungal biomass are positively linked to low leaf and litter quality (e.g. high C:N) and strongly and negatively related to nitrogen in leaf litter (Orwin et al., 2010). The same plant species in the two types of soil can make a significant difference to the abundance of different microbial functioning groups over time. Clay soils have the capacity to hold a greater amount of labile C and therefore attract an abundance of microbes and decompose plant material faster compared to sandy soils (Setia et al., 2012).

The species of cover crop used can result in different rates of microbial activity. The biomass provided by legumes (e.g., clover) that fix nitrogen and have a low C:N ratio, provide a ready source of labile carbon. This is particularly attractive to gram-negative bacteria (Kallenbach et al., 2016; Maul et al., 2014). Plant materials with low content of difficult to decompose, structural carbohydrates can also be quickly incorporated into soil. The plants with high C:N ratio such as the turnip and radish had a significantly high abundance of fungi. Figure **7.1** shows some of the interactions explained above. The time taken for mineralization to occur is important

when deciding on the optimum time for cover crop destruction; too soon and the nitrogen can be lost through leaching, too late and the plant matter is not available to the cash crop.



1. There are several cover crops species that provide different ecosystem services e.g., mustard provides a biofumigant, turnip provides winter fodder, clover fixes nitrogen, black oats deep roots to reduce compaction.
2. All plants, including weeds such as thistles, provide a root system. Roots create a symbiotic relationship with microbes in the rhizosphere.
3. Mycorrhizae infect roots and produces glomalin which glues carbon together to form aggregates. It also produces a network of fine, hairlike hyphae which extend the reach of the root to nutrients and water.
4. Gram-negative bacteria take up labile carbon and accelerate the flow of carbon out of the soil. Gram-positive bacteria are associated with recalcitrant carbon.
5. Glyphosate is used to desiccate a cover crop. The addition of glyphosate increases the bacterial activity and accelerates the flow of carbon out of the soil. It can also have a negative effect on the worm population.
6. Biodegradation of plant litter depends on the microbial community, seasonal climate, and soil type.

Figure 7.1 A summary of the interactions between cover crops, glyphosate, and soil microbial community

The cover crop most commonly used by respondents to the survey was Phacelia (*Phacelia tanacetifolia*). In the pot trials (Chapter 5) where clay soil was treated with SPM from Phacelia, the total PLFA after 3 months was lower than the control treated with water. Phacelia has a high biomass and low C:N, and is easily destroyed by frost (HDRA, n.d.). It was suspected that as the crop was collected immediately following a hard

frost, the decomposition process may have started early on negatively affecting the results. Plant senescence by frost can lead to increased nitrogen being released into soil which may be vulnerable to leaching during the winter months (Storr et al 2020).

7.2 The impact of inoculant and glyphosate on carbon flow

7.2.1 Impact of inoculant

In the field experiment a biological inoculant was introduced to soil to promote the growth of a cover crop biomass, and add AM and other beneficial microbes to the soil to encourage aggregate formation. Its application was not typical of its commercial use, which is to boost cash crop yield for financial gain. As revealed in the farmer survey, a cover crop is already considered to be an added expense with no perceived cash return, therefore investing in cover crops plus inoculant is not common practice. After 2 years the plots that were not inoculated produced 3 times more fungal biomarker group than the inoculated plots (Figure 4.9). Cover crops with the inoculant had a smaller presence of the fungal biomarker than the cover crop only plots (Figure 4.8). Where inoculant and glyphosate were used together, the microbial community appeared to be unaffected or stable.

The above results are difficult to compare with much of the research on inoculants because the focus was on the changes in the soil and not the yield of the crop. The subsequent growth of wheat on the treated plots did not show any significant differences in yield. With regard to increased crop growth, research shows the effect of inoculants is mixed with some giving positive results (Ceballos et al., 2013; Emam, 2016; Middleton et al., 2015) and others showing no effect (Pellegrino & Bedini, 2014). The addition of microbes possibly introduces competition and a lack of change in the community could be the consequence of a priority effect (Fukami, 2015). Little is known about the short and long term effects of inoculants in healthy soil. It may create negative effects through competition within an established microbial community which could affect future yields (Herzberger et al. 2015; Hart et al. 2018). However, others have suggested it may potentially offer wider benefits of resistance and resilience to sudden changes such as addition of chemicals, or extreme weather conditions(Santos et al., 2019).

7.2.2 Impact of glyphosate

Many herbicides and pesticides are applied to agricultural land and their cumulative effect on the soil is not well understood (Hart et al., 1996). Glyphosate is a widely used to desiccate some crops prior to harvest or to clear weeds before drilling new seed. In some rotations, a field could receive more than one dose per year. In the field trial, there was a significant increase in gram-negative bacteria group where the plots were treated with glyphosate. Gram-negative bacteria are associated with sources of labile carbon which, in this case, could have been supplied by the glyphosate itself (Lane, 2011). The effects did not appear until 2 years after the glyphosate was applied. Of greater concern, was the significant negative affect on the worm count where glyphosate was used supporting findings in other research in field and laboratory (Correia & Moreira, 2010; Gaupp-Berghausen et al., 2015; Pochron et al., 2020; Zaller et al., 2014, 2021). Not only has glyphosate been found to be linked to a decrease in worms it has also been found to reduce the abundance of AM resulting in poor water retention in the soil (Zaller et al., 2021).

7.3 Limitations

The field trial was limited by the short timescale to complete the research. In addition, the cover crop remained in the soil for 2 years and this is not a typical use of a cover crop but was designed this way to extend the period of time that the rhizosphere, and associated microbes could be observed and measured with roots intact. As a consequence, it potentially limited the amount of plant biomass being incorporated back into the soil each year and limited the amount of glyphosate that would ordinarily be applied each year to desiccate the crop.

Investigating the effect of biomass from different crop species was also limited by the time available and relied on the SPM in place of natural decomposition of the biomass in soil. If time was available, a more informative experiment would be to analyse the microbial community following a long term, in-field or in-vitro experiment, where different species could be grown from seed under the same conditions and either desiccated with glyphosate and turned into the soil or allowed to naturally decompose. This would be more representative of natural events.

7.4 The use of cover crops by farmers

Evidence provided by the farmer survey in Chapter 6, showed a positive uptake of cover crops with many (44 %) having used them for more than 5 years. A majority (88 %) of the respondents that used cover crops were using them to increase the SOM and improve soil structure. The positive benefits for the use of cover crops are described in many documents and has the support of policy makers including DEFRA and the European Commission (DEFRA, 2020; Smit, 2019). There are still perceived barriers over their use, including which species, timings for planting and destruction, and equipment required, as highlighted in Chapter 6. These findings are supported by other surveys citing financial barriers, and lack of knowledge on planting and termination (Storr et al 2019; Roesch-Mcnally et al. 2021). Research about the use of cover crops is being regularly published however, any new evidence relevant for decision making regarding the use of cover crops does not always reach the farmer and therefore is potentially slowing down uptake. The science needs to be communicated in a way that makes it understandable and accessible to farmers. From the survey results (Chapter 6) it was apparent that farmers are finding information from various sources including social media, network groups, conferences and directly from their agronomists. The scientific findings in this work for example, are complex but could be advantageous to farmers that want to understand more about the soil biology and how the use of commercial inoculants and herbicides can have an impact. Roesch-McNally et al (2021) found that there were opposing contradictions about the use of crops amongst farmers and that many of these barriers were linked to their use as part of a whole farm system making it difficult to make decisions about their use. A focus group approach enabled farmers to find answers and solutions to potential barriers.

7.5 Recommendations for further research

Farmers need to have a better understanding about the impacts of using cover crops and how the use of herbicides, and inoculants together with cover crops can impact on the soil, positively and negatively.
The effect of inoculants on different soils needs to be investigated further. The residual effect of adding the inoculant to a crop and therefore the soil, could create unforeseen consequences. Understanding the long term effect on different soil types with a range of SOM, from impoverished to organic rich, together with the resultant plant yields including cover crop species, would help farmers make decisions on its short and long term benefits. There is little research on the microbial response where inoculants are used in rich soils

compared to poor soils in an agricultural context or more specifically, the interactions between glyphosate and inoculant.

Understanding the flow of carbon and the relationship with nitrogen is key. If in future, the financial rewards are towards increased carbon storage, the cover crop mix needs to provide a high C:N ratio and include species that strongly encourage AM. In addition, If fertilizer costs increase, then the crop mix could provide a nitrogen fixing service using plants that offer a readily available source of nitrogen. However, the balance between the availability of C and N is crucial - higher nitrogen will accelerate the rate of carbon out of the soil system but provide higher cash crop yields (Mulvaney et al., 2006). It would also be useful to know if leaving a cover crop in for a long term (e.g a year) allowing the rhizosphere to remain intact is more beneficial in adding to the soil carbon pool, than destroying and incorporating the biomass after winter growth. The financial cost-benefits of each of these scenarios should be considered too.

Temperature effects the microbial activity. During this research, the cover crop was subject to both unusually warm temperatures in 2018 and 2 serious flood episodes in the winter of 2019 and 2020. In the UK, cover crops are generally used over the cooler winter months. As climate changes and extreme weather patterns occur, the resilience, resistance and appropriateness of the cover crops need to be considered. This includes knowledge about the chemical and biological changes that occur in the soil and the potential to reduce climate change impacts on the soil health.

Finally, the research described in this thesis highlights the complex relationship between plant traits, soil, and agricultural management techniques. Cover crops can play an important role but this is a long terms strategy in the enrichment of the microbial community, and as more is discovered about the role of bacteria and fungus, the better prepared we will be to have healthy and productive soils.

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Appendix 1 - Results of PLFA analysis of soils treated at Hilley Farm 2018 - 2020

PLFA data for field trials at Hilley Farm for 2018,2019 and 2020. Soil samples taken from 0-23cm horizon. Values in bold are significantly different (p. <0.05)

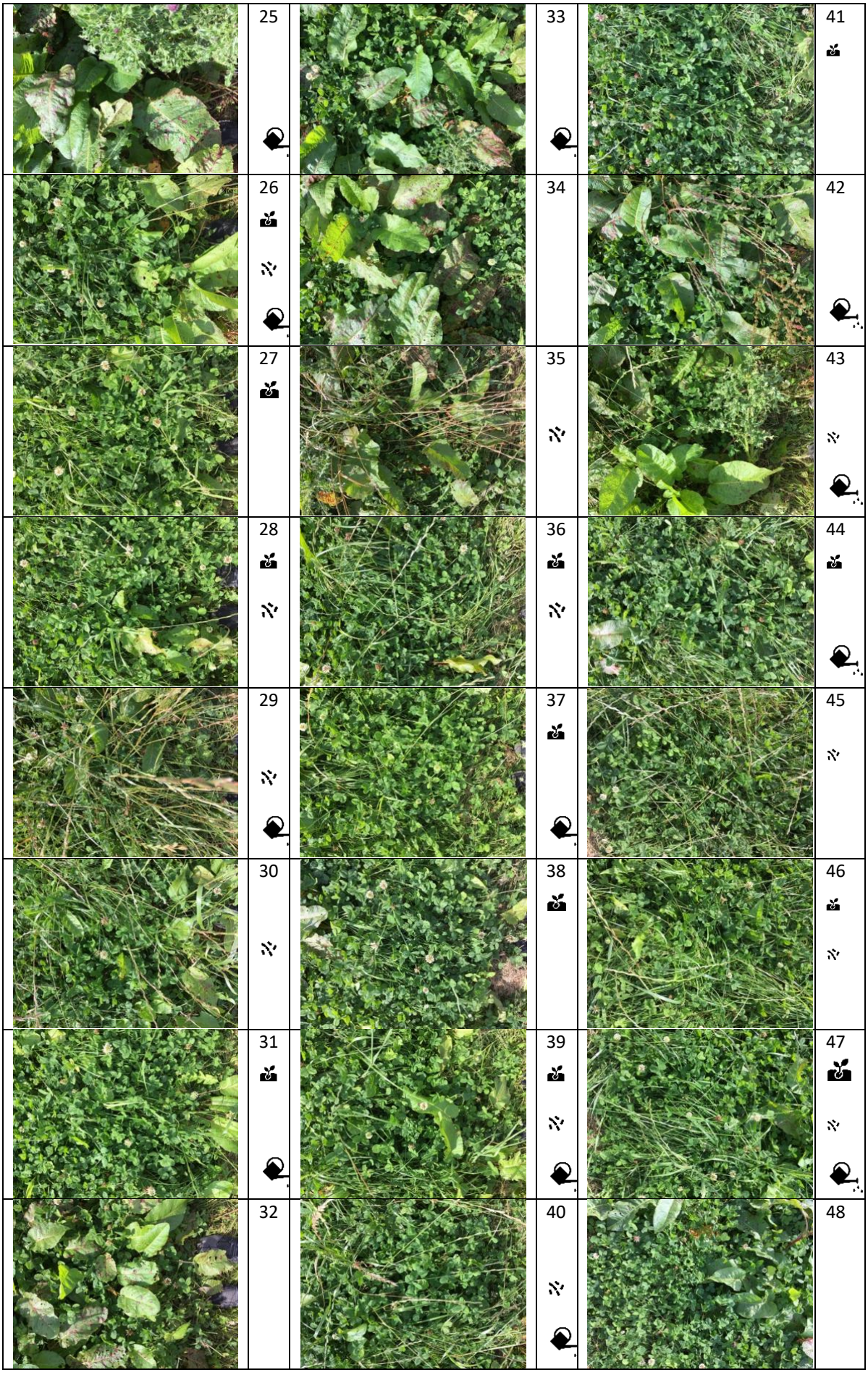
				Year 1			Year 2			Year 3			Year 1/2	Year 1/2/3
Treatment	**Treatment**	**Treatment**	**Measurement**	**Mean**	**SD**	**P**	**Mean**	**SD**	**P**	**Mean**	**SD**	**P**	**P**	**P**
Cover crop			Total PLFA	25.249	3.858	0.982	19.881	8.111	0.525	51.135	53.151	0.071	0.846	**0.037**
Glyphosate			Total PLFA	22.799	9.488	0.142	18.304	1.979	0.895	21.051	4.405	0.810	0.110	0.934
Inoculant			Total PLFA	23.665	6.695	0.854	19.131	3.398	0.724	26.564	8.883	0.166	0.857	0.074
Cover crop	Glyphosate		Total PLFA	21.093	5.532	0.506	20.555	2.810	0.642	54.955	60.529	0.729	0.776	0.747
Cover crop	Inoculant		Total PLFA	25.708	4.929	0.633	19.880	4.653	0.327	25.668	9.731	0.089	0.257	**0.052**
Glyphosate	Inoculant		Total PLFA	23.043	7.997	0.645	20.505	3.889	0.704	24.224	6.457	0.861	0.563	0.978
Cover crop	Glyphosate	Inoculant	Total PLFA	22.801	4.875	0.936	18.466	3.146	0.578	26.927	4.390	0.896	0.550	0.992
YEAR			Total PLFA				17.838	1.909		26.319	23.579		**<0.001**	**<0.001**
Cover crop			Total fungal	1.394	0.159	0.432	1.629	0.954	0.434	2.291	2.123	0.843	0.358	0.957
Glyphosate			Total fungal	1.682	1.548	0.488	1.534	0.541	0.577	1.813	2.128	0.943	0.127	0.836
Inoculant			Total fungal	1.526	1.025	0.592	1.936	0.816	0.168	1.494	2.037	**0.044**	0.331	0.016
Cover crop	Glyphosate		Total fungal	1.112	0.486	0.315	1.861	0.713	0.519	3.953	5.127	0.247	0.348	0.179
Cover crop	Inoculant		Total fungal	1.912	0.913	0.416	1.788	0.469	0.896	0.786	1.645	0.816	0.175	0.874
Glyphosate	Inoculant		Total fungal	1.625	1.373	0.848	1.827	0.573	0.742	1.498	1.936	0.733	0.775	0.840
Cover crop	Glyphosate	Inoculant	Total fungal	1.302	0.282	0.662	2.155	1.625	0.549	1.331	1.991	0.369	0.782	0.463
YEAR			Total fungal	1.664	0.491		1.325	0.286		4.639	6.926		0.086	0.296
Cover crop			Total bacterial	12.070	2.077	0.602	9.668	4.002	0.730	20.579	13.173	0.081	0.712	0.045
Glyphosate			Total bacterial	10.668	3.817	0.183	9.081	0.959	0.975	11.183	3.630	0.516	0.160	0.500
Inoculant			Total bacterial	11.195	2.565	0.698	9.044	1.411	0.856	10.296	1.640	0.164	0.777	0.099
Cover crop	Glyphosate		Total bacterial	10.293	2.805	0.387	9.922	1.154	0.347	24.466	32.546	0.915	0.807	0.910
Cover crop	Inoculant		Total bacterial	12.573	2.219	0.829	9.717	2.162	0.308	10.743	2.020	0.097	0.374	0.065
Glyphosate	Inoculant		Total bacterial	10.814	3.461	0.936	9.923	1.990	0.631	12.101	4.479	0.857	0.694	0.962
Cover crop	Glyphosate	Inoculant	Total bacterial	10.767	2.678	0.922	8.386	1.496	0.340	12.291	3.501	0.887	0.429	0.990
Control			Total bacterial	11.200	3.506		8.841	0.923		9.156	3.280			
YEAR													**<0.001**	**0.006**
Cover crop			Fungal: Bacterial	0.117	0.012	0.336	0.148	0.087	0.592	0.146	0.110	0.277	0.345	0.437
Glyphosate			Fungal: Bacterial	0.143	0.122	0.527	0.169	0.054	0.393	0.186	0.268	0.508	0.149	0.330
Inoculant			Fungal: Bacterial	0.128	0.057	0.975	0.212	0.068	0.109	0.130	0.151	**0.039**	0.115	0.020
Cover crop	Glyphosate		Fungal: Bacterial	0.107	0.030	0.827	0.186	0.055	0.265	0.174	0.126	0.280	0.377	0.456
Cover crop	Inoculant		Fungal: Bacterial	0.150	0.062	0.282	0.184	0.028	0.561	0.091	0.200	0.388	0.571	0.695
Glyphosate	Inoculant		Fungal: Bacterial	0.138	0.076	0.802	0.189	0.068	0.960	0.098	0.099	0.579	0.947	0.775
Cover crop	Glyphosate	Inoculant	Fungal: Bacterial	0.123	0.017	0.518	0.273	0.250	0.395	0.109	0.162	0.497	0.376	0.621
Control			Fungal: Bacterial	0.171	0.112		0.149	0.022		0.395	0.447			
YEAR													**0.002**	0.185

				Year 1			Year 2			Year 3			Year 1/2	Year 1/2/3
Treatment	**Treatment**	**Treatment**	**Measurement**	**Mean**	**SD**	**P**	**Mean**	**SD**	**P**	**Mean**	**SD**	**P**	**P**	**P**
Cover crop			Gram +ve	5.785	1.202	0.560	4.522	1.647	0.821	9.991	5.923	0.122	0.707	0.080
Glyphosate			Gram +ve	5.071	1.852	0.134	4.182	0.239	0.840	5.861	4.487	0.673	0.118	0.742
Inoculant			Gram +ve	5.234	1.330	0.862	4.062	0.612	0.949	3.184	2.540	**0.037**	0.868	**0.013**
Cover crop	Glyphosate		Gram +ve	4.780	1.276	0.427	4.453	0.347	0.179	8.158	11.162	0.535	0.997	0.898
Cover crop	Inoculant		Gram +ve	5.911	0.760	0.638	4.403	1.033	0.268	3.786	2.597	0.245	0.255	0.276
Glyphosate	Inoculant		Gram +ve	4.977	1.586	0.811	4.756	0.895	0.904	4.175	4.058	0.816	0.870	0.941
Cover crop	Glyphosate	Inoculant	Gram +ve	5.146	1.049	0.939	3.929	0.635	0.328	4.806	2.957	0.528	0.430	0.582
Control			Gram +ve	5.408	1.945		4.074	0.349		4.084	3.693			
YEAR													**<0.001**	0.091
Cover crop			Gram -ve	6.202	1.308	0.582	4.978	2.274	0.738	10.512	7.664	0.086	0.655	0.063
Glyphosate			Gram -ve	5.413	2.066	0.277	4.800	1.068	0.938	5.180	3.247	0.474	0.226	0.434
Inoculant			Gram -ve	5.785	1.285	0.524	4.883	0.803	0.850	7.031	1.286	0.469	0.697	0.422
Cover crop	Glyphosate		Gram -ve	5.330	1.422	0.320	5.328	0.799	0.524	15.919	21.044	0.571	0.607	0.656
Cover crop	Inoculant		Gram -ve	6.512	1.509	0.923	5.140	1.180	0.322	6.956	3.507	0.066	0.551	0.043
Glyphosate	Inoculant		Gram -ve	5.726	1.999	0.985	5.053	1.141	0.335	7.894	1.549	0.677	0.503	0.830
Cover crop	Glyphosate	Inoculant	Gram -ve	5.506	1.645	0.867	4.318	0.902	0.423	7.421	2.192	0.511	0.456	0.739
Control			Gram -ve	5.540	1.659		4.561	0.769		5.004	2.542			
YEAR													**0.005**	**<0.001**
Cover crop			Gram +ve: Gram -ve	0.962	0.250	0.795	0.965	0.166	0.825	1.029	0.384	0.742	0.972	0.929
Glyphosate			Gram +ve: Gram -ve	0.973	0.276	0.759	0.933	0.342	0.791	3.730	5.925	0.421	0.913	0.356
Inoculant			Gram +ve: Gram -ve	0.906	0.114	0.456	0.835	0.069	0.662	0.517	0.519	0.591	0.910	0.868
Cover crop	Glyphosate		Gram +ve: Gram -ve	0.900	0.056	0.983	0.843	0.064	0.367	0.583	0.522	0.810	0.427	0.934
Cover crop	Inoculant		Gram +ve: Gram -ve	0.928	0.130	0.263	0.864	0.120	0.705	7.145	16.609	0.107	0.541	0.097
Glyphosate	Inoculant		Gram +ve: Gram -ve	0.877	0.074	0.754	0.952	0.139	0.186	0.530	0.481	0.669	0.377	0.957
Cover crop	Glyphosate	Inoculant	Gram +ve: Gram -ve	0.966	0.160	0.396	0.922	0.103	0.636	0.721	0.540	0.321	0.955	0.414
Control			Gram +ve: Gram -ve	0.964	0.174		0.918	0.206		7.661	17.412			
YEAR													0.530	0.163

Appendix 2 - Photographs of each plot and details of treatments

	1		9		17
	2		10		18
	3		11		19
	4		12		20
	5		13		21
	6		14		22
	7		15		23
	8		16		24

LEGEND Cover crop Glyphosate Inoculant



LEGEND Cover crop Glyphosate Inoculant

Appendix 3 – Farmers survey

Use of cover crops

I'm Mandy Stoker, a PhD student at Rothamsted Research Institute and University of Lancaster studying the benefits of cover crops in agriculture.

I am approaching you as a farmer, to participate in this short survey. My aim is to get a better understanding why farmers do or don't use cover crops. If you do use cover crops, I'd like to know your main reasons for using them. If you don't use them, is there a specific reason for not using them or do you need further evidence to show the benefits of using them. I should be grateful if you would take part.

This survey comprising largely multiple choice answers, should take no longer that 10 minutes to answer. There are 5 sections, however, depending on your answers only 3 sections need to be completed. There is an opportunity for you to provide further information or opinion in your own words if you wish to.

You are under no obligation to complete the survey and can stop at any time. You can withdraw from the survey at up to a week of submission by emailing me at the address below.

If you would like to receive a summary of all of the results received, please tick the box below and this will be sent to your the email address if provided. Once the results have been sent to you, your email address will be deleted.

If you have any questions about the survey, please email me at m.stoker@lancaster.ac.uk

The research has been approved by the Faculty of Science and Technology Research Ethics Committee. The deadline for completion and submission of the survey is 1st December 2020. The results of the study will be presented in my PhD thesis. Your anonymity is guaranteed and it will not be possible to identify you from my presentation of the results.

If you have concerns about the survey please contact the project supervisor Dr Carly Stevens at c.stevens@lancaster.ac.uk or the Head of Department , Professor Barker at p.barker@lancaster.ac.uk.

For further information about how Lancaster University processes personal data for research purposes and your data rights, please visit our webpage: www.lancaster.ac.uk/research/data-protection

* Required

1. Please check the box to confirm that you are happy to proceed with the survey with the understanding that you consent to the following: *

CONSENT FORM

Project Title: Adding evidence to decision making regarding cover crops

Lancaster University

Name of Researcher: Mandy Stoker

Email: m.stoker@lancaster.acuk

•I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily

•I understand that any information given by me may be used in future reports, academic articles, publications or presentations by the researcher/s, but my personal information will not be included and I will not be identifiable.

•I understand that my name/my organisation's name will not appear in any reports, articles or presentation without my consent.

•I understand that data will be kept according to University guidelines for a minimum of 10 years after the end of the study.

•I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. If I withdraw within 1 week of submission of the form, my data will be removed.

Check all that apply.

- [ ] I'm happy to proceed

About your farm

I'd like to know about your farm.

2. Where do you farm - County? *

_____

3. Type of farm- tick all that are relevant *

Check all that apply.

- [ ] Livestock
- [ ] Arable
- [ ] MIxed
- [ ] Poultry
- [ ] Organic Livestock
- [ ] Organic arable
- [ ] Organic mixed

Other: [ ] _____

4. About the land you farm - are you.... *

Check all that apply.

- [ ] Tenant
- [ ] Owner
- [ ] Own and rent

5. Have you ever used cover crops on your farm? *

Mark only one oval.

- ◯ Never *Skip to question 13*
- ◯ Yes

About your cover crops

In this section I'd like to know why you use cover crops

6. How long have you used cover crops? *

Mark only one oval.

- ◯ 1-2 years
- ◯ 3-5 years
- ◯ More than 5 years
- ◯ Not using them at the moment

7. What cover crops are you using (tick all that apply)? *

Check all that apply.

- [ ] Phacelia
- [ ] Black oats
- [ ] Stubble turnips
- [ ] Raddish
- [ ] Mustard
- [ ] Clover
- [ ] Hairy vetch
- [ ] Rye grass
- [ ] A mix suggested by your agronomist/seed supplier
- [ ] Don't know
- [ ] Other: _____

8. Why do you use cover crops? (tick those that are most relevant) *

Check all that apply.

- [ ] Winter soil cover
- [ ] For grazing
- [ ] Increasing organic matter
- [ ] Increase nitrogen
- [ ] Improve soil structure
- [ ] Fumigation
- [ ] Weed control
- [ ] Control soil erosion
- [ ] Other: _____

9. How do you prepare for the next cash crop *

Check all that apply.

- [ ] Plough in the cover crop no spraying
- [ ] Dessicate and plough in
- [ ] Roll and direct drill
- [ ] Other: _____

10. How did you first find out about cover crops *

Mark only one oval.

- Farming magazine
- Agronomist
- Agricultural event
- Information from neighbour or friend (word of mouth)
- Radio
- Television
- Podcasts
- Other: _____

11. Is there a down side to using cover crops and if so what?

_____

_____

_____

_____

_____

12. If you want to tell me anything else about your experience of using cover crops please write it here.

_____

_____

_____

_____

_____

Skip to question 18

Barriers to the use of cover crops

I would like to know a bit more about why you don't use cover crops and what evidence you would like to make you reconsider their use.

13. Do you feel you know plenty about the use of cover crops?

Mark only one oval.

	1	2	3	4	5	6	7	8	9	
I don't know anything	◯	◯	◯	◯	◯	◯	◯	◯	◯	I know a lot

14. What are the main reasons for not using cover crops? Tick any of your reasons *

Check all that apply.

- [ ] It's too expensive
- [ ] I'm not interested
- [ ] I don't feel they are of benefit on my farm
- [ ] I don't know which cover crop to use
- [ ] I don't know enough about it
- [ ] I've heard they create problems in the following crop
- [ ] I don't have the time
- [ ] It doesn't fit in with my rotations
- [ ] I don't have appropriate equipment

Other: ☐ _____

15. If you had more evidence about the benefits and use of cover crops, how likely are you to give them a go? *

Mark only one oval.

	1	2	3	4	5	6	7	8	9	
Not likely	◯	◯	◯	◯	◯	◯	◯	◯	◯	I would try it

16. If you were financially rewarded to increase the carbon in your soil would you use cover crops as a first choice solution? *

Mark only one oval.

- ◯ Yes
- ◯ No
- ◯ Maybe

17. If there's anything further you would like to say about why you don't use cover crops or have specific things you would like to know, please write below.

Sources of information - science and technology in agriculture ?

This section explores how you would normally find out about new ideas about farming and what your preference is for finding out more information about cover crops.

18. Which of the following do you regularly use to find out latest farming ideas? Tick any that are relevant. *

Check all that apply.

- [ ] Farmers weekly
- [ ] Other farming magazines
- [ ] Advice from agronomists
- [ ] TV eg Countryfile
- [ ] Farm shows eg Cereals, Groundswell
- [ ] Invitation to special events
- [ ] Farm facilitator group
- [ ] NFU
- [ ] Farm podcasts
- [ ] Radio programmes eg farming today
- [ ] Farming friends and family
- [ ] At the local pub
- [ ] Seed /chemical suppliers
- [ ] Government websites e.g DEFRA
- [ ] On the internet
- [ ] Land agent
- [ ] Consultancy
- [ ] University departments
- [ ] Trade Associations eg Organic Farmers & Growers
- [ ] LEAF
- [ ] Red Tractor scheme
- [ ] Customers e.g Waitrose, Tesco, Sainsbury
- [ ] Specialist farm tools - usually online

Other: ☐ _____

19. Are you aware of the target set in the Paris Agreement to increase the soil organic matter by 0.4% per year? *

Mark only one oval.

- ◯ Yes
- ◯ No

20. On a scale of 1 to 10 how likely are you to implement new agricultural techniques to increase the organic matter content in your soil? *

Mark only one oval.

	1	2	3	4	5	6	7	8	9	10	
Unlikely	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	Highly likely

21. If you would like a copy of the summary of results please provide your email address(optional)

_____