RACHEL HASLER

Effects of organic matter additions on the soil microbial population and associated stoichiometry in horticultural systems

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
PhD Environment and Agrifood

PhD
Academic Year: 2018 - 2023

Supervisor: Dr Mark Pawlett
Associate Supervisor: Professor Jim Harris
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This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

Organic matter addition influences soil function (respiration/ decomposition) and community structure (PLFA/ NLFA community profiles) which result in changes to the provision of soil derived ecosystem services. Additions of organic matter to gardens (e.g. composts, mulches, soil conditioners) is a widespread practice both globally and in the UK. Research on the long-term cumulative impact of annual additions to UK gardens is limited. A field trial, set-up in 2007, at the Royal Horticultural Society's Wisley Gardens site, was used to examine the changes to soil chemical, physical and biological soil quality indicators following 12 years of different soil organic matter additions. Additions varied in carbon-to-nitrogen ratios (C/N), physical structure and macro/ micro-nutrient profiles. The amendments used in the trial included composted bark, bracken, stable manure, garden compost, spent mushroom growing compost, peat, fertiliser (rate adjusted each year to meet plant growth requirements) and controls; no organic matter addition and no plant sown, no organic matter addition and plant sown. Lab studies were designed following analyses of amendment legacy effects in field trial plots to further examine the effects of addition on microbiological indicators of soil function, health and quality. Reviewing long term chemical and physical data from the field trial highlighted significant effects of organic matter additions on soil micro- and macro-nutrients. Recommendations for garden industry were made from the findings and include the need for labelling standards for compost material packaging to reduce unwanted environmental impacts of use. Organic matter treatment to clay-loam textured soil significantly increased microbial respiration and shifted microbial community structure. The effects were distinct and dependent on composition of the organic matter applied. The horticultural sector has outlined targets for reducing the impact of garden practise on drivers of climate change. This study aligns with literature which seeks to understand common practise in order to improve economic and environmental sustainability.

Keywords:

soil microbiology, organic matter, microbial respiration, compost, gardens, nutrients

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To me. I’d like to acknowledge me. I did this, as a working-class single mother, through burning field trials, storms that lifted polytunnels and a global pandemic. I just think that’s worth acknowledging.

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LIST OF EQUATIONS

LIST OF ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
C	Carbon
DEFRA	Department for Environment, Food and Rural Affairs
FAME	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organisation (United Nations)
Fe	Iron
GHG	Greenhouse Gas
Gov UK	UK Government
G+/Gram+	Gram positive bacteria
G-/Gram-	Gram negative bacteria
Ha	Hectare
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
Kg	Kilogram
LM	Linear model
LSF	Life Support Function
mg	Milligram
Mg	Magnesium
MSIR	Multiple Substrate Induced Respiration
N	Nitrogen
NLFA	Neutral Lipid Fatty Acid
OM	Organic matter (prior to application to soil – in form of compost/ manure/ composted material to be used a soil conditioner)
P	Phosphorus
PCA	Principal component analysis
PLFA	Phospholipid Fatty Acid
RHS	The Royal Horticultural Society
SOC	Soil organic carbon
SOM	Soil organic matter

T0	Samples taken from organic matter material (prior to application to soil)
T1	Sample time 1 – field trial soil samples taken prior to the year’s organic matter addition
T2	Sample time 2 – field trial soil samples taken approximately 1month after the year’s organic matter addition and incorporation
TC	Total carbon
TOC	Total organic carbon
Yr	Year

1 Introduction

Chapter synopsis

This chapter introduces the research project and frames the work in the wider context of UK garden management. The specific relevance of soil processes is outlined before a description of the aims, objectives, and structure of the thesis.

1.1 Background and context

1.1.1 Garden specific land management

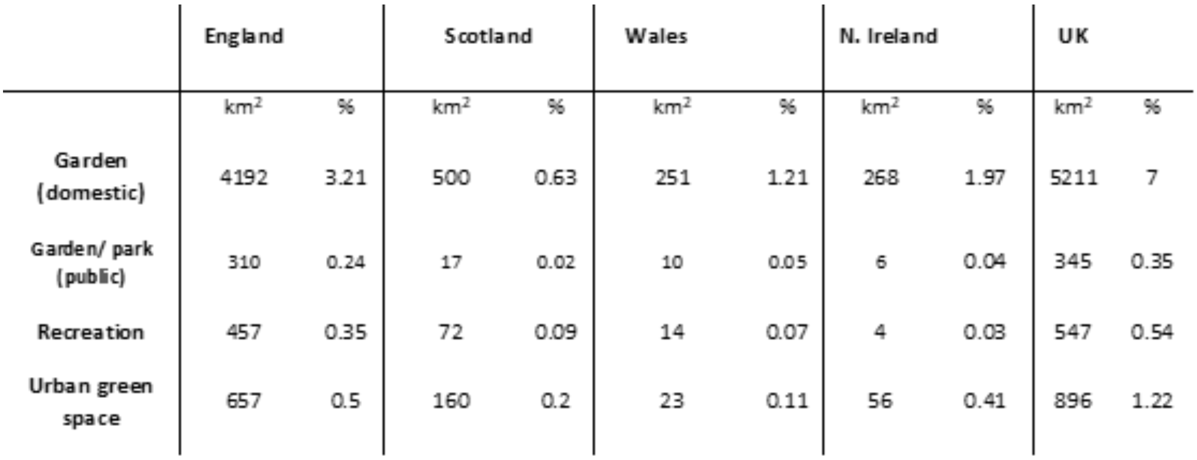
Gardeners add organic matter to soils based on tradition, cultural practices, habit, and locally available resources (Andersen et al., 2010; Edgerton et al., 2009). In the UK, public gardens, urban green spaces, parks and recreation grounds total nearly 7000 km^2 (2.9%) of total UK land area (Rickson et al., 2015,). Science backed advice for best practice of soil management in gardens is likely to be predominantly adapted from agricultural and horticultural studies or from anecdotal and habitual practice (Alexander, 2009). These managed areas are a valuable asset in terms of ecosystem service provisioning and offer opportunity to improve how we manage carbon cycling functions through informed decision making (Camps-Calvet et al., 2016; Mimet et al., 2020).

According to the People and Nature Survey of 24,994 UK adults, from April 2020 and March 2021 conducted by UK Gov, over 98% of people reported they had access to a garden, allotment or outdoor space (GovUK, 2022). Reasons for spending time in these garden spaces were for fresh air (65%), to participate in gardening or maintenance (42%), health and wellbeing (34%), to take a break (27%) and to connect with nature and wildlife (22%). 35% of respondents said they take actions to encourage wildlife into their gardens and outdoor spaces (GovUK, 2022). This survey highlights the multitude of drivers for people participating in gardening or wanting to spend time in allotment or outdoor spaces for non-agricultural purposes and goes someway in highlighting the number of people that science-backed gardening advice would benefit.

Knowledge translated from academic spheres into gardening culture benefits the wider community. Soil (and our management of it) provides opportunity for adapting to IPCC predictions of global change (IPCC, 2022). Improved garden management can mitigate carbon losses and provide ecosystem benefits and services for surrounding land and people. To do this it is essential gardeners are supported and valued by industry and academia towards best practise models of management (RHS, 2020). This study provides specific recommendations for organic matter applications and outlines factors that should be considered by organisations developing gardening advice.

Multidisciplinary modes of mitigating the impacts and drivers of climate change is increasingly a focus for a wide range of industries. Agricultural land use to produce food, fuel and fibre is essential for supporting life and society, but is also responsible for land degradation, loss of biodiversity and increases in GHG emissions (Power, 2010; Tilman, 2002).

Table 1-1 Data from Rickson et al, (2016) Final Report to Defra, detailing breakdown of garden land, parks and public green spaces, recreation grounds and urban green spaces in total KM^2 and as a percentage of total UK land.



	England		Scotland		Wales		N. Ireland		UK	
	km^2	%	km^2	%	km^2	%	km^2	%	km^2	%
Garden (domestic)	4192	3.21	500	0.63	251	1.21	268	1.97	5211	7
Garden/ park (public)	310	0.24	17	0.02	10	0.05	6	0.04	345	0.35
Recreation	457	0.35	72	0.09	14	0.07	4	0.03	547	0.54
Urban green space	657	0.5	160	0.2	23	0.11	56	0.41	896	1.22

Agriculture and all connected industries now have opportunity to collaborate and provide interdisciplinary research on the impacts of land management (Fedele et al., 2019). The context of this study is non-agricultural practice but aligns with the agricultural industry's drive to make management more environmentally and economically sustainable (El Bilali et al., 2019).

Industries, such as the horticultural industry, are also promoting environmentally sustainable practises. For example, the Royal Horticultural Society (RHS) updated their sustainability strategy to specifically aim to incorporate science backed gardening advice. Reasons for this are included in their 2020 strategy publications “The RHS Sustainability Strategy: Net positive for nature and people by 2030”, “Greening Grey Britain” and “RHS Science Strategy 2020-2025” (RHS, 2021; RHS, 2015; RHS, 2020). Notably there is demand from the RHS membership base for ensuring gardening is resilient to climate change effects. Garden organisations have the capacity to lead the way in adaptation and ensure an environmentally and economically “future-proof” sector.

A report published by Defra and the Office for National Statistics (ONS) stated that the value of ornamental horticulture increased from £1.4 billion in 2020 to £1.6 billion in 2021 (Defra, 2022). A parliamentary report informed by the Chartered Institute of Horticulture reported that the UK horticultural industry is worth £5 billion per annum and recently (2022) debated the role of the sector in protecting the environment (Winchester, 2022). The RHS estimate that 29.5% of Great Britain's urban land area is residential garden land (RHS, 2020b). Considering the uptake of gardening by non-professionals since the 2020 pandemic and the value of the recreational horticulture sector a series of experiments were designed to address the gap in the literature on garden-specific land management and its influence on the soil microbiome and associated functions.

1.1.2 UK horticultural industry and organic matter management

Gardeners habitually add organic matter to soils, either purchased from national suppliers or garden centres, made from kitchen and garden waste, or collected from local materials and businesses such as stables. Though alternatives are available, peat-based composts are still widely sold in many UK garden retailers and are due to be phased out for amateur gardeners by 2024 (Gov UK, 2022). Composts, (and some types of soil conditioners and soil improvers such as bark-based products) - all marketed at gardeners, consist of material composted over time and intended to be added to garden soils for a variety of benefits (e.g. improved tilth, nutrient provision, mulch).

Organic matter additions have varying C/N ratios and other characteristics which interact with soil biology and influence soil characteristics both in the short term and accumulatively over time (Hoffland et al., 2020). Though there are industry standards for testing composted materials (such as BSI PAS 100 quality assurance (CCS, 2018)), it is rare for any of this information to be included on compost packaging when sold, and it is not mandatory to include this information to purchasers. If compost is produced in the garden, it can be considered unlikely that the majority of gardeners producing compost will have the material tested regularly to understand the chemical, physical and biological characteristics (or the interactions of these characteristics) prior to adding it to soil.

Decomposition of organic material depends on interrelated factors that affect the rate of decomposition and resulting products (plant available nutrients/ carbon available to microbes) of the decomposition process (Zhang et al., 2019). Environmental/ abiotic factors (e.g. temperature, moisture, pH), substrate quality (e.g. chemical and physical characteristics) and soil microorganisms (bacterial and fungal decomposers, soil fauna responsible for moving and separating organic matter) (Barrios, 2007; Kaiser et al., 2014; Lehmann and Kleber, 2015) are all integral to the composition of the material and the rate of decomposition.

Carbon to nitrogen (C/N) ratio is a key parameter in measured in soil quality analyses (Arshad & Martin, 2002; Dong et al., 2021). During the composting process C/N ratio of an organic material can reduce three-fold (Ho et al., 2022).

As the material is decomposed, nutrients are released and there is a reduction in total C (Yang et al., 2022). Recommended C/N ratio for organic material to be directly added to soil (as a soil conditioner or improver) ranges from 25-35:1 (El-mrini et al., 2022; Rynk, 2022). Though not a direct measure of "quality" as is found in soil quality indices, C/N can be a measure of the end use of an organic matter soil amendment. The composting process can be defined as the transformation of fresh organic matter through several phases of decomposition (mesophilic, thermophilic, second mesophilic or colling, maturation, Figure 1-1) for the production of an organic matter material to be incorporated into soils as a soil conditioner (Insam and de Bertoldi, 2007). Although there are industry-approved standards for the production process of organic matter soil conditioners (Environment Agency, 2012) the range of products and their influence on soil function once applied to soils is understudied in a gardening context.

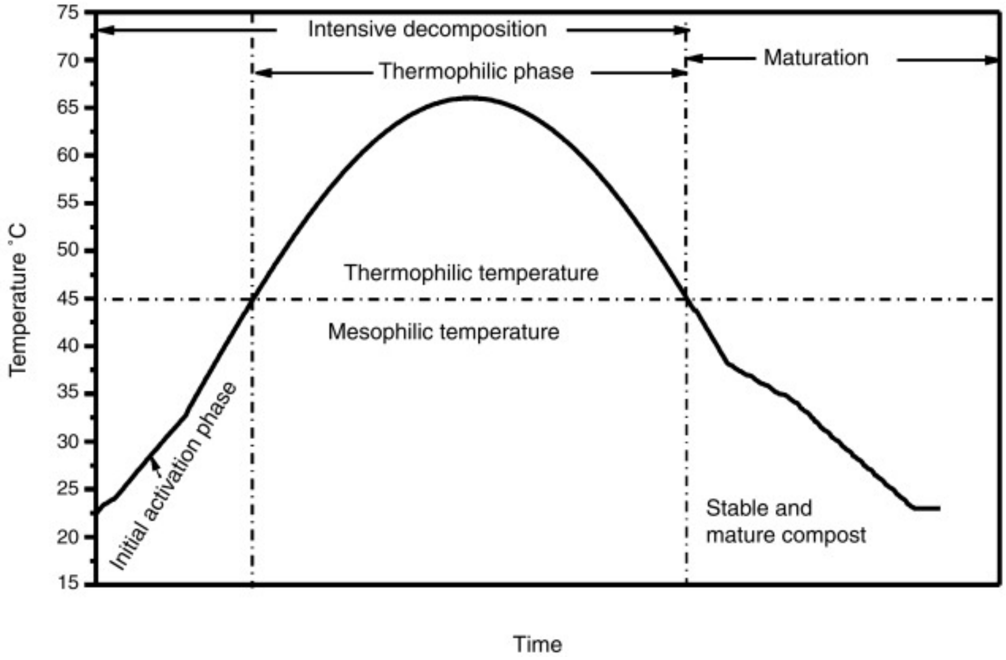


Figure 1-1 Simplified depiction of the stages of the composting process from Akratos et al. (2017)

Fresh material, or material which is early in the composting process (with a higher C/N), may be recommended for use as a mulch (applied to soil surface) where there is a reduction in potential for nutrient immobilisation when incorporated below the soil surface. Organic matter material which is more advanced in the composting process is generally added as a soil conditioner, with the intention of improving soil texture and/or to break-down slowly and release nutrients into the soil through further decomposition (Ho et al., 2022). For the purpose of this research the focus is on soil conditioners produced from fresh organic materials for incorporation into the soil.

Ensuring a diverse range of microorganisms and mesofauna are present in soil means that recalcitrant materials (such as lignin) can be decomposed or transformed and provide plant available nutrients and resources for soil microbiology to continue cycling further inputs (de Boer et al., 2005; Duddigan et al., 2022; Maron et al., 2018). There is evidence that even humic substances, (or refractory fractions of soil organic carbon (Ueno et al., 2016)) which can be considered resistant to microbial degradation when physically or chemically protected, can be altered, transformed, and degraded if specific microbial life is present and in proximity (Kluczek-Turpeinen et al., 2005; Ueno et al., 2016). Organic matter decomposition can be considered a dynamic and progressive process reliant on the structure and functionality of soil biology (Lehmann and Kleber, 2015).

Though all organic inputs may be decomposed, the time-frame of the process may vary widely. Rate of decomposition is not constant and organic material is not homogenous (Hoffland et al., 2020). Focusing on long term monitoring of the effects of organic matter addition to soils and the changes in microbial community composition helps to breakdown the effects of practises which are assumed to be beneficial but may have subsidiary beneficial or detrimental effects on soil functions. What may be considered a low nutrient or low-quality input initially may have different composition, characteristics, or qualities after ten years of applications, which may or may not be beneficial for management aims. Material which has been considered recalcitrant, or resistant to decomposition due to high molecular weight, in short-term monitoring due to seasonal fluctuations in soil moisture and temperature may be altered over decadal time-scales (Sullivan et al., 2022). Transformation of organic matter has been shown to be dependent on the structure and presence of the soil microbial community, abiotic factors (temperature, O_2 availability and soil moisture content), and time-scale (Lehmann and Kleber, 2015). For this reason it is essential that research on SOM dynamics consider soil biology as a central component of soil physiochemical dynamic processes and increase long-term monitoring of different stages of SOM cycling processes.

1.1.3 Soil organic matter and carbon transformation

A number of different soil quality indices exist and are a useful framework with which to examine soil. Mukherjee and Lal (2014) highlighted that a soil quality index should encompass three essential areas of focus; environmental quality, agronomic sustainability, socio-economic viability. However, in gardens these essential elements vary to those considered in agricultural systems. At the time of writing there are no garden-specific soil quality indices, nor any transferable indices used to measure the “quality” of composts and organic matter amendments.

Developments in carbon fractionation methods, such as thermogravimetric methods (Tokarski et al., 2020) and use of Rock-Eval pyrolysis methods (Cécillon et al., 2021; Sebag et al., 2016) have improved the collective understanding of the identity and location of belowground carbon. Identifying the mechanisms, performed by the microbiological community, that transform carbon inputs within the terrestrial carbon cycle furthers understanding of soil as functional system (Hoffland et al., 2020).

The functions and processes of the SOC system are mediated by soil biology (Liang et al., 2017). Immobilisation and mineralisation are microbially governed, continuous processes involving constant feedbacks between biological agents and abiotic factors (Kaiser et al., 2014). Rate of mineralisation can change depending on the C/N ratio or the chemical composition of C and N in the substrate added as microbiota adapts to input (Bankó et al., 2021). Short-term influence of inputs with a high C/N ratio (containing little nitrogen) can limit N resources for decomposer organisms, and result in nitrogen immobilisation. As the C/N ratio decreases then mineralisation can occur. The dynamics of microbial functional adaptation to input are essential for understanding soil geochemical changes (Kaiser et al., 2014). Aside from influencing dynamic changes to mineralisation/ immobilisation, adding substrates with varying C/N/P to soils contributes to the build-up of organic matter and influences measures of soil quality. Soil structure is improved by increasing soil organic matter (Annabi et al., 2007). There are also interactions with other geo/ physio-chemical parameters such as the effect of tillage on soil aggregation and interactions of this with carbon storage as outlined by (Cooper et al., 2021). (Girkin et al. (2019) highlight the importance of considering substrate availability as on spatial variation of methane emission. The work presented here retains a focus on the biological element of these processes but does recognises the importance of a multidisciplinary approach to further understanding of key soil processes.

Primary consumers of the soil food-web are microbes (bacteria, fungi, actinobacteria) (Geisen and Bonkowski, 2018). These microbes are responsible for most of the chemical alteration of organic matter which is added to soil. It is widely established that in most ecosystems fungi are the most abundant (in terms of biomass) of the decomposer community and are responsible for the decomposition of lignin and the majority of more recalcitrant inputs (Sassi et al., 2012; Wagg et al., 2019). Human land management, such as the addition of organic matter to soil, is considered to have wide ranging impacts on the relationship between bacteria and fungi. Land management alters of natural conditions, such as substrate or nutrient limitation or abundance that affects competition between these two domains. Organic matter additions, if managed appropriately, can support a diverse microbiological network which is resilient and maintains function despite disturbance or increased competition (Barrios, 2007).

1.1.3.1 Soil microbial community: linking function to management

The soil microbiome has seen a dramatic increase in scientific, academic, and public attention in recent years. The increased accessibility of next generation sequencing (NGS) has shed light on the abundance and diversity of microbial life found in soils (Leite et al., 2022). Estimates have been made of more than 1trillion (10^{12}) microbial species on Earth (Locey and Lennon, 2016). Estimates of number of microbial species in soils vary due to the immense variability of soil types. Trevors (2010) label soils as a “biochemical gene library” and uses the example of a single g of dry soil containing up to a billion bacterial cells. This in turn has led to calls for more collaboration between researchers to ensure progress in classifying and understanding the diversity of microbial life, the functions that they complete and the impact of global changes on their function (Locey and Lennon, 2016; Zhou et al., 2020).

1.1.3.2 Soil biological functions in UK gardens

Tiedja et al. (1999) highlighted the massively underestimated scale of soil biological diversity. The existing literature includes detailed taxonomic work focused on classifying soil microbial diversity and quantifying the roles, functions and interactions of this diverse network of organisms. Though valuable for classification the number of species present in soils can mean focus on taxonomic classification steers attention away from understanding a diversity-function relationship of soil microbes and how this applies to field (or garden) contexts (Wagg et al., 2019).

Other key work has highlighted the importance of soil microbially-derived functions to ecosystem services (Costanza et al., 1997; Farber et al., 2002). Biological functions are responsible for many key soil derived ecosystem services such as water regulation, soil formation, support of primary productivity, refugia and reservoir of genetic diversity and resources as well as underpinning many of our recreation and cultural areas of importance (Adhikari & Hartemink, 2016; Barrios, 2007; Dalgleish et al., 2007).

Microbial diversity is essential to ensuring functions are maintained. Many microbes perform multifunctional roles reducing redundancy within the system (Maron et al., 2018). The relationship between fungal and bacterial populations has been highlighted as one of the key belowground associations which ensure ecosystem performance (Wagg et al., 2019).

1.1.3.3 Garden management feedbacks with microbial functions and processes

How fungal and bacterial populations are affected specifically by garden management is not well explored within the existing literature. Common garden management has been highlighted as having benefits to soil health and/or soil quality such as increased SOM in studies such as that of Dobson et al. (2021). What is less well understood is the soil microbial role in this and how these potentially beneficial management practises are affecting the structure and function of the garden soil microbial population.

Human land management becomes a filter through which the soil microbial population is forced to adapt (Louca et al., 2018). The microbiome is considered a dynamic system, characteristic of surrounding conditions, consisting of functionally relevant keystone species and diverse networks of microbes filling ecological niches and delivering specific functions (Banerjee et al., 2018; Berg et al., 2020). Considering this soil microbiome as sum of its living parts and a functional ecological unit, or holobiont, removes focus from individual species and allows examination of entire and multi-ecosystem resilience and the factors which stimulate changes in its structure and function (Rainer and Luttge, 2013).

The soil biological community is both influenced by and is an influence on organic matter inputs. The process of organic matter decomposition provides resources (energy and nutrients) for the microbial community, which adapt and cycle the inputs through microbial biomass-necromass in an effort to return to an equilibrium. This process is dynamic and influenced by input and abiotic factors.

Essential soil functions such as carbon and nutrient cycling and soil organic matter (SOM) formation would not be possible without a diverse and abundant microbial community (Fatichi et al., 2019; Porporato et al., 2003). The functions completed by belowground microbial life are the building blocks of many, if not all, essential "ecosystem services" (Adhikari & Hartemink, 2016; Aubert et al., 2006; Barrios, 2007).

Supporting or manipulating the soil microbial population through informed decision making can ensure soil functions are maintained. Due to the lack of garden-specific peer reviewed information, it is possible that some management practises contribute to soil degradation in garden systems, such as contributing to nutrient accumulation (Lorenz, 2017). Presented in the following chapters is a series of system-specific experiments, the results of which can contribute to how decisions in gardens, allotments and urban green spaces are made and help to mitigate any negative implications of organic matter management. The experimental design and resulting discussion examine the relationship between key domains (bacteria and fungi) in the soil microbiome and characterises shifts in microbiome structure in response to changes to surrounding abiotic factors.

1.2 Research focus

1.2.1 Application of research and underlying themes

As a background thread between the chapters it is proposed for the consideration of the reader that that "quality" (of soil and/or organic matter) is subjective within a garden context and even the lowest "quality" of organic matter can be a useful input if the appropriate microorganisms are able to facilitate decomposition into plant-available forms of nutrients or be useful in specific garden settings.

Using a long-term, garden-specific field trial enabled this work to address a specific gap in the existing literature. Highlighting changes to nutrient stoichiometry and microbiome structure and function as influenced by organic matter additions commonly applied in UK gardens.

Research on the influences of organic matter applications on soil biology and associated stoichiometry predominantly focus on the comparison of farm-yard manure, mineral fertiliser regimes, and/ or incorporation of agricultural materials such as rice husk/ wheat straw (Diacono and Montemurro, 2010; Francioli et al., 2016; Qasim et al., 2022). The variety of organic matter types used in gardens is diverse and distinct from agricultural organic matter additions, more detail of the types of organic matter used in gardens can be found in later chapters. Research is required to evaluate the seasonal and long-term impacts of the use of organic matter additions both in the garden and on wider environmental and ecological scales. Most commonly studied agricultural organic matter inputs include farmyard manure and crop residues, though stable manure is common in gardens kitchen/ garden waste and peat-based composts are not common in agricultural systems.

Studies which provide insight, from long-term data, into soil processes over decadal to century long time-spans help to increase understanding of the dynamic nature of soil and provide valuable data for soil ecosystem models (Sullivan et al., 2022).

Careful and informed application of any resource to soil is essential for reducing losses from systems that do not produce essential food/fuel/fibre. Clearly many gardens and allotments that use organic matter additions do produce vegetables and fruit on a non-commercial scale, but the context of this work is non-intensively managed systems and is centred around a long-term field trial provided by the Royal Horticultural Society (further detail in section). A central focus of existing literature on the effects of organic matter additions to soil use an agricultural frame of reference. To address this imbalance direct-sown, annual, ornamental plant production has been used to represent garden management and as an indicator of the ability of soil to support plant growth and to ensure focus is on microbial function and related soil stoichiometry.

In Chapter 3 there is an assessment and comparison of the legacy effects of ten years of annual organic matter additions on soil stoichiometry plant yield (measured DW/ m^2). The effects of organic matter amendments on plant growth and nutrients were determined and provide a basis for the development of experiments in Chapter 4 and 5. Each chapter or experimental design considers the below-ground effects of amendments as more than a support system for plant growth.

This work is intended to be transferable and applicable to UK garden systems using organic matter amendments. Informing recommendations for gardeners looking to understand the soil microbiome and related functions such as carbon sequestration and nutrient cycling.

Gardeners (including those responsible for allotments) have been shown to be implementing management which is in-line with best-practise recommendations for soil health (Dobson et al., 2021). By focusing on one specific garden practise (organic matter addition) the intention has been to improve best-practise recommendation for gardeners through quantitative analysis of the effects of repeated application of distinct types of organic matter.

There remains opportunity for further work exploring other garden management techniques such as tillage/ digging methods, irrigation, crop and companion cropping, pest and weed control. However, we acknowledge that there are crossovers between many of these methods. For example, the use organic of matter as mulch to supress weed growth or use of companion cropping reducing the spread of pests and disease.

1.2.2 Research objectives

1.2.2.1 Knowledge gaps

Long term field trials provide valuable in-situ data. The Royal Horticultural Society (RHS) provided use of an organic matter field trial at the RHS Wisley Gardens site which commenced in 2007 (more detail of the field trial site is outlined in Chapters 3&4). Long-term data from the trial (2007-2019) was used for the evaluation of legacy effects of organic matter applications in Chapter 3. Sampling of the site in 2018/19 was used for the experiments in Chapter 4. Organic matter samples from the trial were used in Chapter 5. The experiments, data analysis and interpretation of results have been used to address the knowledge gaps identified from a critical review of the literature (Chapter 2) and outlined below:

- Lack of existing research specific to UK garden contexts, including lack of analysis on specific garden management techniques (such as organic matter/ compost addition: type of compost, method by which applied)
- Limited understanding of feedbacks between soil stoichiometry and microbiome structure and function as influenced by non-agricultural management.

- Mechanistic understanding of soil microbially mediated functions as affected by varying inputs to system (such as organic matter addition)
- Analysis of in-situ effects of repeated land management e.g. annual compost addition over long-term timeframes (e.g. >5yrs).
- Comparison of different organic matter additions. Commonly studied examples include farmyard manure and household/ municipal waste. Widely used in gardens but less common in the literature include composted bark, recycled waste products such as compost produced from mushroom cultivation substrates, substrates specific to certain locations e.g. composted bracken and novel products (biochar).
- Quantification of the effects of management on specific soil functions such as respiration.

1.2.2.2 Aim

Based on the literature review presented in Chapter 2 and the knowledge gaps identified the aim of this research is:

To evaluate the long-term and within season effects of garden specific organic matter application on soil microbial community structure and functions associated with nutrient and carbon cycling.

1.2.2.3 Hypothesis

Each experimental chapter (chapters 3, 4 and 5) has its own specific hypothesis. The overarching hypothesis of this research and thesis is:

Distinct types of organic matter applied to soil will influence microbial community functionality through varied carbon and nutrient availability, and long-term application can result in excessive available nutrients, increase in CO_2 output or build-up of soil organic matter depending on changes to microbial community structure.

1.2.2.4 Objectives

To address the knowledge gaps the following objectives were developed to test the previously outlined aim and hypothesis.

1) Complete a critical review of existing research into organic matter additions commonly made to UK garden systems and studies that link input to microbial mechanisms of decomposition functions.

2) Synthesise and interpret fluctuations in soil stoichiometry as influenced by long-term (10years) annual organic matter addition.

3) Evaluate the legacy effects of long-term, annual organic matter input to a garden specific field trial and select treatments for further analysis based on distinct features of input relevant to UK garden contexts.

4) Establish the impact of changes to garden soil function (respiration) as influenced by feedbacks between repeated organic matter applications and soil microbial community structure following 10years of organic matter amendment.

5) Quantify the benefit of organic matter addition on soil microbiological function by applying inhibitory pressures to the microbial population and quantifying resilience of soil function provided by organic matter addition.

6) Provide future recommendations applicable specifically to UK garden organic matter management.

1.3 Summary of thesis structure

This thesis has been written using a paper-format. Each chapter has been developed from a distinct set of analyses and/or experiments with the intention of submitting for peer-review (where indicated). The following synopsis provides a summary of each chapter and an indication of intellectual contribution and relevance to the aims and objectives of the project.

1.3.1 Chapter 2. Carbon cycling processes: A horticultural perspective

This chapter presents a critical review of existing literature on carbon cycling processes in relation to organic matter inputs to UK gardens. As so little peer-reviewed work is available on UK gardens specifically, highlighting a key knowledge gap addressed in later chapters, the review was expanded to include horticultural studies, relevant agricultural studies with transferable data (e.g., farmyard manure applications) and non-UK garden studies.

1.3.2 Chapter 3. Legacy effects of annual organic matter additions on soil organic matter (SOM) and associated nutrient stoichiometry in a 10-year horticultural field trial

Chapter 3 assessed the legacy effects of a long-term RHS field trial (2007-2019) predominantly using a dataset provided by the RHS from an organic matter field trial at RHS Wisley Gardens. Linear modelling and multiple regression analyses have enabled consideration of the interactions between input (organic matter) and output (products of microbially mediated functions). Sampling in the final year of the trial (2019) and analysis of carbon and hot water extractable carbon were completed by the author and incorporated into the data analysis of previous sampling (2007-2018), which was completed by RHS technicians. Lab analyses 2007-2018 were completed externally by NRM laboratories. The hypothesis development, synthesis, and interpretation of 13years of soil chemical and physical data presented in Chapter 3 enabled robust recommendations on organic matter applications in UK gardens.

Other studies have been published from the trial and the analysis in Chapter 3 supports these studies and further contributes to more thorough understanding of the effects of organic matter addition. This would not have been possible without the work of RHS technicians in sampling and data collection, who we specifically acknowledge for their contribution in Chapter 3 and the acknowledgments of this thesis.

1.3.3 Chapter 4. Organic matter additions to horticultural soils: Management for enhancing soil microbial function

This chapter develops on the findings of Chapters 2 and 3 and. The RHS provided use of the field trial (2019-2020) and the author is responsible for all field trial sampling, analyses, analysis of data and interpretation. This chapter considers the impacts of organic matter addition on microbial function (measured respiration, multiple substrate induced respiration - MSIR) and microbial community structure (phospholipid fatty acid - PLFA and neutral lipid fatty acid – NLFA community profiles) as well as microbial biomass. Whilst the focus of this chapter is on the soil microbiome, the microbiome cannot be analysed in isolation from soil chemical and physical characteristics, as such comparisons to the characteristics of the soil and of the organic matter inputs themselves were considered integral to the interpretation of results.

1.3.4 Chapter 5. Allocation of soil respiration to soil fungi and bacteria under different organic matter treatment using selective inhibition

Chapter 5 presents a novel method for implementing inhibitory effects on soil microbial domains by using antibiotics in combination with multiple substrate induced respiration (MSIR - Microresp©). Changes to the structure of the microbial population are quantified and resilience/ maintenance of population structure and function, to inhibitory effects as a result of organic matter treatment, are evaluated and discussed.

This chapter utilised a different soil type (clay-loam) from the RHS field (sandy-loam) in combination with two organic matter types (composted stable manure and composted garden waste) provided by the RHS to provide information on the relevance of results presented in previous chapters on soils with a higher clay percentage.

1.3.5 Chapter 6. Synthesis and conclusions

The final chapter summarises and relates findings of the project to the wider industry context. An assessment of how each chapter relates to the original aim and relevant objectives is presented. An outline of the contribution to knowledge and routes for further work are discussed. Limitations of the study and the findings which enabled formation of conclusions and recommendations from previous chapters are revisited.

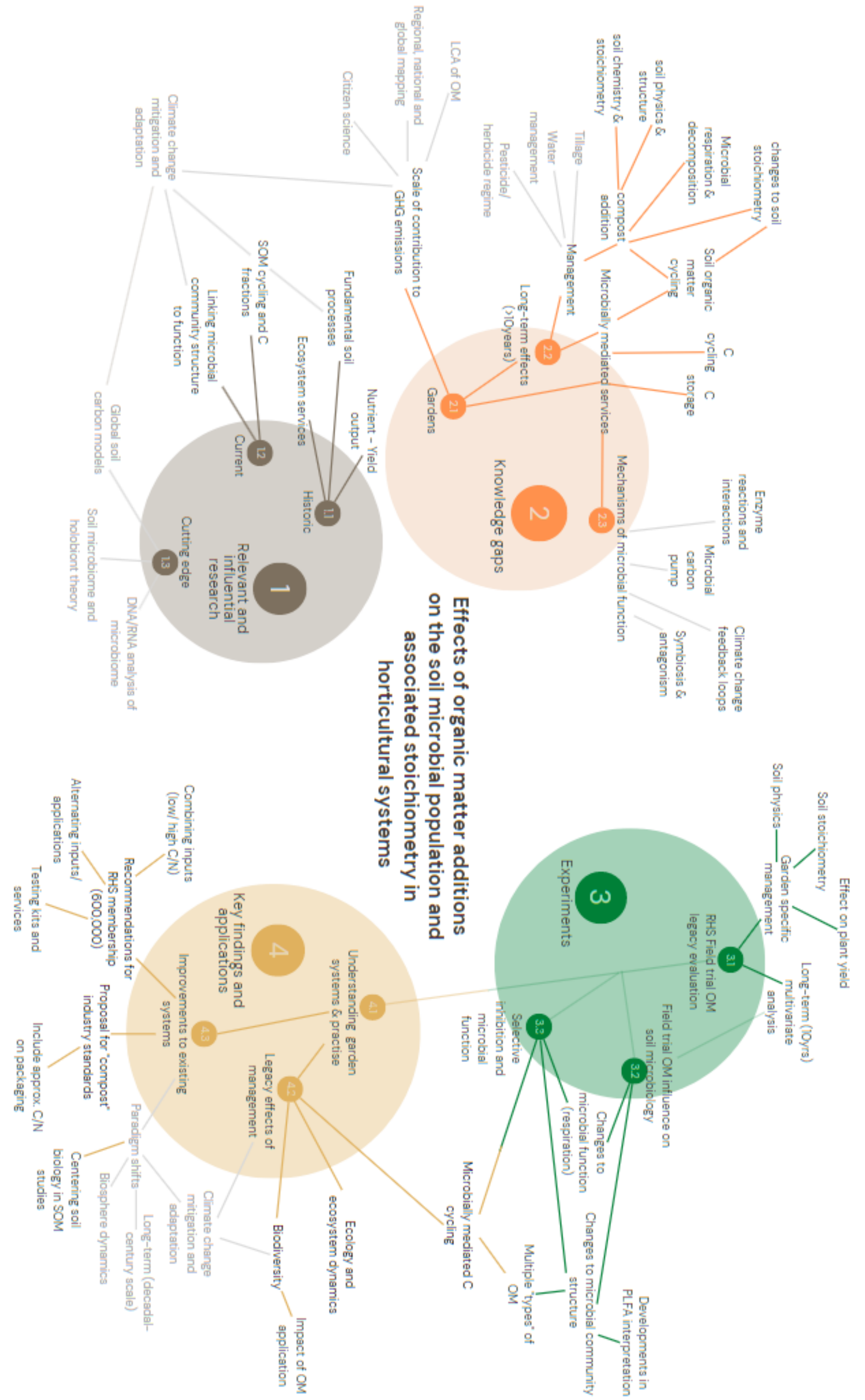


Figure 1-2 Graphic abstract: Map of research framework including - 1. Existing and influential research. 2. Knowledge gaps in existing research. 3. Experiments designed to address knowledge gaps. 4. Key findings from experimental results.

1.4 Definitions

Soil quality - "*…the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health*. Doran and Parkin (1994)"

Soil health – "*Soil health is the capacity of soil to function as a vital living system…*(Doran and Zeiss, 2000)*"*

Soil fertility - *"the ability of the soil to supply essential plant nutrients and soil water in adequate amounts and proportions for plant growth and reproduction in the absence of toxic substances which may inhibit plant growth* (FAO, 1976)*"*

Long-term – For the purpose of this research long-term is considered a time period that encompasses several growing seasons, generally >10years.

Organic matter - any material produced originally by living organisms (plant or animal) that is returned to the soil and goes through the decomposition process (FAO, Bot and Benites, 2005).

Organic matter inputs/ amendment/ addition – material applied to soils for a range of benefits including but not limited to improvements in soil texture, provisions of nutrients, increasing soil organic matter %, enhancing microbial function.

If in relation to the RHS field trial or subsequent experiments, then the following definitions are of terms used to describe the organic matter materials used –

- **Peat** – A commercially made Irish peat-moss compost.
- **Bark** – A commercially made composted bark product, sold and marketed as a "soil conditioner" or "soil improver".
- **Bracken** – A commercially made compost of 100% composted bracken.
- **Mushroom compost** – A commercially made compost made from the substrate remaining after commercial mushroom production.

- **Manure** – A manure-based compost commercially screened and packaged and sold as “composted manure”.
- **Compost** – A garden waste compost produced from garden materials such as grasses, brash and plant material as a result of pruning.

1.5 Research contribution

Following the literature review gardens were identified as increasingly important and under-studied systems. A lack of studies which consider gardens as an integrated land-use with a collective impact on wider environmental systems can be attributed to a lack of evidence-based guidance and long-term monitoring of common practise.

Within garden systems and allotments there is a need for science-backed advice on common practises. As such experimental studies which provide data on long-term and dynamic within -season effects of organic matter or compost addition on soils are necessary. Guidance for decision making based in quantitative research that can be disseminated into publication within the horticultural community will ensure land management influences on global carbon dynamics across all sectors, are adapted to be more resource and carbon efficient.

Novel contribution of this research includes:

1. Identification, through review, of a lack of science-backed gardening advice on compost additions which specifically account for:
    - Variability of organic matter materials
    - Long-term effects (over 10 years or more)
    - Feedbacks and trade-offs between (garden) management and soil microbiology
2. Interpretation of data on the impacts of 12 years of organic matter addition on field trial plots.
3. Development of recommendations for organic matter applications in UK gardens

4. Identification of the need for a framework of minimum standards for labelling of compost material bought and sold to UK gardeners.

1.6 Dissemination from PhD thesis

This section outlines the intended publications, conference presentations and other dissemination of the research presented in thesis.

1.6.1 Journal publications

Publication of Chapters 3 and 5 are in preparatory stages.

Chapter 4 is awaiting review at the European Journal of Soil Science.

1.6.2 Conference presentations

This research has been presented and shared in conferences, seminars, symposiums and group workshops, details of which are listed below:

Hasler, RJ Organic matter additions to horticultural soils: Management for enhancing soil microbial function presented at European Geosciences Union General Assembly (May 2020 - Vienna – moved online due to COVID-19 lockdown restrictions).

Hasler, RJ Organic matter amendment effects on soil microbiology and soil stoichiometry presented at the Royal Horticultural Society’s Annual Symposium (October 2022 – RHS Wisley Gardens)

Hasler, RJ Long-term implications of using woodland by-products as mulch and biochar presented at the Landworkers Alliance Regional Annual Assembly (September 2022 – Coed Esgair Las, Wales)

Hasler, RJ The soil microbiome in garden systems – concepts for consideration when planning a garden. Zero Carbon Britain (2022 – Centre for Alternative Technology, Wales)

Hasler, RJ Organic matter amendment effects on soil microbiology and soil stoichiometry presented at The Forestry Hub Forum (October 2022 – The Forestry Hub, Wales)

Hasler, RJ Organic matter additions to horticultural soils: Management for enhancing soil microbial function to be presented at the Global Soil Biodiversity conference (2023 – Dublin, Ireland)

1.6.3 Other dissemination and impacts

Once published there is opportunity to summarise the findings for grey-literature published by the RHS. The findings will be incorporated into future RHS planning and discussion for their in-house management decisions and for advice provided to membership base and general public.

The findings and information from this thesis are to be used for teaching, training and workshop material for organisations improving their soil management and monitoring processes such as Coed Cymru (Welsh Woodlands), The Centre for Alternative Technology and the Canal and River Trust.

2 Carbon cycling processes: A horticultural perspective

Chapter synopsis

This chapter presents a critical review of existing literature on carbon cycling processes in relation to organic matter inputs to UK garden soils. Key areas are outlined which require future work to improve understanding of gardens and microbially mediated carbon cycling.

2.1 Abstract

The soil microbiome is the epicentre of multiple ecosystem functions; if managed effectively it can serve to mitigate the effects of anthropogenic disturbance on drivers of climate change effects. Land management decisions have a significant impact on the structure and function of the soil microbiome. Yet the general trend within the existing literature is towards evaluating the benefits of the soil microbiome to professional management strategies, while largely omitting potential benefits to gardens and non-professional horticultural systems. Gardening knowledge and experience can help to identify underexplored areas for future research aimed at utilising soil functional benefits, such as microbiome-derived ecosystem services. This review focuses on non-professional horticultural land management decisions, specifically the repeated application of composts or organic matter to soil, and the interaction of this practise with microbially mediated carbon cycling and sequestration. Governing essential biogeochemical processes, the soil decomposer population is integral to the terrestrial carbon cycle. The structure and function of this community has profound direct and indirect effects on plant productivity.

2.1.1 Keywords

Soil microbial population, carbon cycling, horticulture, gardens, organic matter, compost

2.2 Introduction

The soil microbial community mediates key ecosystem services, such as carbon cycling (Jiang et al., 2020; Li et al., 2019; Maron et al., 2018a), and it responds to the stressor or selection pressure created by various management techniques and climates (Louca et al., 2018). Understanding the effects of land management decisions on the soil microbiome is therefore key for evaluating the climate change mitigation potential of soil microorganisms (Jansson and Hofmockel, 2020; Schloter et al., 2018). The dynamic relationship between carbon input and carbon use by microbes in the terrestrial carbon cycle is not fully understood (Louca et al., 2018). However, the field of research on functional processes that are completed by the microbial community in terrestrial system functioning is well established (Jin et al., 2018). Horticultural systems utilise annual inputs of carbon in the form of organic matter (soil conditioners) and provide an excellent frame of study for the effects of this on ecosystem structure and function (Alexander and Bragg, 2014; Sharma and Alam, 2013). Within soil modelling the multitrophic interactions which result in key soil functions are often necessarily simplified (Soliveres et al., 2016). The complexity of the soil biological system may have both positive and negative effects on plant productivity, depending on the system (Mezeli et al., 2020). What prompts changes from mutualistic to antagonistic microbial associations is less well explored. This missing link is essential to our understanding of why soils and microbial decomposition and respiration can result in changes from carbon sink to source (De Deyn, 2017).

Soil carbon stocks are estimated at more than five times the atmospheric carbon stock (USGCRP, 2018). In order to ensure carbon losses from terrestrial stores to the atmosphere are reduced we must understand the complex relationship between microbial stabilisation and mineralisation (Bowden et al., 2004; IPCC, 2022).

Predicting the effects of interactions between aboveground inputs and belowground functions is an evolving debate in soil ecology, plant physiology, geosciences and other related fields of research (Sokol and Bradford, 2019). Understanding garden management factors that influence life cycles and strategies of organisms *in situ* is a multidisciplinary effort. Microorganisms mediate carbon cycling functions, quantifying feedback between domains and the inputs which influence their community structure assist management decision making. This knowledge can minimise trade-offs and enhance synergies across a range of systems (de Vries et al., 2013; Deyn, 2017).

The relative abundance of antagonists and mutualists within the soil food web can be changed by the provision or restriction of resources – making this a manageable characteristic of soil. For example, adding organic matter can increase or decrease competition for nutrient resources, increase disturbance effects on the microbial population following incorporation of organic matter, or change the relative abundance of functional groups (e.g. bacteria and fungi) (Deyn, 2017; Grime, 1977; Martínez-García et al., 2018a).

Useful tools for exploring the ability of an ecological community to cope with stress or resource restriction date back to behavioural or lifestyle strategy frameworks developed by Tilman (1982) and Grime (1977), later reviewed by Sauter et al. (2021). The Competitor - Stress Tolerator - Ruderal (CSR) relationships described by Grime (1977) are explored further in Figure 2-1 (adapted from Ho et al., 2017). Originally used to describe plant communities, the three **C-S-R** axes provide a way of interpreting the lifestyle strategies that organisms within a population utilise. **C**ompetitors function in moderate nutrient availability/low disturbance where they compete with each other for nutrients by varying foraging strategies, **R**uderals are adapted to high nutrient/disturbance regimes and **S**tress tolerators to low-nutrient or adverse environmental conditions (e.g. drought).

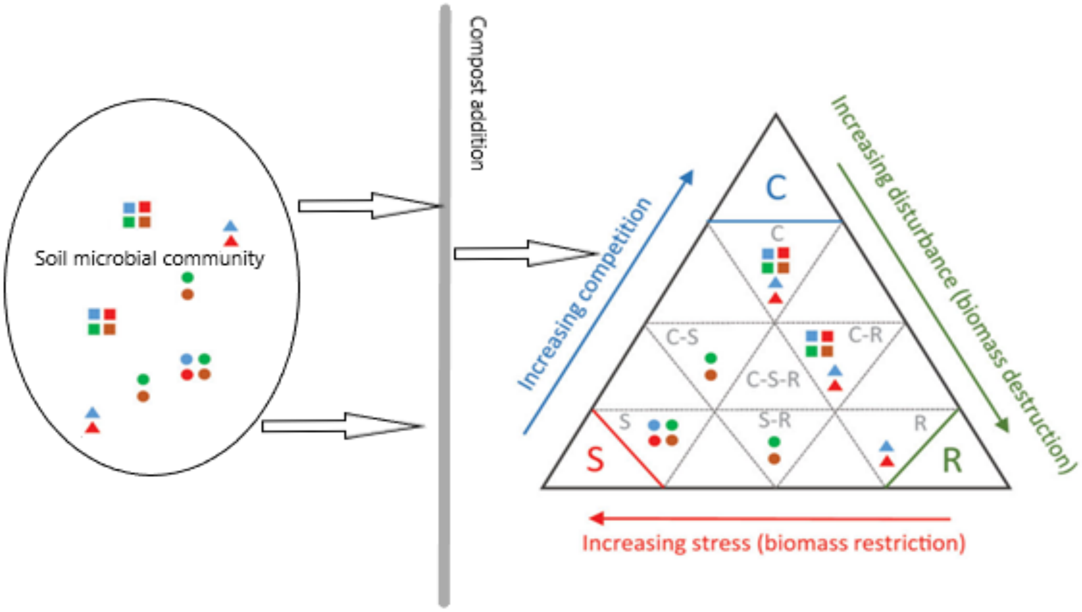


Figure 2-1 Schematic diagram representing C-S-R lifestyle strategies (adapted from Ho et al., 2017). Coloured shapes denote different soil microbial functional groups and their associations, this community is pushed through a "filter" (in this case compost addition) and the structure of the community changes to adapt to the input as competition (for carbon or nutrient resources in the addition), disturbance (as a result of incorporation of the incorporation of the input) and stress (due to composition of the input).

Other theories to describe dynamic ecological communities have been developed and updated as more is understood about adaptation and lifestyle strategies of living organisms (Díaz-Sierra et al., 2017). Goldberg and Novoplansky (1997) highlighted that these theories, originally used to examine plant lifestyle interactions, often make assumptions on continuity of resource availability, a dynamic process which is mediated by soil organisms. Using the same theories to assess microbial lifestyle strategy, or incorporating soil microbial processes in these theories relates to more recent work considering the soil microbiome or plant holobiont (Lemanceau et al., 2017). Land management, stress, disturbance and direct organic matter or nutrient inputs to the soil system all influence the life cycles of the soil microbial population (Deyn, 2017; Díaz et al., 2016; Ho et al., 2017).

This review focuses on changes to the soil microbial community structure and function created by garden land management, such as compost and organic matter addition (Figure 1-2). Ways of utilising the potential of the microbial community in a transition towards more sustainable land management are outlined as recommendations and conclusions.

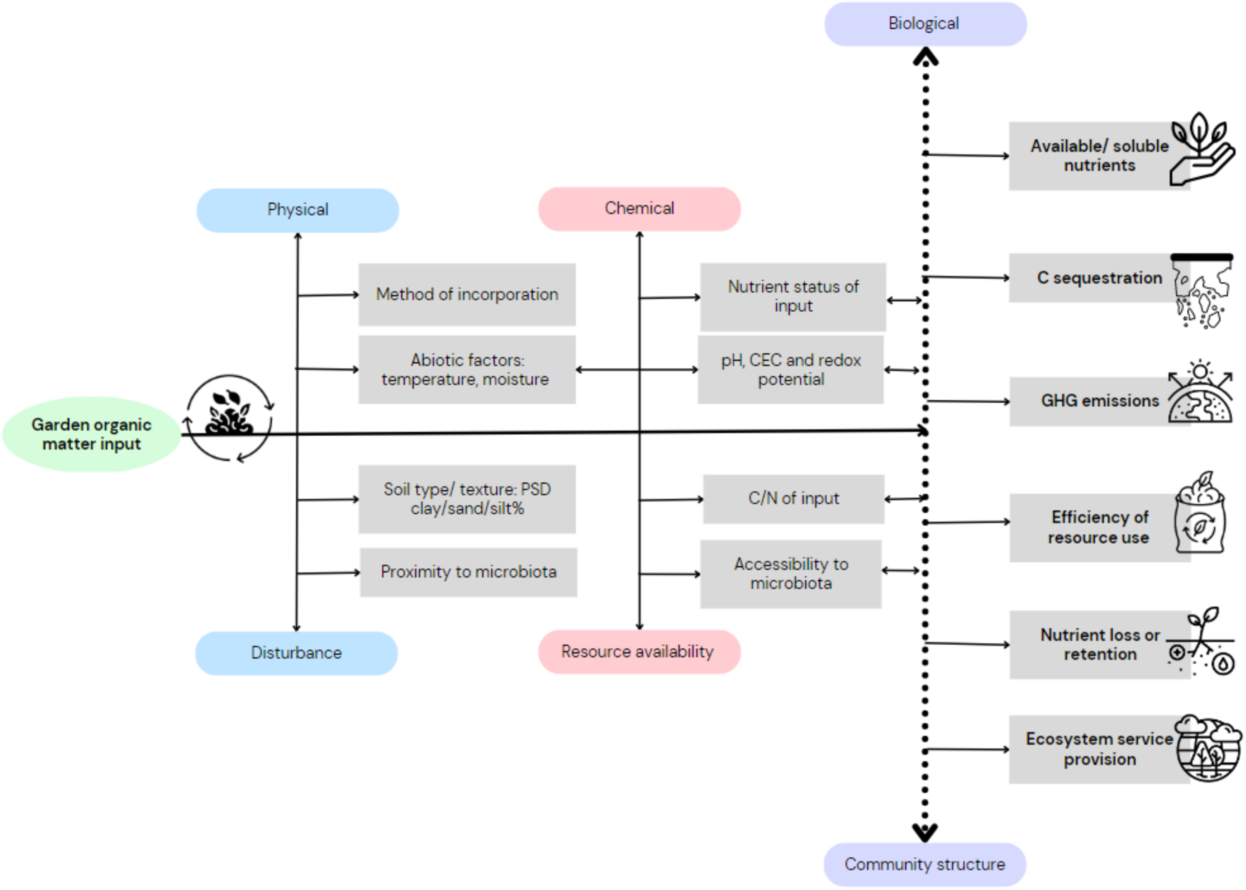


Figure 2-2 Schematic diagram of review focus – the impacts of organic matter additions in UK gardens.

2.2.1 Horticultural soil management

A growing UK population with increased awareness of climate change impacts has led to a rise in conservation activities, particularly among non-professionals (Dehnen-Schmutz et al., 2010; Mimet et al., 2020). The contribution of non-agricultural systems to ecosystem service provision is increasingly included in valuations and models of Earth's systems (Kendal et al., 2012). According to Defra, total UK land used for gardens, allotments, recreation areas and urban green spaces is over 10% of total UK land area (Defra, 2018). This is a significant proportion of UK land managed and influenced by society, culture and local community. Ensuring that non-agricultural land management decisions are climate focused and informed by peer-reviewed research requires increased focus on the multitudinal benefits non-agricultural land can have on surrounding ecosystems, services and society (Mimet et al., 2020).

Research focused on non-agricultural systems such as ornamental and recreational horticulture shifts emphasis towards plant quality traits and ecosystem functioning rather than just production (yield). The UK ornamental industry is worth an estimated £1.4 billion per annum, a 4.8% increase on 2016 (Defra, 2018). Fruit and vegetable production combined are worth approximately £2.3 billion per annum in the UK (Defra, 2018).

An Oxford Economics report commissioned by the Royal Horticultural Society (RHS), Horticultural Trades Union (HTU), Agriculture and Horticulture Development Board (AHDB) and the National Farmers Union (NFU) concluded that ornamental horticulture contributed £24.2billion to UK GDP in 2017 (Oxford Economics, 2018). The report also highlighted that more than half of UK adults have some involvement with gardening and projected this to increase in line with government investment in public green spaces (Oxford Economics, 2018). Overall this represents a large portion of the UK population directly or indirectly influencing land management and a significant contribution to the UK economy. Preserving productivity and improving environmental sustainability of the horticultural industry can be facilitated by understanding key microbially mediated soil functions in horticultural practice.

Aside from supporting production, the soil may be investigated as a functional system which delivers multiple services benefitting humanity and the natural environment (Gomiero, 2016). More specifically, there is evidence that domestic gardeners may have both detrimental and supportive effects on ecosystems and the environment. Common issues include adverse environmental effects from inappropriate use of pesticides and mineral fertilisers through inaccurate identification of the problem or application of the product (Cameron et al., 2012), offsite degradation of ecosystems such as peat bogs (Alexander et al., 2008), nutrient losses as a result of compost and fertiliser inputs (Small et al., 2019). Beneficial effects of gardens can include provision of havens for diverse wildlife, buffering against flooding and improved air quality (Cameron et al., 2012). There are numerous studies which report benefits of garden ecosystems for human physical and mental wellbeing (Millenium Ecosystem Assessment, 2007; Sirakaya et al., 2018). Cultural heritage is also closely linked with local ecosystems and this relationship is important to how we value our natural capital and manage our relationship with it (Costanza et al., 1997; Farber et al., 2002; Riolo, 2019). With this in mind, and projections for increased urban spread, it is important not to exclude amateur gardeners from discussions on ecosystem functions, services and environmental impacts.

2.2.2 Climate change

The IPCC has projected various scenarios of the impacts of climate change, which depend on the effectiveness of human efforts at curbing CO_2 output and global temperature increase (IPCC, 2018). In order to mitigate or adapt to these changes it is imperative that we work with our natural resources and value them as an integral part of our survival. Projected and current effects of climate change include loss of biodiversity resulting in reduced ecosystem functioning and provision of services, increased CO_2 emissions, increased potential for soil erosion, increase in prolonged rainfall events and an increase in drought occurrence (Borrel et al., 2018; Bowden et al., 2004; Chapin III et al., 1998; Lal et al., 2011; Lowe et al., 2019). Horticultural systems, such as gardens, when managed for biodiversity can help to improve the connectivity between other habitats that exist in intensively managed agricultural landscapes, parks, woodlands, nature reserves and within urban areas and the wider landscape (Mimet et al., 2020).

Land management decisions, such as digging or turning over soils in horticultural settings, have been shown to increase the loss of CO_2 from stable or inert pools into more dynamic atmospheric pools (Sokol and Bradford, 2019). The result of this is a feedback loop whereby land systems are increasingly forced to cope with the effects of a rapidly changing climate whilst contributing to the same changing cycle (Crowther et al., 2015b; Nottingham et al., 2019). To move beyond land management decision-making based on coping with the symptoms of climate change towards a model where Earth's assets, resources and organisms are utilised to their full potential requires increased understanding of the microbial world, and in particular the carbon cycling fraction of the microbial community (Gougoulias et al., 2014; Maron et al., 2018a). How we value our place in Earth's ecosystems and how ecosystem functioning is resilient to anthropogenic climate change is an evolving field within ecology. Soil related research disciplines are expanding to involve increased monitoring of human impact and reliance on natural resources (Brevik et al., 2015; Sykes et al., 2019).

2.2.3 Valuation of Earth's systems and resources

Ecosystems have been defined as

"…*a dynamic complex of plant, animal, and microorganism communities and the non-living environment, interacting as a functional unit"* (Dalgleish et al., 2007; "Millenn. Ecosyst. Assess.," 2005).

Most research defines ecosystem services according to function (de Groot et al., 2002). In de Groot's method, functions and processes are defined and considered "ecological sub-systems" behind the benefits and services. It is possible that garden systems are of high value using this approach, but more research is needed to quantify the multifunctionality of gardens. De Groot and colleagues (2002) emphasised a need for a unified and consistent way of comparing the value of different ecosystems and suggest a move away from the use of the term "value" in a solely economic context. Value is referenced as being ecological, socio-cultural, and inevitably economic (de Groot et al., 2002). Twenty years later current literature reflects this emphasis and the trend for more descriptive, nuanced studies and definitions of ecosystem services (Abson et al., 2014; Gimona et al., 2023; Mandle et al., 2021). The need for integration of standardised definitions for valuation across disciplines is increasingly highlighted as important for reducing the fragmentation of related content from different areas of expertise (Abson et al., 2014; Plaas et al., 2019; van Bruggen et al., 2019a). Holism and the classification of ecosystems as entire functional units, including the human facet of management, is a strong theme in the emerging literature on how we value land systems (Castree, 2016). In particular there is growing interest in agroecological theory and the potential for agricultural systems to partition focus to ecological gains (Anderson et al., 2020).

More recently research into ecosystem services, particularly those provided by soils, has included new terminology such as “Life Support Functions (LSFs)” rather than the more widely used “ecosystem services” (Schloter et al., 2018). This new terminology is intended to better represent the reliance of living ecosystems on specific processes. Linking these functions to provisioning of life support rather than to the word “services” denotes the essentiality of soil preservation.

LSFs relate to specific functions, however the broader terms soil health and soil quality, and indicators of both, are discussed extensively in the literature (Karlen et al., 2019). The role of the soil microbiome is integral to both soil health and soil quality (Dubey et al., 2019). Soil quality is a term which is defined in a multitude of ways depending on discipline, research focus, country or industry. A common definition used for soil quality is taken from Doran and Parkin (1994):

“*…the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health*. (Doran and Parkin, 1994)”

Soil health can be considered the result of chemical, physical and biological factors which result in a soil’s ability to function (Tully and McAskill, 2019). However Tully and McAskill’s definition does not centre the biological element that has become more essential to the concept of “soil health”. Doran and Zeiss (2000) centre biology as the key component of soil health with their definition:

“*Soil health is the capacity of soil to function as a vital living system…*(Doran and Zeiss, 2000)*”*

These definitions encompass some aspects of the description of LSFs. However, the generally accepted description of soil health could be considered as broader, encompassing all the potential functions of soil rather than a select set of essential LSFs. These functions are essential for the delivery of soil derived ecosystem services including water cycling and purification, carbon sequestration and organic matter cycling, biodiversity, support of plant growth and health (Doran, 2002; Lal, 2004; Miguel A. Altieri, 1999; Porporato et al., 2003).

2.2.4 Carbon cycling and soil organic matter (SOM)

Trends in land management such as increased tillage and cultivation have increased the loss of carbon from soil to atmosphere (Gougoulias et al., 2014). However, belowground microbial carbon cycling functions, and the drivers for related fluxes are not well understood. Effective steering of microbial carbon use efficiency is a promising tool for managing these soil carbon fluxes (Kallenbach et al., 2019; Ye et al., 2019). Different management approaches, such as crop rotation (Kallenbach et al., 2019) and pesticide use (Dornbush and von Haden, 2017), are known to impact microbial carbon use efficiency (CUE) (Ye et al., 2019). However, information in the existing literature on how microbial CUE is affected by organic matter inputs to soil in garden systems is limited. Substrate "quality" (composition, C/N, and ratio of labile to recalcitrant material) is known to be key in initiating microbial metabolic pathways that involve the decomposition or stabilisation of organic matter of varying recalcitrance (Qiao et al., 2019). Common horticultural practice, such as the repeated application of organic matter as soil conditioners for improvement of soil organic matter (SOM), have an impact on carbon sequestration and carbon storage. These two terms are differentiated; the former outlines removal of atmospheric CO_2 into stable belowground stores (Chenu et al., 2019). The latter emphasises the long-term result of sequestering carbon (>100years) in stable soil carbon fractions (Lorenz and Lal, 2015).

The persistence and quality of SOM involves feedbacks with the soil microbial community (Schmidt et al., 2011). Soil organic matter amendments improve soil moisture holding capacity, decrease bulk density, increase nutrient cycling and support microbial communities by providing energy sources (Aguilera et al., 2013; Bronick and Lal, 2005; Martínez-García et al., 2018a; Schmauder, 2007). The emergent properties are dependent on whether the application has been considered in relation to the specific soil type, weather, location and composition of the organic matter itself (Borrel et al., 2018). Organic matter amendments influence soil microbial community structure and function by changing the availability of resources or changing the structure of habitat (Martínez-García et al., 2018a). Changing the chemical composition of resources available to the microbial community results in changes to soil respiration (Cleveland et al., 2007; Martínez-García et al., 2018a). Small changes in soil respiration can result in larger collective impacts of garden land on global CO_2 emissions (Bowden et al., 2004).

Legacy effects of organic matter addition can have significant influence on microbial decomposition rates through manipulation of soil C/N, pH, water holding capacity (Lal, 2004; Muriithi and Yu, 2015). For example, repeated addition of peat can have an acidifying effect, addition of composted bark can change soil tilth and increase the C/N of the soil facilitating fungal groups (Dissanayake and Hoy, 1999). Changes can be short-term or within season and/ or result in legacy effects on soil properties. Lee et al. (2021) found that applications of peat-moss to soil had a significant acidifying effect on soil within two weeks of application and which lasted the duration of their trial. More specific discussion on the legacy effects of different organic matter additions to soils is provided in chapters 3 & 4. Organic matter addition can provide plant nutrients and reduce losses of nutrients (e.g. ammonium and available nitrate) from soil, changing the C/N (Latifah et al., 2019). Addition of compost can significantly reduce the need for artificial fertilisers (Silvenius et al., 2016). This depends on the composition of the substrate, method of application and other factors in gardens such as conservation tillage methods, more commonly referred to by gardeners as “no-dig” methods. Benefits of conservation tillage methods in horticultural systems include increases in sustained soil moisture, reduction in surface runoff and a reduction in weed competition with cultivated plants (Swenson et al., 2004). All these factors have significant implications for soil microbial functioning and recent literature is also changing our understanding of how abiotic factors interacting with microbial lifestyles result in changes to terrestrial carbon cycling.

The composition of substrate added to soil as organic matter inputs has been shown to have a more significant effect on soil carbon stocks than temperature (Oldfield et al., 2018a). Though temperature is not to be discounted as an important factor driving SOC fluctuations a new paradigm which links input and turnover of SOM to microbial activity and biomass/ necromass is an interesting development in the literature (Crowther et al., 2015a; Oldfield et al., 2018a). Microbial necromass contributions to soil carbon are increasingly seen as a key area of research in recent literature (Crowther et al., 2015a; Luo et al., 2020; Miltner and Kästner, 2020; Qiao et al., 2019).

The quality of organic inputs to a soil can have a significant bearing on soil carbon stocks (Kallenbach et al., 2019). In addition, inclusion of the proximity of soil organic matter to microbial life in soil carbon models is helping to explain the significance of soil biology in terrestrial carbon cycling (Dungait et al., 2012). Harnessing SOM formation through understanding the effects of the organic matter inputs made in managed systems is an essential development when considering the role of horticultural land in buffering some of the trade-offs between agricultural land management or high input production and ecosystem service provisioning (Villarino et al., 2019).

2.3 Knowledge gaps and emerging trends

2.3.1 The soil microbiome

Food-web mapping and expertise on specific microbial groups are abundant but the complexity of the soil microbiome means there are a wealth of characteristics not understood (Bongiorno et al., 2019; Curry and Schmidt, 2007; Ferris et al., 2001; Louca et al., 2018; Scheu and Falca, 2000). When species or taxa are considered in isolation from wider soil system dynamics there is detailed understanding of their behaviour and lifestyles (de Vries et al., 2013). Emerging from the literature is exploration of links between taxa within complex microbial food webs and ecosystem response to climate change and human management (van Bruggen et al., 2019a). Soil biology is often still considered a black box within soil modelling (Dubey et al., 2019; Tiedje et al., 1999). Specific data to inform more mechanistic modelling is lacking, even where the process or function completed is well documented (Fatichi et al., 2019; Louca et al., 2018; Schmauder, 2007). While soil characterisation provides a baseline for the understanding of soil processes, it is the interaction of this with land management and climate change effects which provides more detailed understanding of the myriad of microbial interactions found in garden systems (Dewaelheyns et al., 2013; Mimet et al., 2020). Pressures from outside the food web are considered for the purposes of this review as garden management (specifically organic matter addition).

The necessity for increased understanding and awareness of microbial ecology has, in part, been prompted by the IPCC projections of climate change effects (Bradford et al., 2016; Hoegh-Guldberg, O., D. Jacob et al., 2018; IPCC, 2022).

The effects of these projections, such as temperature increases or increased flood potential, requires improvements on predictions of how organisms mediating key functions such as carbon sequestration will adapt to, or contribute to, adverse effects of climate change scenarios (Bradford et al., 2016; Cavicchioli et al., 2019).

2.3.2 Soil microbiome and global change interactions

The extent to which the biological carbon decomposer community is resilient to climate change is the link between soil microbial ecology and global climate change mitigation strategy (Borrel et al., 2018). Predictions include a significant influence of warming effects on the fungal decomposer population (Crowther et al., 2012), warming effects which increase decomposition and respiration rates resulting in carbon losses from soil into the atmosphere (Cavicchioli et al., 2019) and thawing of permafrost making more carbon available for microbial cycling (Hoegh-Guldberg, O., D. Jacob et al., 2018).

Increased precipitation and more erratic rainfall events can result in water-filled pore space and anaerobic conditions (Allan and Soden, 2008). This causes a shift in processes completed by the microbial community such as increased methanogenesis and denitrification (Jansson and Hofmockel, 2020; Louca et al., 2018). The global release of carbon from microbial decomposition of soil organic matter is estimated at 50-75 Pg annually (Crowther et al., 2015b). Relevant studies improve the accurate prediction of how microbes evolve and adapt their physiological strategies to cope with temperature increase, drought, waterlogging and flood (Cavicchioli et al., 2019; Crowther et al., 2015b; Lal et al., 2011).

Potential of carbon sequestration of current agricultural systems is estimated at more than half of the carbon lost from productive systems historically (Lal, 2004). The value of work examining agricultural management for improving carbon storage in degraded soils is well acknowledged (Lal, 2004; Lal et al., 2011). Novel ways of assessing the GHG output from agricultural systems such as that of Rosenzweig *et al.*(2020) combine GHG emissions from supply chains with those from within specific farming systems. They estimate that GHG emissions from agriculture are 21-37% of total anthropogenic GHG emissions (Rosenzweig et al., 2020). However there is a clear opportunity for research on the potential of garden, allotment, park, and urban greenspace soils managed by gardeners to complement existing efforts, and to contribute to carbon sequestration targets.

2.3.3 Organic matter in gardens

Gardeners are often anecdotally or culturally guided (Lee and Matarrita-Cascante, 2019a, 2019b). This repeated and experiential methodology can be a good way of maintaining and improving gardening practice (de Oliveira and de Lima, 2020; Kirkpatrick and Davison, 2018). For example, if a benefit is noticeable, such as reduced bulk density or improved tilth from addition of organic matter, then the action tends to be repeated (Snyder et al., 2016). However, issues can arise when there is little monitoring of the long-term effects of land management decisions on ecosystem processes and properties. The addition of organic matter to gardens may improve soils' tilth, but the rate of decomposition and how inputs are transformed into stable SOM pools depends on how easily soil decomposers can assimilate the inputs (Oldfield et al., 2018a; Schloter et al., 2018).

Stable, or slow-cycling SOC, has been shown to be 50-80% microbially derived (Sokol and Bradford, 2019). Stable or heavy soil carbon fractions are generally considered as having multidecadal turnover rates and labile or light soil fractions have, at a maximum, decadal (Neff et al., 2002). These feedback loops represent knowledge gaps to advance our understanding of ecology by examining non-agricultural systems and systems without a focus on primary productivity, such as gardens.

Home composting often helps to create closed systems by utilising material that would otherwise be considered waste. Material such as garden waste, wood chippings and locally available plant material such as bracken, all have considerably different chemical composition and effects on the soil once applied.

2.3.4 Future research focus

An emphasis on studies which recreate non-agricultural management in repeatable experimental designs is required to improve data on the biological element of soil models (Fatichi et al., 2019). Though there are good examples of this using agricultural practise as a framework (eg. Rothamstead, Saxmundham, IBERS) the equivalent for horticultural and garden soils would be of value. By doing this the scientific community can provide mechanistic information for linking function to robust indicators. Developments in genetic technologies have deepened understanding of the role of soil microbes in governing biogeochemical processes (Kuijken et al., 2015); while improved carbon fraction analyses have been linked to microbial respiration and decomposition functions (Keskin et al., 2019; Oldfield et al., 2018a).

Focusing on returning native organic matter to managed soils will facilitate closed-loop systems utilising organic waste materials for ecosystem benefit and carbon sequestration (Nayak et al., 2019). Other interesting experimental variables for exploring the response of the soil decomposer population to garden management choices include tillage methods, use of cover crops and mulches, bio-control and pesticide applications, and the use of bio-stimulants or microbial inoculants (Bongiorno et al., 2019; de Souza et al., 2016; Mahnert et al., 2018; Martínez-García et al., 2018a).

2.3.5 Assessment of non-agricultural soils

Soil includes liquid, gas and solid phases, incorporating organic and inorganic fractions within the soil matrix (Bünemann et al., 2018; Martínez-García et al., 2018a). Environmentally, soils are essential to many processes. Socially, soils are used for multiple purposes and benefit. More studies are required which account for the multifunctionality of soils by examining processes in situ and in a variety of contexts (Bünemann et al., 2018). This includes both agricultural and non-agricultural land, different soil characteristics, different and opposing management regimens and influences of different climates (Schulte et al., 2015).

Horticulture and garden land-based studies provide valuable data on large areas of managed land. The average garden is a dynamic space and source of a multitude of ecosystem service benefits which go beyond the traditional consideration of gardens as a culturally significant or ornamental space (Mimet et al., 2020). Behind these systems is a diverse and dynamic microbiome which holds significant capabilities for adaptation to a range of unpredictable scenarios (Chenu et al., 2019; Ho et al., 2017). Knowledge of the microbiome will help us become more adaptable as gardeners of the future. Further it ensures that zones dedicated to preserving ecosystem service provisioning are efficient and well placed among intensively managed zones (Mimet et al., 2020). Thus increasing habitat and ecosystem connectivity noted in the literature as essential to robust ecosystems (Bagstad et al., 2013).

Microbes and their interactions as influenced by horticultural management are explored as advances in technology become increasingly accessible. The abundance, magnitude of species diversity in a soil ecosystem is quantifiable with the use of low cost genetic sequencing (Kuijken et al., 2015). The use of this information is valid from more than a taxonomic perspective. It is known that the soil microbiome influences plant phenotype and is influenced by land management (Li et al., 2019). Functional metagenomic methods as well as functional group analyses which link microbial community composition to functional attributes are providing increased levels of understanding of the relationship between soil communities and their environments (Garibay-Valdez et al., 2019). Considering the soil characteristics surrounding the microbial population enables adjustments to be made to the abiotic conditions of those surroundings through change of management (Fan and Liang, 2015; Oldfield et al., 2018a). To do this multiple studies are required that manipulate conditions and analyse the changes in population function and structure (Mezeli et al., 2020).

2.4 Conclusions

Discussions surrounding the value of a living soil are abundant in the literature on soil processes, functions and structures. In part this is due to the evidence provided by an increase in interdisciplinary collaboration. The assimilation of this information by industry has resulted in more informed practise such as the inclusion of measures of microbial biomass and community structure in soil quality assessments. The importance of the soil microbiome in maintaining stability of the terrestrial carbon cycle has become clearer and the techniques for understanding this relationship are more available.

This review made a critical evaluation of the available literature on garden land. What is clearly lacking in the literature is a systemic investigation of land which is used daily and managed yet is not solely focused on production. Garden land has the potential to benefit society beyond an economic context. Optimising our influence on microbial function may help meet the required reduction in carbon emissions outlined by the IPCC. Through targeted research efforts, the scientific community can help gardeners to harness the efficiency of biological processes by adapting garden management of microbially mediated carbon cycle to increase soil carbon stocks, soil quality and soil health.

3 Legacy Effects of Annual Organic Matter Additions on Soil Organic Matter (SOM) and associated nutrient stoichiometry in a 10-year horticultural field trial

This chapter presents the analysis and interpretation of long-term soil and plant data collected from the RHS Wisley Gardens field trial (2007-2019). It provides the background for the experiments in subsequent chapters and the concludes with findings based on cumulative effects of 12years of annual organic matter additions. The influence of different types of organic matter applied to field trial plots on soil stoichiometry are explored and related to knowledge gaps and the formation of recommendations for industry.

3.1 Abstract

Improving soil organic matter (SOM) in productive systems is an important objective for land managers globally. Addition of a range of organic matter types to soil on an annual basis is a common gardening practice. A long-term field trial based at RHS Wisley gardens was used to assess the chemical and physical changes of annual applications of common (UK) garden organic matter additions over a 10-year period. Organic matter (six types: stable manure, peat, composted bark, composted bracken, garden waste compost, spent mushroom production compost) were compared to an unamended control and a mineral fertiliser treatment (N, P, K, Fe, Mg, applied at a rate prescribed for optimal plant growth of each years' cultivar) which was used as a comparison for management with solely organic matter input. A flowering plant was cultivated each year and measurements of yield (DW kg/m^2) were taken. Organic matter treatment resulted in significant legacy effects on nutrient status, bulk density, pH and plant yield with the result dependent on organic matter type. The quantity of SOM increased and plateaued by the middle of the trial in manure, bracken, garden compost, mushroom compost treatments. Peat treated plots continued to accumulate organic matter. Significant crop failures hindered the final years of the trial. Correlations of this with the build-up of nutrients (K, Mg, Fe) to levels detrimental for plant growth which are outlined and explored in the results and discussion sections. It was concluded that legacy effects of long-term annual organic matter amendment result in excess available nutrients with implications for unwanted environmental impacts. Applications should be alternated, or materials combined to enhance the desired effects of addition and mitigate detrimental effects of organic matter addition on the wider environment.

Keywords: Field trial, organic matter, plant available nutrients, legacy effects, soil organic matter

3.2 Introduction

Domestic garden soils are an important and abundant land-use category, it is estimated that 30% of UK urban areas are categorised as garden land (Environment Agency, 2021). According to the Office for National Statistics (ONS) functional green space (parks, gardens, playing fields, golf courses and allotments) total 124,800 ha of UK land and publicly accessible green space (parks, gardens, playing fields and allotments) comprises 84,610 ha of UK land (ONS, 2019). Gardening is often cited in grey literature as being an environmentally friendly past-time with gardeners participating for a wide range of reasons (Chalmin-Pui et al., 2021). Gardens account for a significant landmass both in the UK and abroad. Following the Covid-19 pandemic there has been a widespread uptake of gardening and increased interest in garden products and services from the 24million recorded gardens in the UK (GovUK, 2022). This demand requires increased provisioning of best practise guidance backed by research specific to gardens. This will ensure that the environmental effects of an increase in popularity results in beneficial impacts for ecosystems, society, and industry. Gardens support ecosystem service provisioning and are a well-established and embedded part of UK life. Considering this it is surprising that literature specifically focused on garden soils and management specific to gardens is limited. Garden systems are an integral part of the UK patchwork of land-uses which include urban, agricultural and industrial (Chalmin-Pui et al., 2021). Gardens can be found in and around all other land-uses. Addressing the lack of quantitative data on this specific land-use will improve our understanding of soils in non-agricultural settings, enabling improved management of a large body of UK land.

The majority of gardeners have access to organic matter inputs, and it is common for these inputs to be applied annually. A Flemish study included a survey of domestic gardeners and found that 49% of the respondents used chemical or mineral fertilisers in their gardens (Dewaelheyns et al., 2013). Aside from carbon storage and the effect of this on GHG emissions which contribute to global warming, increasing SOM has been shown to improve soil structure, water holding capacity and support of soil microorganisms (Hoffland et al., 2020.). Nutrient availability and preserving or improving soil organic matter (SOM) are important aspects of gardening practice and horticultural systems (Tresch et al., 2019). Common garden inputs can increase nutrient provisioning from organic matter inputs, made in house or local to garden systems (Dewaelheyns et al., 2013). Preserving or limiting losses of soil organic carbon from soils, and adding carbon to soils, is highlighted by the IPCC as a cost-effective method of strengthening the global response to climate change, providing co-benefits across industries (IPCC, 2022). Loss of SOM contributes to pollution of water courses through soil erosion and run off, decreased soil carbon status and reduced productivity (Hoffland et al., 2020). The agricultural industry is affected by world events which drive the cost of fertilisers (Harari et al., 2022). There is a need for increased assessment of how alternatives perform in a variety of UK systems to adapt to rising costs and potential shortages, particularly in systems not responsible for provision of food, fuel and fibre.

Applications of organic matter to garden soils can have both beneficial and undesirable effects. Of benefit is the reported increase of soil fertility, reduction in nutrient losses from soil, enhanced activity of beneficial microorganisms and improved soil structure and stability (Abujabhah et al., 2016; Annabi et al., 2007; Fungenzi et al., 2021; Latifah et al., 2019). Unwanted effects of compost addition can include build-up of excess available nutrients or contribution to nutrient losses due to overapplication and potential import of micro-/ nano-plastics to soil systems (Small et al., 2019; Small et al., 2023; Bandini et al., 2022). There is a lack of studies which compare a variety of inputs and the legacy effects of these inputs (Ho et al., 2022; Yang et al., 2022). Legacy effects of yearly applications of a single, specific organic matter to soil can result in the build-up of available phosphorus leading to nutrient pollution in localised areas (Small et al., 2019). It is therefore essential to consider the fate of the nutrients contained in the materials applied to soil and to consider a multitude of effects from organic matter applications. Adding organic matter with a single aim, e.g. increasing SOM% or improving soil structure, may lead to outcomes which are detrimental to plant health or have negative implications for the wider environment. This study used a data-set taken from a long-term trial, comprised of six different organic matter applications made annually over a 12year period. The use of this long-term field trial data set allowed a comparison of legacy effects which differed between treatments and enabled evaluation of how common garden organic matter applications drive changes in soil stoichiometry and physical parameters. The data analysis positively confirmed the following hypothesis:

Annual additions of the same organic material to garden soils will result in a build-up of available nutrients which will be dependent on the type of organic matter applied and concurrent with increases in SOM% as a direct result of OM applications.

3.3 Materials and methods

Data from a field trial at RHS Wisley Gardens (Woking, Surrey) enabled analysis of how these additions drive changes in nutrient status and availability as well as other key parameters such as organic matter build-up, pH and bulk density over a ten-year period. Carbon/Nitrogen (C/N) ratios of garden inputs and effects on field trial soils treated with organic matter with distinct C/N ratios correlated with effects on SOM% of the field trial plots. The data-set was provided by RHS Wisley gardens and all data from 2007-2018 was produced from soil and organic matter samples taken by RHS Wisley technicians and analyses completed by NRM laboratories. Soil and organic matter sampling from 2019 were completed by Rachel Hasler and analysed at Cranfield University. Plots were assessed with the aim of providing prescriptive advice for UK garden systems based on the inputs readily available to UK gardeners. Each year a direct-drilled, annual, flowering plant was sown and measures of yield, plant health (disease and pest damage) and germination rate were taken, more detail of the effects on plant health within specific years (2007 and 2012) are outlined in Duddigan et al. (2022). Other work has focused on valuable areas of research such as finding sustainable alternatives to peat-based compost (Alexander & Bragg, 2014), carbon fractionation (Duddigan et al., 2019), and horticultural soil amendment effects on soil C fractions and plant health (Duddigan et al., 2022). As such this analysis focuses on long-term trends in soil physiochemical changes driven by organic matter amendment. No measure of labile carbon was taken in the initial years of the trial, however HWEC data was compared between treatments and to the control at the end of the trial, showing a significant increase in HWEC in all treatments. A significant within season increase of labile carbon was noted for two treatments, horse manure and garden compost.

Combining explorative findings from the ten years of the field trial with knowledge on garden land area from organisations such as Landis, RHS and UK Government statistics helps to build a picture of where improvements and targets for management decisions can be focused. Long-term field trials help to provide important insight into how repeated management changes key soil characteristics in situ. Garden organic matter amendment affects biogeochemical processes that contribute to or can mitigate environmental issues associated with nutrient deposition and green space management.

The field trial was located at RHS Wisley gardens and is described by Alexander and Nevison (2015). The site location is Deers Farm Wisley (51.323428◦ N, −0.474392◦ W) and an aerial photo of trial is included in Figure A-1 of the appendices. The trial is situated on Bagshot formation underlying lithology and soil is characterised as a sandy loam (Appendices - Figure A-2). A randomised block design of 3x3m plots (eight blocks and eight replicates) consisting of 6 treatments and 2 controls was treated with amendments of either peat, bracken, bark, green compost, spent mushroom compost or a fertiliser, the layout of which can be seen in the treatment map in Figure A-3 in the appendices. Within garden and horticultural systems it is common practise for organic matter amendments to be applied on the basis of volume or depth, as such 70L of each input was added to each relevant treatment plot to equate to a 5cm layer of amendment material on the surface of the plots, which was later rotavated to a depth of 15cm (Duddigan et al., 2022). An annual, direct sown, ornamental flower plant was cultivated each year (Table 3-1). Control plots had the same plant sown as all other treatments and did not receive OM amendment or fertiliser. For comparison and to assess the possible effects of leftover plant residues a set of replicates was included with no OM amendment and no plant sown.

Criteria that dictated selection of plant varieties for the trial included – common use in UK gardens, ability to be direct/ station sown, annual flowering/ ornamental species. Selected crops for the trial are included in Table 3-1. Plants were sown by hand at a 30cm spacing and harvested in the September of each year of the trial.

Table 3-1 Table of cultivars selected and sown each year of the field trial

Year sown:	**Crop cultivated:**
2007	*Helianthus annuus* (Sunflower, Little Dorrit)
2008	*Borago officinalis* (Starflower)
2009	*Cosmos bipinnatus* (Cosmos, *S*onta series white)
2010	*Nigella damascena* (Love-in-a-mist, Miss Jekyll)
2011	*Cerinthe major purpurascens* (Honeywort)
2012	*Calendula* (Marigold, Neon*)*
2013	*Phacelia tanacetifolia* (Lady Phacelia)
2014	*Lavatera* (Mallow, Ruby Regis)
2015	*Helianthus annuus* (Sunflower, Little Dorrit)

2016	*Lavatera* (Mallow, Ruby Regis)
2017	*Zinnia elegans* (Common Zinnia, Purple Prince (failed)). Re-sown with *Calendula Officinalis* (Marigold, Neon).
2018	*Borago officinalis* (failed)
2019	*Borago officinalis* (failed)

All organic amendments were purchased from the same suppliers for the duration of the trial, except the garden compost which was made in-house at RHS Wisley's on-site composting facility from plant waste generated at Wisley gardens. RHS Wisley composting facility produced the garden waste compost using the windrow composting method with rows turned monthly for 6-9months (photos of the trial plots are included in the appendices section Figure A-4, Figure A-5,Figure A-6). All amendments were applied in March or April of each year of the trial, with 70L volume of each amendment added to the relevant replicate plots. This resulted in a 5cm (approx.) layer mimicking recommendations made by the RHS to gardeners (RHS, 2022). Amendments were then incorporated into the top layer of soil using a rotavator to 15cm.

Table 3-2 Mean values of characteristics of organic matter samples in 2008, 2013, 2018, 2019.

	pH		C:N		Total C		Total soluble N		Total available P		Total available K		Bulk density		Mg		Fe	
2008		Std err.		Std err.	(% of WW)	Std err.	(mg/l)	Std err.	(mg/l)	Std err.	(mg/l)	Std err.	(kg/m3)	Std err.	(mg/l)	Std err.	(mg/l)	Std err.
Compost	8.7	0.05	15.3	0.35	11.9	1.27	53.4	2.1	46.8	1.75	1112.7	20.09	822.0	37.16	9.5	0.58	13.0	2.67
Manure	8.2	0.07	23.9	0.8	41.9	1	81.6	14.57	24.8	0.3	1558.6	56.85	316.0	6.35	32.6	3.37	2.7	0.22
Mushroom compost	7.1	0.06	12.7	0.07	26.1	0.79	241.8	3.67	102.4	2.28	2689.3	1339.54	463.7	15.96	445.1	18.74	2.1	0.15
Bark	6.3	0.06	50.2	0.97	43.9	2.11	8.8	0.32	33.9	0.52	141.6	5.41	433.3	6.94	2.6	0.09	3.2	0.3
Bracken	8.1	0.06	19.4	1.09	36.6	6.14	8.4	2.17	35.5	1.96	808.4	29.96	440.7	4.18	25.0	0.44	1.5	0.4
Peat	4.7	0.06	36.3	0.18	56.4	0.46	39.0	1.4	0.6	0.03	8.6	0.47	383.0	14.05	24.2	1.31	0.5	0.07
2013																		
Compost	8.7	0.02	16.0	1	21.2	3.48	67.9	5.64	29.3	0.08	853.2	71.99	709.0	5.77	7.9	0.25	17.5	1.06
Manure	8.5	0.10	21.4	0.34	38.8	0.55	118.9	49.51	23.8	3.07	1936.2	221.09	415.0	2.89	51.5	19.66	1.7	0.4
Mushroom compost	8.0	0.13	14.1	0.38	32.7	0.35	335.1	50.18	66.2	9.85	4513.7	674.54	618.7	83.16	385.3	53.02	1.9	0.29
Bark	6.1	0.02	57.1	5.5	60.2	4.06	25.3	0.98	36.4	3.28	130.1	6.8	440.7	13.62	1.1	0.35	1.3	0.74
Bracken	5.9	0.1	23.1	0.69	42.2	2.71	21.6	2.26	2.5	0.73	32.5	4.69	406.7	24.36	0.6	0.01	0.9	0.16
Peat	5.0	0.12	33.2	1.04	56.6	1.03	41.7	13.02	3.1	0.11	2.7	0.64	362.0	9.17	1.9	1.33	0.2	0.06
2018																		
Compost	8.5	0.07	20.7	0.45	18.6	0.87	89.0	13.88	23.0	1.88	1031.7	99.70	750.7	7.22	7.6	0.35	7.2	2.32
Manure	8.3	0.07	24.5	1.79	40.8	0.99	291.1	9.79	23.8	5.9	2092.2	86.54	427.0	9.54	109.7	1.49	3.2	1.28
Mushroom compost	7.5	0.3	15.0	0.32	32.9	0.63	252.8	22.18	55.2	16.66	3418.2	206.95	533.3	36.45	274.1	32.87	1.7	0.43
Bark	6.0	0.12	87.0	3.59	51.9	1.13	11.8	1.59	28.9	0.44	114.1	5.08	420.3	9.77	1.4	0.35	1.6	0.77
Bracken	6.3	0.12	27.2	1.04	34.6	2.28	12.5	2.92	4.7	1.77	46.7	11.01	314.0	25.77	0.8	0.28	1.1	0.12
Peat	4.7	0.07	37.5	0.39	56.3	0.06	17.5	1.43	1.0	0.01	4.4	0.7	441.0	15.95	0.8	0.15	0.3	0.08
2019																		
Compost	8.1	0.03	17.8	0.19	21.7	0.76	101.9	5.1	30.3	2.26	1057.1	41.88	639.3	13.68	9.8	1.13	8.9	4.61
Manure	8.1	0.03	21.0	2.48	34.4	3.72	232.2	19.76	16.9	1.5	2346.6	88.75	373.7	10.59	101.9	5.75	1.7	0.28
Mushroom compost	7.6	0.27	13.6	0.06	31.2	0.91	280.2	15.15	54.1	12.08	4602.1	54.76	493.0	5.13	332.3	17.1	1.2	0.19
Bark	6.5	0.31	61.3	4.98	47.7	0.43	7.0	0.22	33.9	3.96	138.6	23.09	421.0	12	1.9	0.59	1.8	0.46
Bracken	5.5	0.09	21.0	0.3	39.9	1.56	66.3	12.4	13.7	1.53	177.5	22.62	433.7	6.98	3.5	0.58	0.8	0.18
Peat	4.3	0.09	39.5	0.64	56.5	2.27	17.3	1.77	1.0	0.01	2.3	0.46	353.3	12.12	0.7	0.1	0.2	0.02

(n=5)

Soil samples were taken from the central 2x2m of each plot. Each plot was sampled twice in each year; once prior to application of the amendments (T1) and once approximately 1-month post-application and incorporation of the amendments (T2). For the purpose of this study T1 measurements are used for each year to account for the legacy of the previous years' OM application. Incorporation of organic amendments was completed using a tractor mounted rotavator to a depth of 15cm. Plots were sampled using an auger at 0-15cm depth, five times from each plot and samples from individual plots homogenised. A 2m margin surrounded each plot to mitigate any cross-contamination of the treatment plots. Each organic matter input was also characterised each year prior to application. Plots were sampled using a "W" transect, auger and tools were cleaned thoroughly between plots. Three sub-samples were taken from each amendment material (T0 - prior to application to the plots).

Plant yield was quantified as above-ground plant biomass which was weighed at harvest and recorded for each plot. A subsample was then dried at 80°C for 48h to determine moisture content. Dry weight biomass was then calculated for each plot. As different crops were sown for the majority of the trial years comparisons of yield were made within individual years. The only exception being comparisons between years 2008/2018/2019 as *Borago officinalis* was sown for all three years.

Laboratory analyses (2007-2018) conducted by NRM laboratories (Bracknell, UK). Samples were first air dried at no more than 30°C and then sieved to 2mm. The pH was determined potentiometrically after stirring soil with water (ratio 1:2.5). The pH probe and instrument were calibrated at pH4 and pH 7 and soils of known pH intervals were used for quality control (Jackson et al., 1986).

Available P was determined using a 0.5M sodium bicarbonate extractant (Olsen's reagent). P was extracted by shaking for 30 minutes and P concentration determined by flow injection analysis (Olsen, 1954; Pelissier, 1954).

Available (total mineral) N (sum of nitrate, nitrite and ammonium) was determined using 2M potassium chloride extraction. Prior to analysis soils were kept between 2-8°C (to restrict N mineralsation and overestimation of available N). Soil was chopped and homogenised and a portion shaken with 2M KCl. Nitrate N, nitrite N, and ammonium N were then measured colourimetrically. Nitrate and nitrite N determination was based on the formation of a diazo compound (between nitrite and sulphanilamide). This compound was then coupled with N-1-Naptheylethylenediamine dihydrochloride resulting in a red azo dye and measured at 540nm. An open tubular cadmium reactor (OTCR) reduced nitrate to nitrite and the nitrite and nitrate reduction were measured as total oxidised N. Indophenol blue, resulting from ammonium reacting with alkaline hypochlorite and phenol (and a sodium nitroprusside catalyst), was measured at 640nm (March, 1984; Patton and Crouch, 1977; Webster, 2008).

Available K and Mg were determined using an ammonium nitrate extraction. Soils were shaken with 1M ammonium nitrate solution at 20°C for 30 minutes. Following filtration of the extractant the concentration of P and Mg is determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), standards were included in the analysis to ensure accurate instrument calibration (Jackson et al., 1986).

Available Fe was determined using a DTPA solution (ratio 1:2). DTPA solution was added to air dried and sieved samples and shaken for 15 minutes. The extract was filtered (Whatman 42 paper) and Fe concentration determined by spectrophotometry, including standards made in the same DTPA solution as samples (Jones, 1984; Soltanpour and Schwab, 1977).

Organic matter % (loss on ignition) was determined on methods developed from McDonald (1984) and, BS EN 13040:2000. Samples were oven-dried and measures of sample mass recorded before heating to 450°C with the difference in weight post-combustion used as the measure of organic matter % of the sample.

Cation exchange capacity (CEC) was determined based on methods developed from BS EN 13040:2000 for organic matter amendment materials and field soils.

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Further analyses for year 2018/ 2019 were completed at Cranfield University using air dried soil and included measure of pH with a 1:5 ratio of dried soil to water in a solution of 1 mol/l of KCl using a glass electrode (BS ISO 10390:2005). Organic matter % (loss on ignition) (BS EN 13039:2011) following the same procedure as outlined above. Total carbon (TC) and total nitrogen were determined using the dry combustion method (elementary analysis) on oven-dried soil. For determining total organic carbon a 4mol/l hydrochloric acid was added to a sample drop by drop and to remove the organic carbon before continuing with the oxidisation process and measurement of CO_2 released using a thermal conductivity detector (Elementar Vario/// EL analyser) (BS 7755-3.8:1995/ (ISO, 1993)). Bulk density was determined using the British standard method of estimation of oven dry bulk density (ISO, 2017; BS 7755 Section 5.6:1999). Tins of known volume ($222cm^3$) were filled with sample material and oven dried at 105°C for 24 hours. The mass of the sample was then determined by weighing and deducting the mass of the tin.

Hot water extractable carbon was based on the method as described in Ghani et al, (2003) which was modified from Haynes & Francis (1993). Fresh field samples taken in 2019 were used to establish the fraction of C in the field trial plots considered labile. For the purpose of this study labile C is defined as carbon which is easily utilised by soil microbes (Ghani et al., 2003). A two-step extraction process as described in Ghani et al, (2003) was completed on samples taken from the final year of the field trial. Moisture content of a subsample from each treatment was determined by oven drying at 105ºC for 24hrs. Field fresh samples (equivalent of 3g oven dry weight) were used for each treatment and the analysis performed in two stages. The first was to quantify the readily soluble C in a 30min water extraction at 20 ºC followed by centrifuge at 3000rpm and filtering the supernatant. The second was a 16hr water extraction at 80 ºC, centrifuge at 3000rpm and filter supernatant (Ghani et al., 2003). Analysis of the extracted suspension was performed using the Shimadzu TOC-V CSH (model TNM-1 version 1.0).

3.3.1 Statistical analysis

Soil and OM amendment data were tested for Normality using Q-Q plots in Statistica version 13.1.3 or “qqnorm” in R software (version 2022.07.2+576). All data were found to be normally distributed. Soil physio-chemical variables of interest were identified using PCA analysis of data from all years of the field trial (2007-2019). In addition, ANOVA was applied to the PCA scores to identify variables showing significant changes between 2008, 2013 and 2018 or significant differences between treatments. Analyses using R (version 2022.07.2+576) and packages dplyr, GGally, gridExtra, ggplot2, were used to identify correlations between the amendment data and measurements made on samples taken from trial plots. In addition, correlations were calculated between variables of field trial soils and plant data.

Tukey’s Honest Significant Difference (HSD) was used as a post-hoc test. Results were considered significant if $p \leq 0.05$. A statistical input-output model was created to test the effect of C/N ratio of the material added on the rate of build-up of organic matter in the trial. The correlations between C/N ratio and macro-nutrients were also calculated.

For each of the 8 variables selected by the method described above, and for each of the three years 2008, 2013 and 2018, soil organic matter percent was regressed on the variables, using both a straight line and a quadratic model. The p-value for the inclusion of the quadratic term was tested at a 5% significance level using a Bonferroni correction with n=24 (=8 variables x 3 years) tests.

3.4 Results

3.4.1 Long-term effects of C/N ratio of organic matter types on soil organic matter (%)

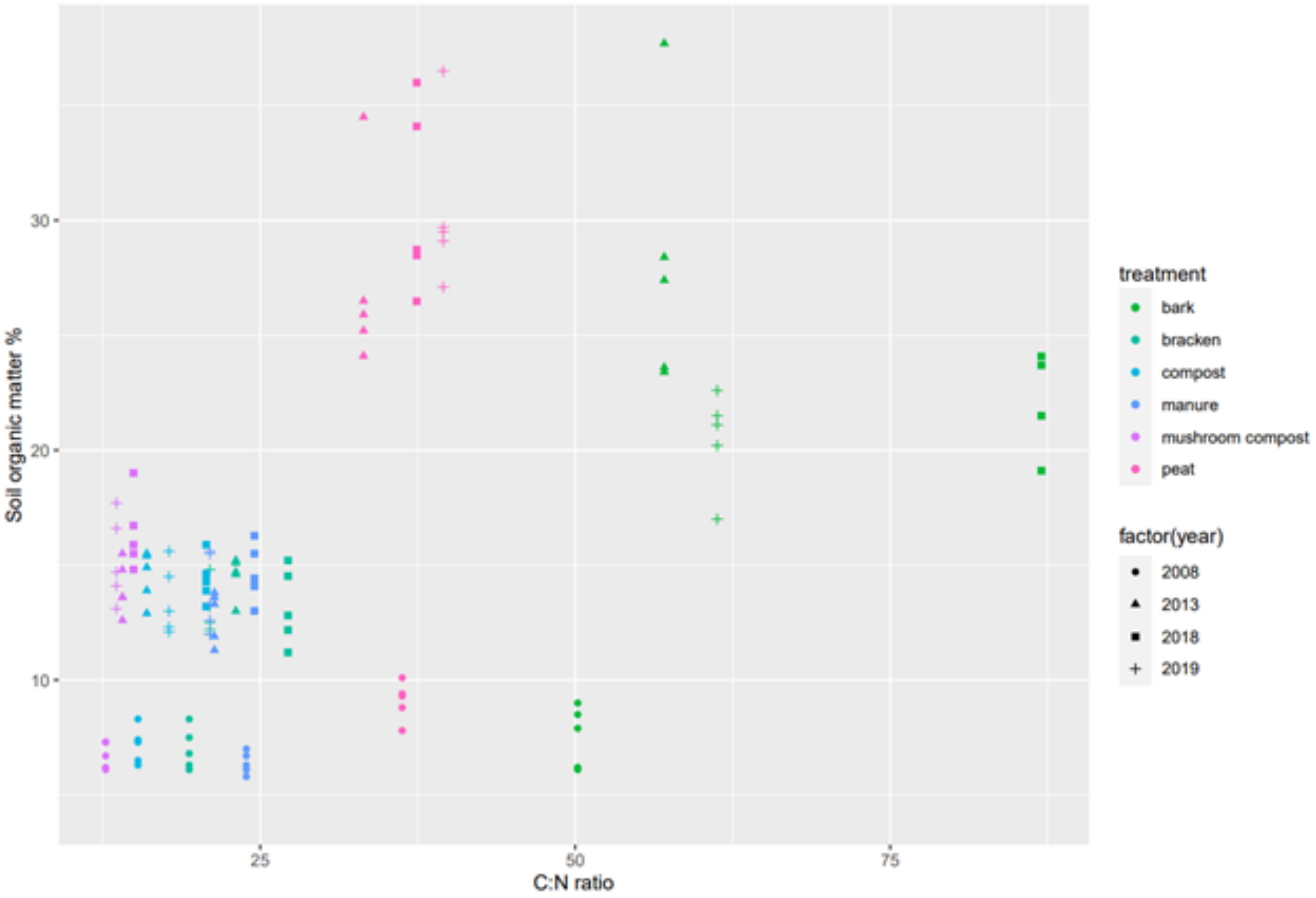


Figure 3-1 Soil organic matter (%) related to C/N ratio of OM input material over time (10years – 2008, 2013, 2018, 2019). 6 x organic matter material applied annually; treatment material C/N measured prior to application to trial plots. Icon shape depicts year of trial in which samples were taken and colour of icon represents different treatments.

Table 3-3 Summary of factorial ANOVA of organic matter treatment material (T0) C/N ratio across years 2008,2013, 2018, 2019 of field trial.

	df	F	P value
OM material	5	387.6	<0.005
Year	3	27.4	<0.005
OM material*year	15	10.7	<0.005
Residual	48		

(n=3)

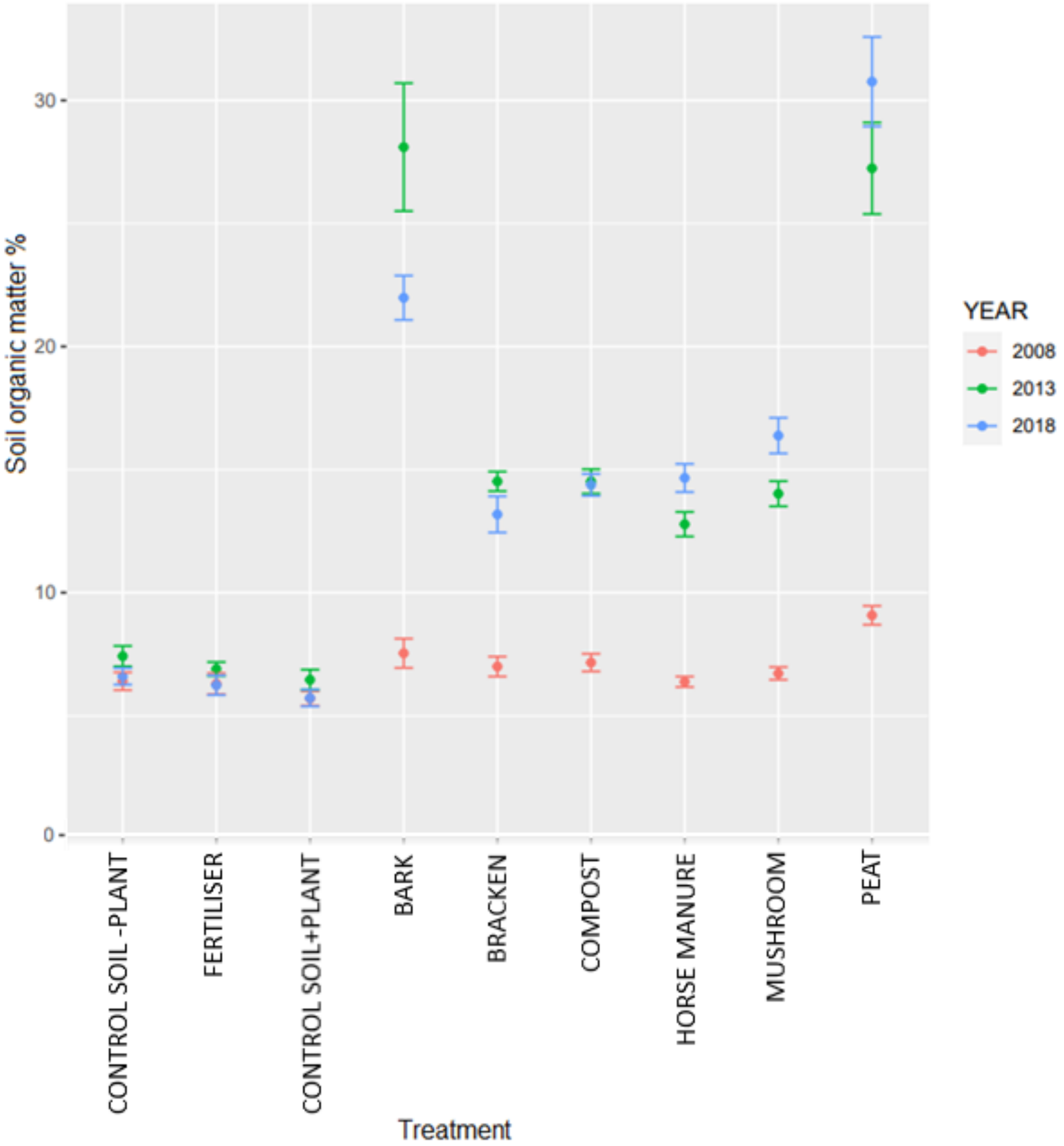


Figure 3-2 Mean SOM% for years 2008, 2013, 2018 from field trial sampled plots. Error bars denote standard error.

Table 3-4 Summary of factorial ANOVA for SOM% in years 2008, 2013, 2018 of field trial.

	df	F	P value
Treatment	8	222.7	<0.005
Year	3	181.2	<0.005
Treatment*year	24	20.8	<0.005
Residual	144		

(n=5)

3.4.2 Long-term effects of organic matter type on plant yield

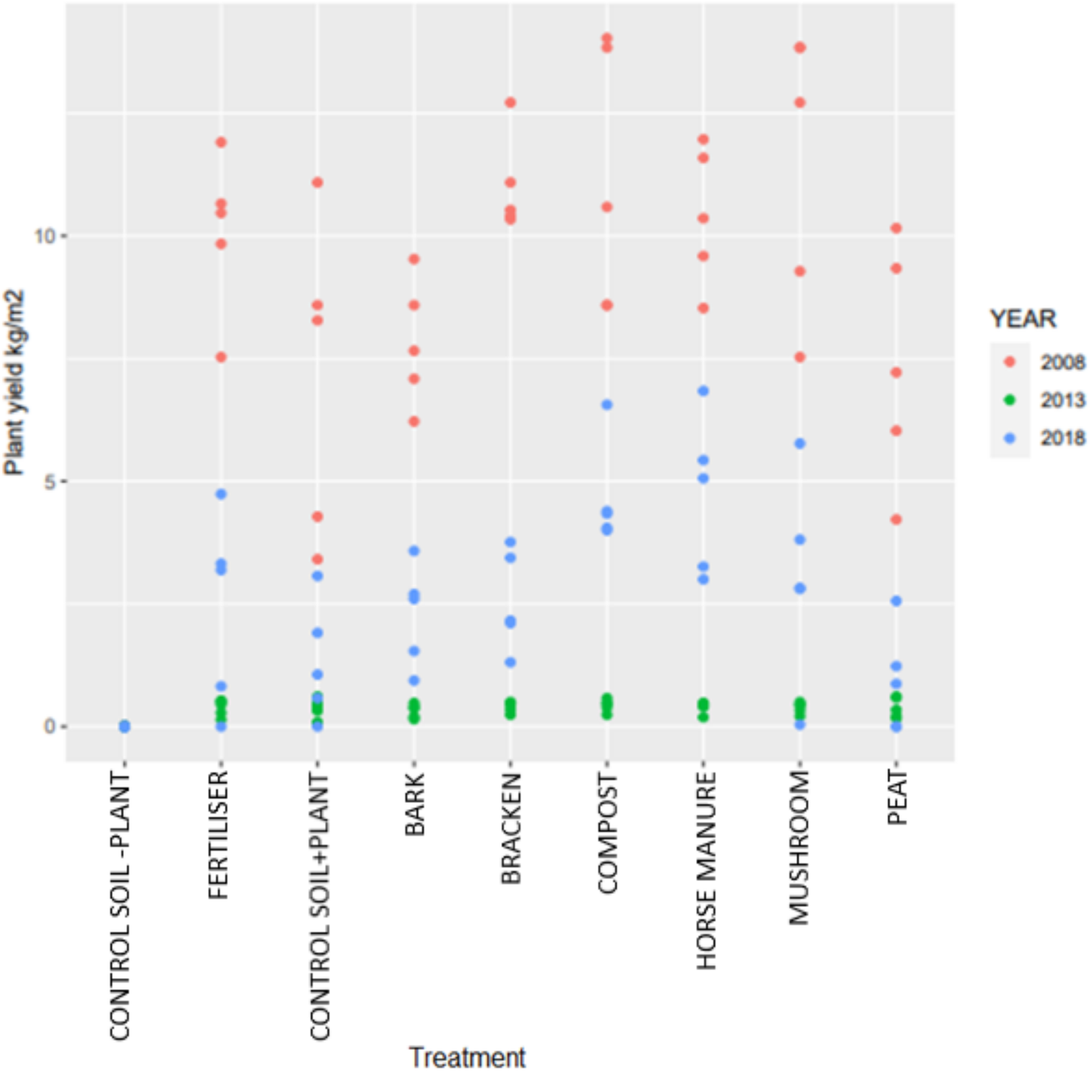


Figure 3-3 Plant yield (DM kg/m^2) for each of the treatments in years 2008/2013/2018. *Borago officinalis* sown in years 2008 and 2018 and *Phacelia tanacetifolia* (Lady Phacelia) in 2013.

Table 3-5 Summary of one-way ANOVA for plant yield (kg/m^2) in field trial plots

	df	F	P value
Treatment	26	41.7	<0.005
Residuals	108		
(n=5)			

The C/N ratio of the organic matter inputs did not significantly differ between years for the individual treatments except in bark and peat treatments (Figure 3-1 $p \leq 0.005$). Bark C/N was significantly higher than all other treatments and peat C/N was higher than all treatments except bark in all years. Bracken /mushroom compost and manure/ mushroom compost were significantly different to each other in 2018 ($p \leq <0.05$). The C/N ratio of the bark input increased from 57 to 87 between 2013 and 2018, possibly due to a change in the feedstock used to create the bark compost (Figure 3-1).

The increase of SOM% seen between 2008-2013 was not identified between 2013-2019 except in the bark treatment in which organic matter% decreased between 2013 and 2018 (Figure 3-2). The decrease of organic matter% in the bark treated plots ceased and no significant difference (increase/decrease) was seen between 2018-2019 (Figure 3-2).

Figure 3-6 (2018) shows the results of fitting straight line and (where appropriate) quadratic relationships between predictor variable soil organic matter% (SOM%) of the field trial plots and response variables; available nutrients N, P, K, Mg, Fe and pH, bulk density and plant yield from 2008, 2013, 2018. In each of the plots, the quadratic term was included provided the p-value in the ANOVA comparing the linear and quadratic fits was lower than 0.05/24, i.e. an overall 5% significance level with a Bonferroni correction for 24 tests (= 8 variables x 3 years).

The gradient and p values are presented in Table 3-6. The equations used for the expression of either a linear or quadratic relationship are shown below. Bonferroni's correction for multiple (n=24) testing was used to determine the significance of the effects of either quadratic or linear regression between predictor (SOM%) and response variables.

$$\text{Linear: } a + b * OM\%$$

$$\text{Quadratic: } a + b * OM\% - c * (OM\%)^2$$

Equation 1 Equation for the linear or quadratic expression of the relationship between organic matter (%) build up as a result of treatment and soil pH, bulk density, nutrient availability, plant yield in Figure 3-4, Figure 3-5, Figure 3-6. In the

above equation a is the intercept, b = linear gradient and c is the quadratic gradient, all of which are found in table 2.

Borage yield was significantly affected by organic matter amendment (). In 2008 all treatments presented significantly higher yield than the control (control soil+plant). At the beginning of the trial a significant quadratic relationship between SOM% and plant yield was found (Figure 3-4). This effect was not noted in the later years of the trial. Figure 3-4, Figure 3-5, and Figure 3-6 show yield data from the beginning 2008, middle 2013 and end 2018 of the trial, statistical comparison between years was completed only between 2008 and 2018 as borage was grown in both years. 2013 plant yield data was analysed separately as *Phacelia tanacetifolia* was cultivated therefore preventing comparison with other years. By 2018 (crop sown=borage) the only treatments to significantly increase yield compared to the control were compost and horse manure treated plots (). In 2013, none of the treatments differed to the control in measurements of plant yield ().

3.4.3 Field trial soil characteristics 2008-2013-2018

Reduction in bulk density significantly correlated with increase in SOM% between 2008-2013-2018 (Figure 3-4, Figure 3-5, Figure 3-6). With significance of the correlation increasing towards the end of the trial. All treatments significantly increased SOM% with the largest increase noted in the peat and bark treatments. Garden compost, horse manure and the mushroom compost did not significantly differ from each other in measures of bulk density in year 6 of the trial though they did all decrease bulk density compared to the control.

Available P significantly correlated with an increase in OM% in the first year of the trial (Figure 3-4), however this relationship was no longer significant by the end of the trial. Mushroom compost and horse manure both increased available P compared to the control and other treatments.

The significant build-up of K, Fe, Mg (and the correlation of build-up with increasing SOM%) in field trial plots increased in magnitude over time. Overall, the regression between these response variables and SOM% increase as a predictor variable was found to be significant. The relationship between SOM% and plant available nutrients was significant. Following this finding regression analyses of correlations between plant yield and accumulation of plant available nutrients (N, P, K, Mg, Fe) were completed to determine if by the middle (2013) and end of the trial (2018) OM addition had driven changes to nutrient status significantly related to plant yield and are presented in 3.4.4.

A significant relationship between organic matter build-up and available Mg was identified in the initial year (2008) of the trial, this effect was highly significant in later years of the trial and was true of all treatments except bare plot+fertiliser. Available N was highest in mushroom compost treated trial plots in all years. Mushroom compost, horse manure and garden compost all increased K significantly, with the effect significantly correlated to plant yield by the end of the trial (Figure 3-7).

In year 6 of the trial (2013) following 5years of organic matter additions, significant ($p= <0.05$) correlations between bulk density, K, Mg, Fe and SOM% were recorded (Figure 3-5). Available P was highest in the mushroom compost amended soils (Figure 3-10). All treatments significantly lowered bulk density when compared with control plots and fertiliser treated plots.

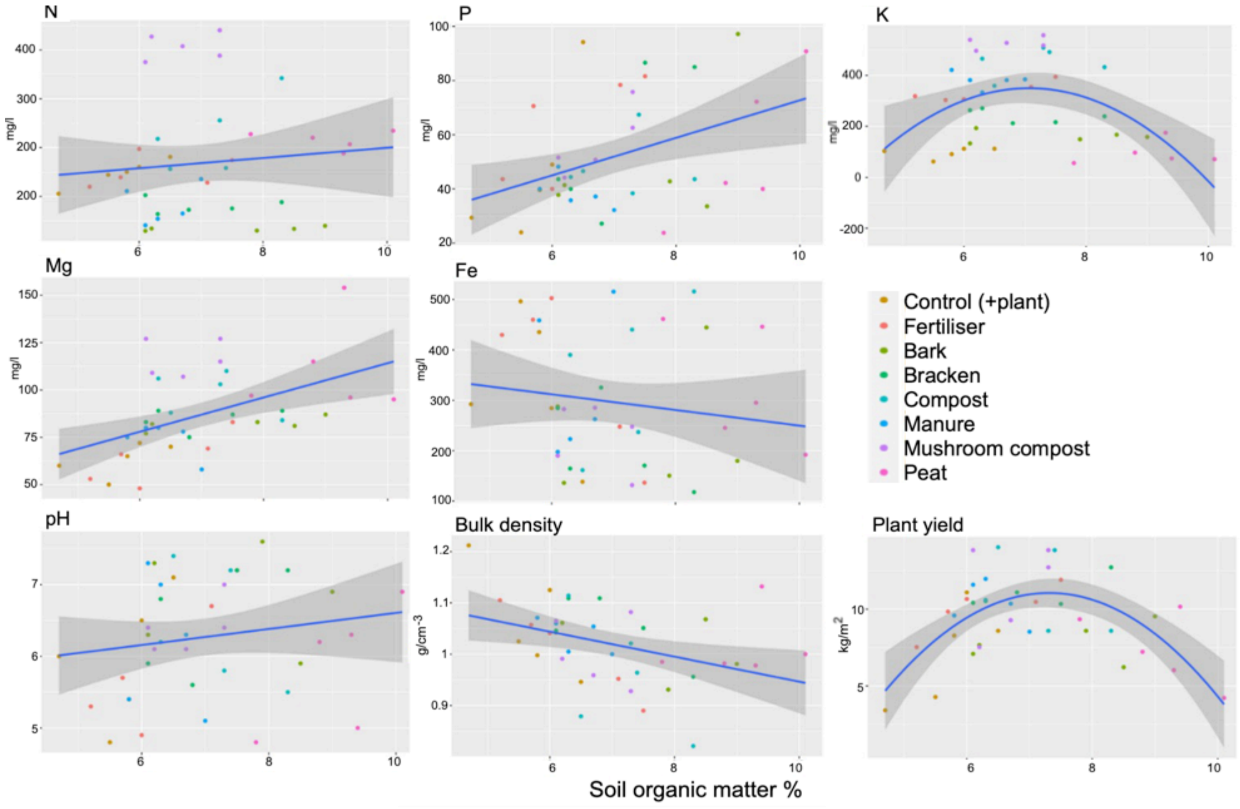


Figure 3-4 Output of linear regression for trial plot characteristics in 2008 against soil organic matter %. Treatment is represented by icon colour and grey shading indicating standard error of line fit.

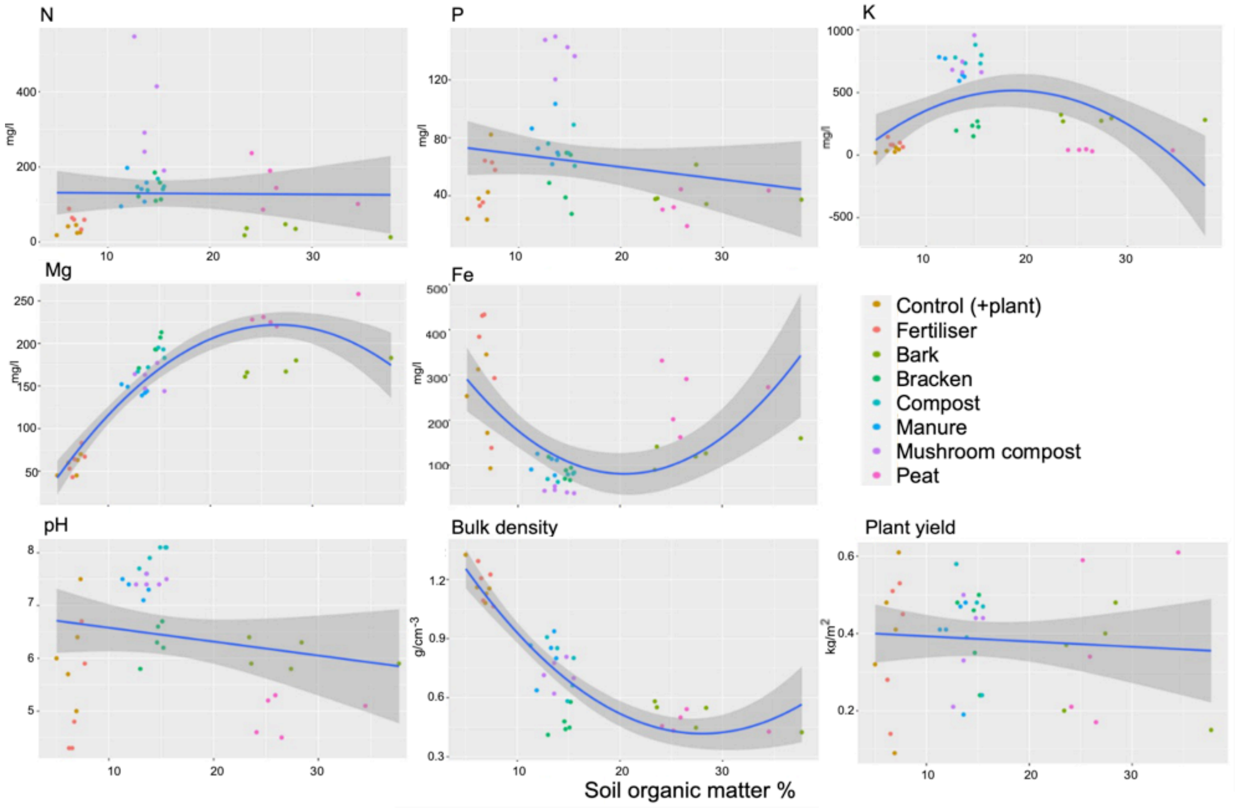


Figure 3-5 Output of linear regression for trial plot characteristics in 2013 against soil organic matter %. Treatment is represented by icon colour and grey shading indicating standard error of line fit.

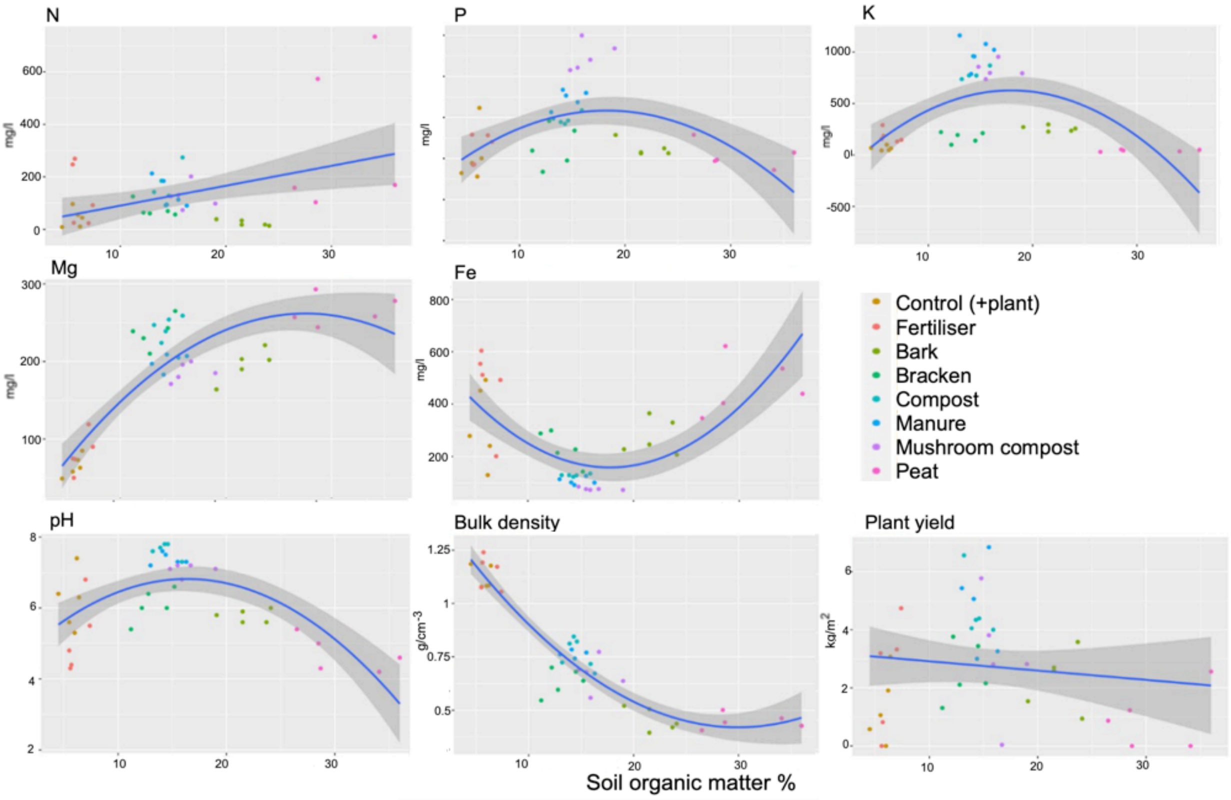
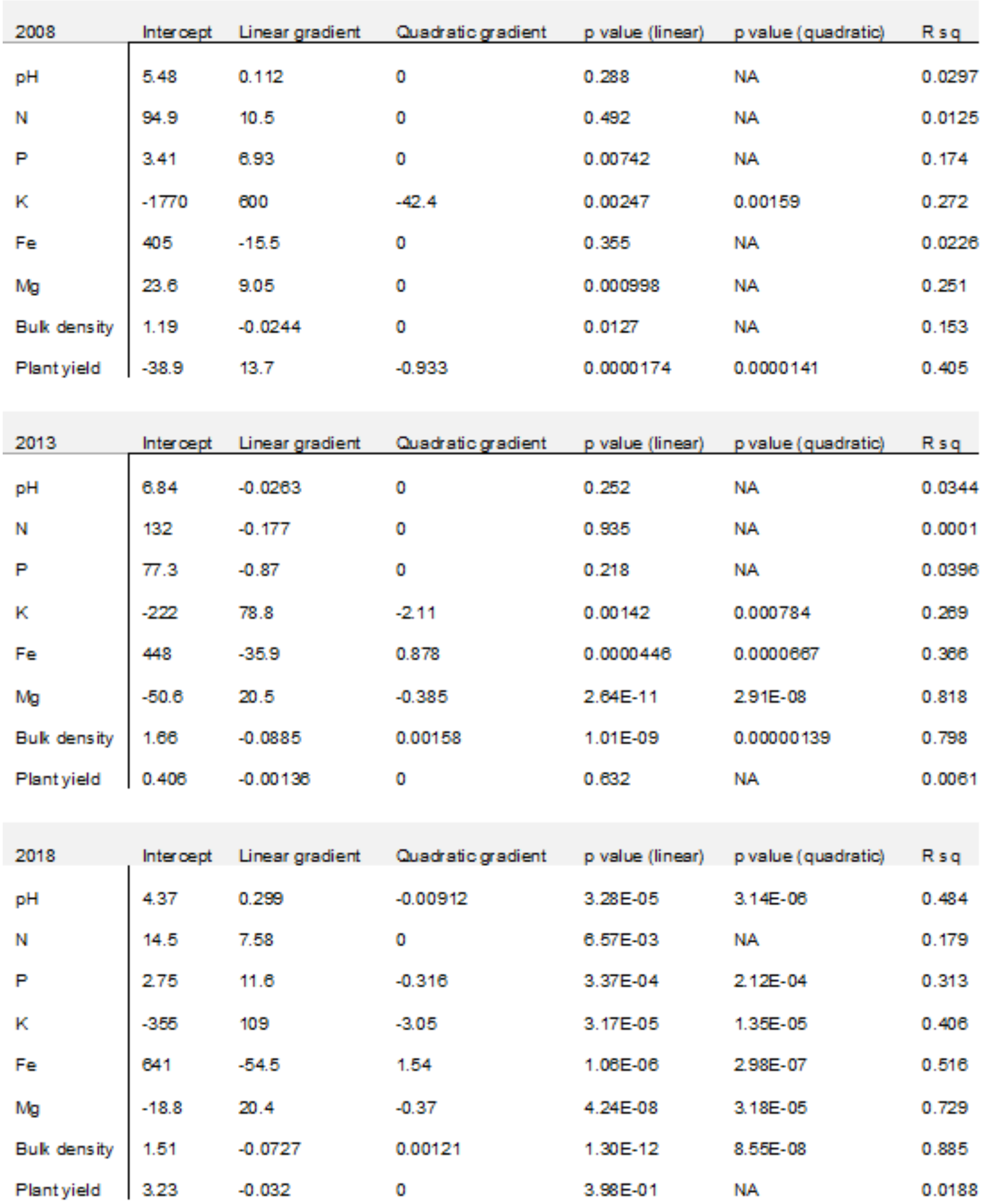


Figure 3-6 Output of linear regression for trial plot characteristics in 2018 against soil organic matter %. Treatment is represented by icon colour and grey shading indicating standard error of line fit.

Table 3-6 Data table of R^2 and p values from linear regression model results depicted in figures 4,5,6



2008	Intercept	Linear gradient	Quadratic gradient	p value (linear)	p value (quadratic)	R sq
pH	5.48	0.112	0	0.288	NA	0.0297
N	94.9	10.5	0	0.492	NA	0.0125
P	3.41	6.93	0	0.00742	NA	0.174
K	-1770	600	-42.4	0.00247	0.00159	0.272
Fe	405	-15.5	0	0.355	NA	0.0226
Mg	23.6	9.05	0	0.000998	NA	0.251
Bulk density	1.19	-0.0244	0	0.0127	NA	0.153
Plant yield	-38.9	13.7	-0.933	0.0000174	0.0000141	0.405

2013	Intercept	Linear gradient	Quadratic gradient	p value (linear)	p value (quadratic)	R sq
pH	6.84	-0.0263	0	0.252	NA	0.0344
N	132	-0.177	0	0.935	NA	0.0001
P	77.3	-0.87	0	0.218	NA	0.0396
K	-222	78.8	-2.11	0.00142	0.000784	0.269
Fe	448	-35.9	0.878	0.0000446	0.0000667	0.366
Mg	-50.6	20.5	-0.385	2.64E-11	2.91E-08	0.818
Bulk density	1.66	-0.0885	0.00158	1.01E-09	0.00000139	0.798
Plant yield	0.406	-0.00136	0	0.632	NA	0.0061

2018	Intercept	Linear gradient	Quadratic gradient	p value (linear)	p value (quadratic)	R sq
pH	4.37	0.299	-0.00912	3.28E-05	3.14E-06	0.484
N	14.5	7.58	0	6.57E-03	NA	0.179
P	2.75	11.6	-0.316	3.37E-04	2.12E-04	0.313
K	-355	109	-3.05	3.17E-05	1.35E-05	0.406
Fe	641	-54.5	1.54	1.06E-06	2.98E-07	0.516
Mg	-18.8	20.4	-0.37	4.24E-08	3.18E-05	0.729
Bulk density	1.51	-0.0727	0.00121	1.30E-12	8.55E-08	0.885
Plant yield	3.23	-0.032	0	3.98E-01	NA	0.0188

3.4.4 End of trial: available nutrients and plant yield

The prediction of plant yield from SOM% was not significant by the end of the trial (2018). However plant yield did correlate with levels of K, P and Fe in 2018 which were driven by OM or fertiliser input (Figure 3-7,Figure 3-8, Figure 3-9). Regression analyses between N, P, K, Mg, Fe and dependent variable "plant yield" for 2013 and 2018 were completed. P, K, Mg, Fe were found to significantly correlate with plant yield in 2013, but by 2018 only P and K positively correlated with plant yield (Figure 3-7 and Figure 3-8) and a negative relationship between Fe and plant yield was determined highly significant by 2018 (Figure 3-9).

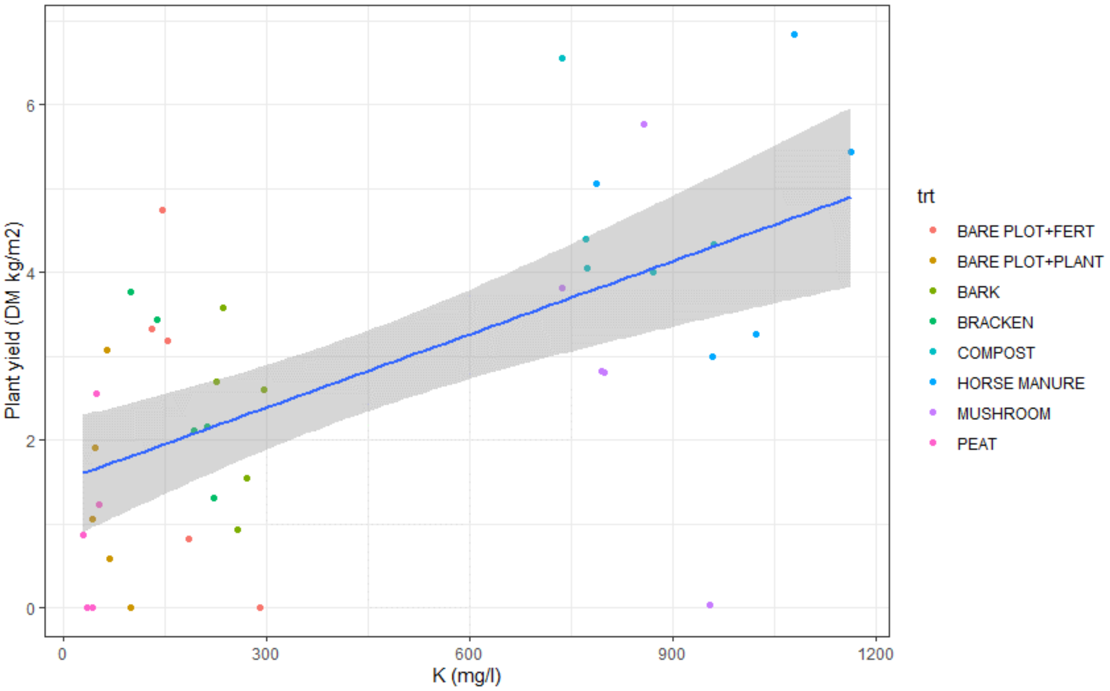


Figure 3-7 Regression output for predictor variable K (mg/l) and response variable plant yield 2018 (DM kg/m2). Bare plots were omitted due to no plant sown. Bare plot+plant used as control.

Table 3-7 Summary of linear regression model of potassium (K) and plant yield.

	df	R^2	F	P Value
K (ind.) vs. plant yield (dep.)	1	0.355	20.92	0.000
Residual	38			

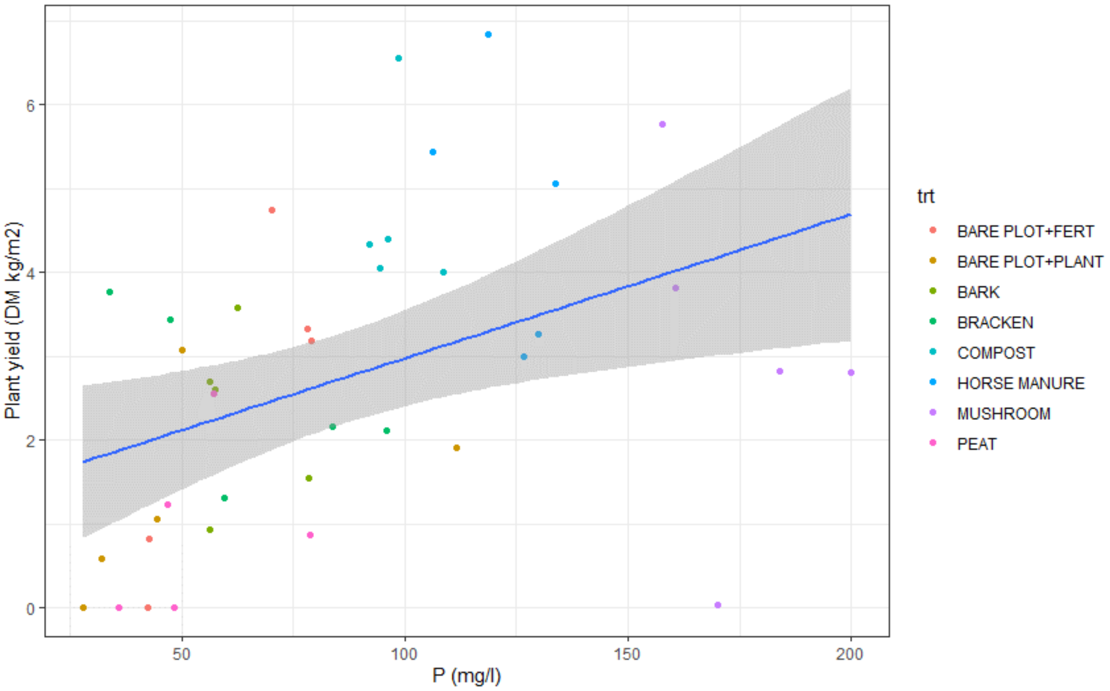


Figure 3-8 Regression output for predictor variable P (mg/l) and response variable plant yield (DM kg/m2) 2018. Bare plots were omitted due to no plant sown. Bare plot+plant used as control.

Table 3-8 Summary of regression output for available P and plant yield (2018).

	df	R^2	F	P Value
P (ind.) vs. plant yield (dep.)	1	0.171	7.875	0.007
Residual	38			

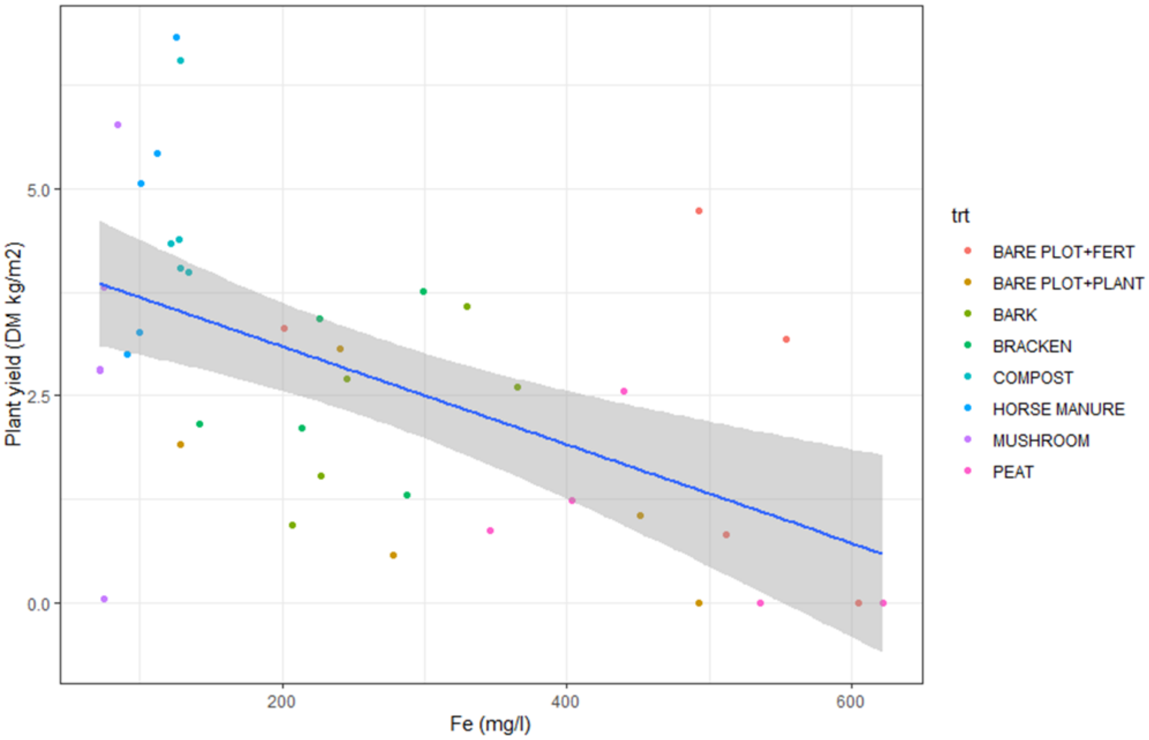


Figure 3-9 Regression output for predictor variable plant available Fe (mg/l) and response variable plant yield (DM kg/m2) in 2018. Bare plots were omitted due to no plant sown and bare plot+plant used as control

Table 3-9 Summary of regression output for available Fe and plant yield (2018)

	df	R^2	F	P Value
Fe (ind.) vs. plant yield (dep.)	1	0.297	16.08	0.000
Residual	38			

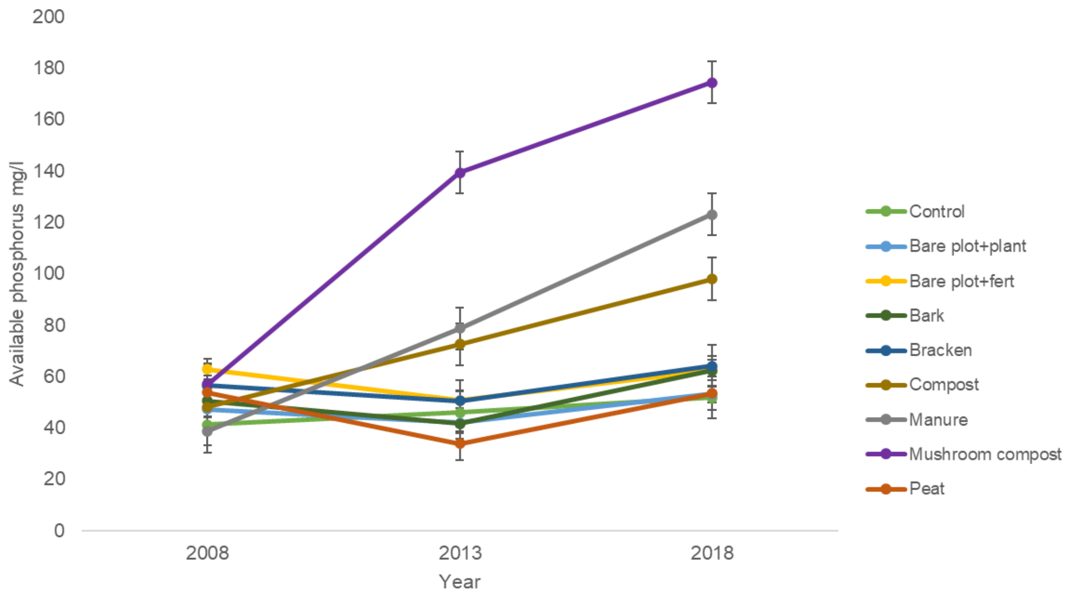


Figure 3-10 Phosphorous (mg/l) as measured in field trial plots for years 2008, 2013, 2019. Error bars denote standard error.

Table 3-10 Summary of repeated measures ANOVA (2008, 2013, 2018) for available P in field trial

Source of variation	df	F	P value
OM treatment	8	10.376	0.000
Residual	36		
Year	2	240.953	0.000
Year*OM treatment	16	52.048	0.000
Residual	72		

(n=5)

Available phosphorus (P) in field trial soils increased significantly in compost, manure, and mushroom compost treatments. Each of the three treatments which resulted in the highest available phosphorus in trial plots were distinct from each other, the control and remaining treatments.

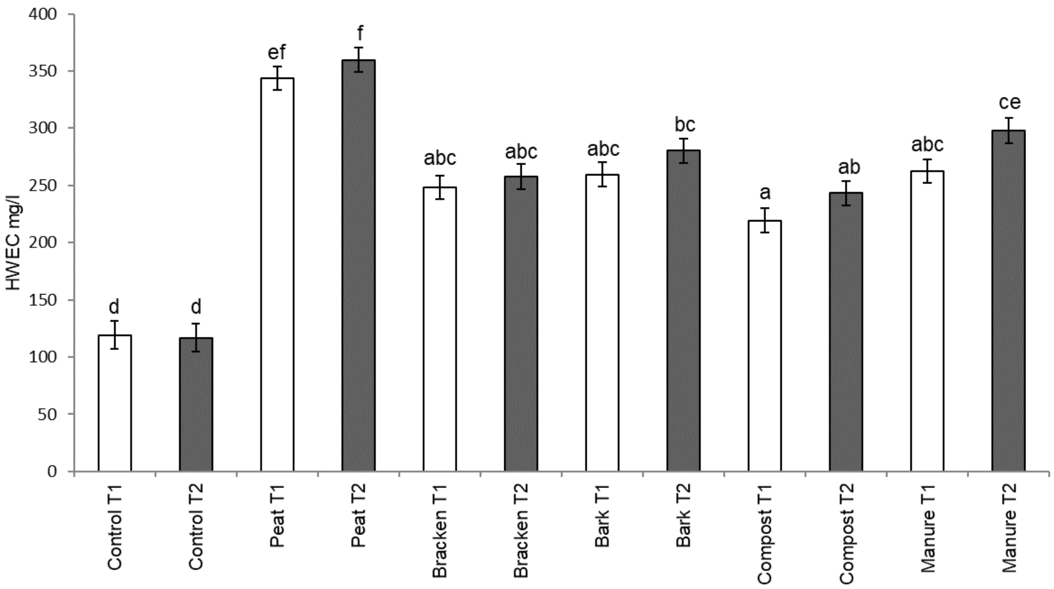


Figure 3-11 Hot water extractable carbon (HWEC) in soil samples taken from the field trial plots in 2019 (T1 & T2). Error bars indicate standard error. Letters above bars indicate significant differences between treatments and time of sampling compared to all other treatments and the control using Tukey's HSD post-hoc test.

Table 3-11 Summary of one-way ANOVA for HWEC in samples from T1 and T2

	df	F	P value
Treatment	11	47.233	0.000
Residual	35		0.000

(n=4)

Hot water extractable carbon (HWEC) was significantly higher than the control in all treatments at both time points (T1 & T2). Peat treated plots had higher HWEC at both sampling times than any other treatment and compost (T1) had the lowest (Figure 3-11).

3.5 Discussion

Gardens are not homogenous sites and are comprised of different crops, areas and planting intentions which have different nutrient, water and management requirements. Despite this, organic matter additions are common in wide range of different gardens and additions are used for multiple benefits (Dobson et al., 2021). Examples include improvement of the water holding capacity of a specific area for gardeners looking to water less in drier summers, application of manures and green waste composts for the supply of nutrients and application of heterogenous “woody” material applied as a mulch to suppress weeds, or composted bark amendments incorporated below the soil surface to improve the workability and tilth of a clay soil (Dewaelheyns et al., 2013; Small et al., 2023; Tresch et al., 2019). Considering each application and choosing based on evidence rather than making habitual applications of a material based on local availability or anecdotal advice is a positive step in reducing unwanted effects of garden management such as nutrient losses causing wider environmental pollution.

This study has evaluated the long-term legacy effects of organic matter addition within a garden system on a sandy loam soil. Results of which may differ on other soil types. OM addition to garden soils can increase contribution of these systems to carbon sequestration and therefore climate change mitigation (Lal and Rattan, 2004). To what extent depends on the type of organic matter applied. Potential recommendations to reduce a “priming” effect on microbial decomposition processes resulting from low nutrient conditions and consequently, microbial mining for resources would be the addition of different organic matter in combination or alternated each year of application to ensure nutrient availability. There is a need to balance the immediate needs of a garden soil (and associated organic matter applications) to support plant growth through nutrient provisioning and the potential for climate change mitigation through carbon sequestration services.

Different organic matter treatments had significant influence on soil chemical and physical parameters which correlated with increased SOM% as driven by organic matter treatment. Thus, confirming the original hypothesis that annual additions of the same type of organic matter to soil would result in a build-up of available nutrients, with this effect being dependent on organic matter type and concurrent with SOM% increase. Overall regression trends between SOM% increase were significant. In the following discussion the results of the data analysis and lab experimentation are evaluated in relevance to the hypothesis.

3.5.1 Organic matter accumulation

Organic matter% of the treatment plots did not significantly differ from the control or from each other in the first year of the trial (2008). By the middle of the trial (2013) all plots treated with organic matter additions had significantly higher SOM% than the control plots and the plots treated with fertiliser. Yearly additions of organic matter to soil were expected to increase the SOM% measured in the trial plots (Chenu et al., 2019). The bark and peat treated plots had significantly higher SOM than all other treatments in 2013 and 2018 (middle and end of trial) and all other treatments did not differ from one another in years 2013 and 2018, despite significant differences in C/N ratios of the material applied (bracken/ mushroom compost 2018, manure/ mushroom compost 2018). This aligns with work which has stated that C/N ratio of the material is not the only driver of organic matter build-up (Ding et al., 2022; Gross and Glaser, 2021).

There were no significant increases (all treatments) or decreases (all treatments except bark Figure 3-2) in SOM% between the years 2013 and 2018, suggesting a saturation point, or equilibrium had been reached between input-output. Existing literature describes possibility that all soils have an inherent total capacity, or saturation point, for C storage dictated by physiochemical properties of the soil system (Craig et al., 2021). Once the upper limit of this capacity has been reached SOM or TOC build-up will cease or even decrease with C being lost from the system. Upper limits of SOC increase following manure applications were recorded as +48%, but only if the initial SOC was below 1% (Gross and Glaser, 2021). The mechanisms behind this are not fully understood (Dungait et al., 2012; Gross & Glaser, 2021). Some studies have identified this limit as a product of physiochemical parameters. If this were the case, it could be expected that each of the treatments would reach a limit at a different time-point due to differences in physiochemical properties of the organic matter amendment material. All treatments (except bark and peat) reached a saturation point by 2013. As such we can propose that other (biological) mechanisms are determining the rate and limit of turnover (Craig et al., 2021; Rasmussen et al., 2018). Further work which would improve understanding of the findings of this analysis should include exploration of microbial mechanisms which mediate soil organic matter decomposition as influenced by different C inputs. With focus on understanding the timeframe for adaptation of a microbial community to changes in environment and abiotic conditions.

Changes to physiochemical parameters are key drivers of changes in biological community structure and function (Craig et al., 2021). Testing the theoretical limit of SOM or C in a specific system in a shorter time than it takes for the community to adapt would help to differentiate whether the saturation point for SOM% build-up in the results presented here (Figure 3-2) is a physiochemical limit or whether the limit is dictated by the speed of microbial adaptation to input.

The only treatment to alter SOM% during the period between the middle and end of the trial was the bark treatment which significantly decreased SOM% but remained higher than all other treatments and the control. This could be due to changes in the structure of the material added, which can be seen by the significant increase in the C/N ratio of the bark material (prior to application) in 2018 (Figure 3-1). This increase in recalcitrance did not result in a reduction of HWEC or labile C when compared to other treatments, which were all significantly higher than the control (and lower than peat), suggesting that decomposition processes could have been maintained even when recalcitrance of input increased as labile C sources were still available to support microbial function.

Although no microbiological analyses of the trial were taken between 2007 and 2018 it is plausible that the microbial community structure was altered between 2008-13 after which time it had adapted to changes in soil chemical and physical conditions (prompted by OM input) and was more able to cycle the form of C added. A lag-effect between 2008-13 may have resulted in the significant accumulation of SOM. The lag caused by the microbial community changing in structure and allowing microbes to be outcompeted by those which can initiate decomposition of a new input into the system (Zheng et al., 2021). Fungal niche adaptation is a potential mechanism by which the bark treatment resulted in a reduction of organic matter from a high C input. Fungi are widely cited as being more capable of decomposing high carbon material than other microbial domains and are vital to early stages in the microbial chain of carbon decomposition (Zheng et al., 2021).

Any potential equilibrium of input-output of SOM in the field trial is influenced by microbial decomposition functions (Craig et al., 2021). Gross and Glaser (2021) highlighted that saturation of soil organic carbon (SOC) was reached sooner in soils with high SOC and Angers et al, (2011) found that sandy soils were more prone to C saturation. The results found from the RHS Wisley trial align with these previous findings as field trial plots reached saturation within the first 6years of the trial.

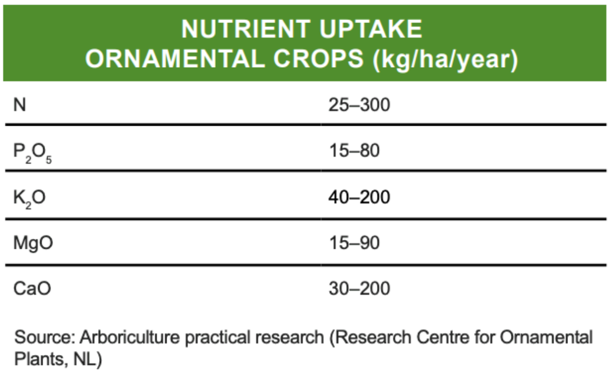
3.5.2 HWEC and organic matter input

HWEC has been cited as a sensitive measure of soil carbon which has been shown to consistently correlate with changes in field management even when no changes are seen in SOC measurements (Ghani et al., 2003). Furthermore HWEC has been shown to positively correlate with measures of microbial biomass (N and C) (Ghani et al., 2003). The complexity of SOM and soil C is not fully understood and the dynamisms of various fractions of soil carbon along an organic matter decomposition continuum are still being examined by the scientific community. This continuum can be considered as stretching between labile, stable and inert fractions of soil C with HWEC representative of the labile fraction thus providing resources for soil organisms which facilitate the release of plant available nutrients through decomposition of OM material (Bankó et al., 2021).

In the RHS Wisley field trial HWEC was significantly increased by organic matter amendment – the amount being a characteristic of the input (Figure 3-11). Peat treated plots had significantly higher HWEC levels than all other treatments (Figure 3-11). Bark and manure treated plots increased labile carbon availability in the second measurement of the year (2019) (Figure 3-11). Though bark had the highest total C of any organic matter material treatment in 2019 (Table 3-2) it is interesting that by the end of the trial it also had the highest availability of HWEC (or labile C). This effect was also outlined by Ghani et al. (2003) who showed that in treatments which increased total SOC, increases in HWEC were significantly correlated. Microorganisms can reduce or negate SOC sequestration through accelerated decomposition in nutrient poor conditions (Kok et al., 2022). All treatments had varying C/N ratios and varying degrees of available nutrients (Table 3-2) and resulted in legacy effects that significantly influenced nutrient availability, potentially through altered decomposition. The increase in labile carbon would suggest that decomposition was affected by the OM amendment.

3.5.3 Available nutrients

Multiple studies have shown that a beneficial effect of organic matter applications is a reduction in nutrient leaching (Latifah et al., 2019; Siedt et al., 2021). The inverse is the potential for a build-up of nutrients to potentially toxic levels which could have alternative negative impacts on soil and plant function (Dhaliwal et al., 2019; Small et al., 2019). The first and main hypothesis, that additions of organic matter to garden soils would result in a build-up of available nutrients, and that this effect would be distinct and dependent on the organic matter applied, was confirmed by the analyses presented. Figure 3-4, Figure 3-5, and Figure 3-6 show the output of linear modelling using organic matter % measurements taken from the field trial plots in years 2008, 2013, 2018 against measures of available nutrients separated by treatment. There were significant increases in available N (peat), P (mushroom compost, horse manure) and K (mushroom compost, horse manure), Mg (all treatments except fertiliser). Available Fe was significantly increased by fertiliser, bracken, bark and peat application.



NUTRIENT UPTAKE ORNAMENTAL CROPS (kg/ha/year)	
N	25–300
P_2O_5	15–80
K_2O	40–200
MgO	15–90
CaO	30–200

Source: Arboriculture practical research (Research Centre for Ornamental Plants, NL)

Figure 3-12 Table from ADAS and RCOP (NL) showing nutrient requirements for ornamental crops (Schoeters et al., 2021).

RB209 provides reference information and fertiliser guidance for agricultural systems such as cereals and tuber crops. Though there is not specific guidance in RB209 for ornamental, direct sown, annual flowering plants such as are common in gardens (UK) there is guidance provided by AHDB on nutrient uptake of ornamental crops in their publication “Sustainable fertiliser use in horticultural crop production” (Schoeters et al., 2021) and on commercial bulb production in Section 6 of RB209. In Figure 3-12 the average nutrient uptake of a commercial, ornamental crop can be seen. Organic matter amendments we applied on a volume basis so as to represent common garden methods for application of composts and soil conditioners (Duddigan et al., 2021). 70L of each organic amendment were applied to 9m2 plots, which equates to approximately 77,778l or 77.8m3 per hectare. None of the application rates were adjusted according to the nutrient status of each input and the nutrient application rates varied widely across the input varieties as can be seen in Figure 3-13.

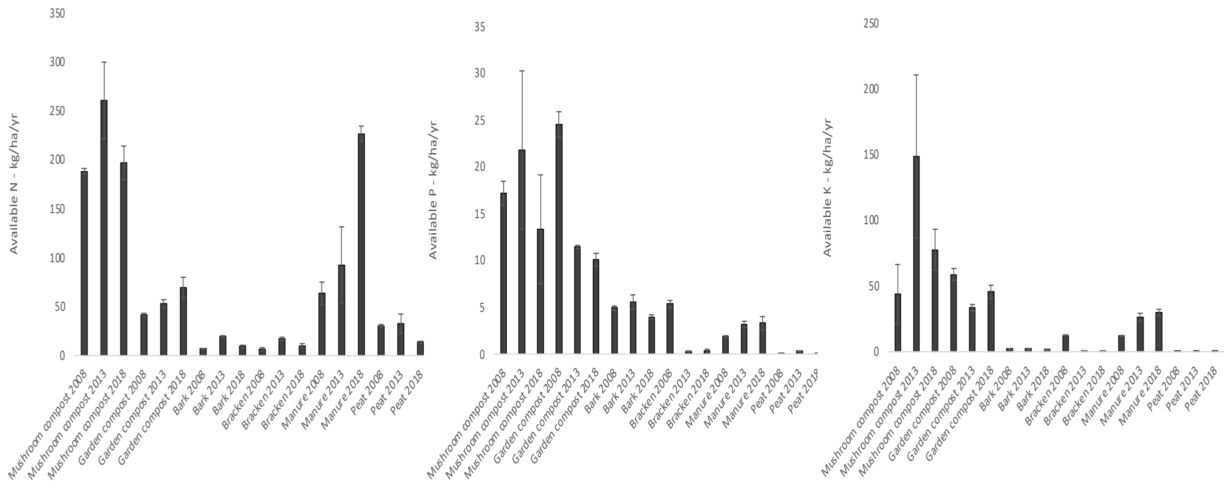


Figure 3-13 Input of available nutrients (N, P, K) from each organic matter amendment material in 2008, 2013, 2018 (kg/ha/yr). Error bars denote standard error of the mean for each treatment.

In comparison the organic matter material characterised in this chapter, which was added on a volume basis (as is common in both horticultural and agricultural systems using organic matter amendments) provided significantly different amounts of available nutrients (Figure 3-13). It is worth keeping in mind that the AHDB figures are for commercial production of ornamental crops. The requirements in garden systems which produce a variety of annual and perennials as well as ornamentals and consumable varieties of plant in the same system result in different requirements than in large-scale systems.

Of the organic matter materials themselves (prior to application to soil) the bark material had the lowest soluble N. The relationship between N and plant yield was, as expected, found to be significant overall. However, the correlation between plant yield, N and the bark treatment was not significant suggesting that other variables influenced yield in the bark treated plots. Research into the drivers behind N immobilisation and mineralisation is a growing area of study (Elrys et al., 2022; Gao et al., 2022). Some studies highlight the relevance of the C/N ratio of input as a regulating force behind microbially mediated N cycling (Ho et al., 2022). There are other studies which posit that labile C content, not C/N ratio, is the overriding driver of microbial N immobilisation and mineralisation (Ding et al., 2022). As all treatments increased labile C we can propose that in this study microbial nutrient cycling was not limited by unavailable N by the end of the trial. Furthermore, we can conclude that N availability was not limited by any of the organic matter treatments, even if very little available/soluble N was imported in the material there was sufficient labile carbon (HWEC) to support decomposition and an increase in plant available nutrients (sometimes excess) in all treatments. The exception to this being the peat treatment group which may have reduced nutrient availability through mechanisms such as lowering pH (Neina, 2019). Further evidence that supports the conclusion that N availability was not a limiting factor in the field plots treated with OM can be seen in , in which plant yield was not significantly negatively affected by OM% increase and no significant negative correlation between plant yield and available N. Despite bark treated plots having low levels of nitrogen the yield from these plots was not significantly lower than many other treatments which had higher N availability. Peat which had the highest N had some of the lowest plant yields.

The reduction in N noted in the mushroom compost treatment in 2018 compared to 2008 and 2013 may have been due to changes in the compost itself (Table 3-2). Compost varies year to year and analysis of the composts highlights some variability in the material (Table 3-2). The pH of the peat material was significantly lower than other treatments in all years, garden compost and manure were the highest. Despite garden compost, peat, bracken and bark materials having the lowest levels of Mg, peat treated plots saw the largest accumulation of Mg and this significantly correlated to SOM% increase. The same was true of Fe, peat material had the lowest levels of Fe in the material but the high levels in the plots by 2018 (Table 3.2 and Figure 3-6). Mechanisms moving the measurable and available Fe may have included fluctuations in ground water, as noted in the introduction the site is characterised as having a high-water table. Interactions of the groundwater and redox interactions influencing Fe availability in soils are examined in Schuth et al. (2015) and outline that organic-rich, top-layers of the soil horizon are a “dynamic reservoir” for available Fe (Schuth et al., 2015).

Through the decomposition process or by the altering of parameters (such as pH), the organic matter amendments are driving changes to soil stoichiometry as the soil microbial population adapts to the input and strives to return to a nutrient equilibrium (Ng et al., 2014). By 2018 reduction in plant yield was significantly related to Fe availability in trial plots (Figure 3-9). Composts have been shown to have higher concentrations of trace elements than (agricultural) soils (He et al., 2005). Fe is essential to plant growth and application of trace elements to improve plant symptoms of deficiency are common (He et al., 2005). Availability of Fe and other trace elements is impacted by parameters such as redox level. Fe is less available to plants in raised pH conditions. In field trial plots pH was increased by organic matter input, however in peat plots Fe was significantly related to plant yield reductions and repeated application of peat lowered pH. High levels of Fe in soils have also been shown to result in deficiency of other essential nutrients such as molybdenum (Mo), particularly in soils with high organic matter content (He et al., 2005). Without measurement of plant nutrient uptake, it is not possible to conclusively say what the interactions behind the relationship between plant yield and Fe levels in the field trial plots were. However, despite being an essential nutrient for plant growth, excessive Fe is widely accepted to have potential negative implications for plant health due to interactions with oxygen. Additions of composts have been shown to interfere with plant defence mechanisms for Fe toxicity at various stages of the plant life cycle, including increased sensitivity during seed germination (Ravet et al., 2009). Which would support the anecdotal reports from the RHS that in later years of the trial germination rates were low and 2017, 2018 and 2019 were noted as crop-failures due to poor establishment.

Both horse manure and mushroom compost demonstrated a significant build-up of P by 2018 (Figure 3-10). Soil available P positively correlated with plant yield throughout the trial, suggesting that it was never a limiting factor for plant growth (Figure 3-8). Ideal levels of available P in soil in order to support plant growth have been cited as 0.003-0.3mg/l soil (Mnthambala et al., 2022). This suggests an excess of plant available P which can cause problems for plant health and have unwanted environmental impacts (Small et al., 2019). Various studies have outlined issues resulting from excess P runoff into surrounding systems leading to issues with eutrophication (Qin et al., 2020; Small et al., 2023; Holman et al., 2008). Furthermore long-term excess P additions to soils have been shown to result in changes to soil properties, such as dissolved organic carbon, and influence changes to microbially mediated P cycling functions (Liu et al., 2022). Symptoms of P excess stress on plants have been outlined as changes to osmotic pressure, decreases in stability of essential elements (particularly Zn, Cakmak and Marschner, 1987) within plant cells and reductions in enzyme reactions (Hu et al., 2021). Considering the significant increase of available P in several of the treatments (mushroom compost, garden compost and manure) it can be assumed that by 2018 these treatments were having some effect on plant health and localised environmental pollution. It is essential that these effects (tripling of P in manure treated plots 2008-2018), be considered by gardeners when applying organic matter.

A significant increase in available K significantly correlated with plant yield in 2018 (Figure 3-7). K is an essential nutrient for plant growth and health, key in photosynthesis, stomatal movement, protein synthesis and stress response (Römheld & Kirkby, 2010; Wang et al., 2013). Despite both P and K being essential for plant growth and health, an excess of either can be lost from the system and cause unwanted environmental effects such as localised pollution.

By 2013 significant build of Mg was noted in the plots, the effect correlating with organic matter treatment accumulation (Figure 3-5). From this analysis it is possible to theorise that crop “failures” recorded by the RHS in the later years of the trial (2017, 2018, 2019) were due to changes in soil stoichiometry driven by the input of organic matter, which were not noted in fertiliser treated plots. Unfortunately, as the crop was considered to have been unable to outcompete weeds in these years no data was recorded for 2019 and limited data collected in 2017 and 2018. No measurements of necrosis or symptoms of plant nutrient uptake or stress were recorded.

Nutrient interactions, particularly the response in uptake due to excess of a particular nutrient are well documented in agricultural studies and have been highlighted in some studies focused on manure and slurry applications (Aguilera et al., 2013; Ding et al., 2022; do Carmo et al., 2016). Despite being well explored, soil biochemical-plant interactions are complex and vary depending on both biotic and abiotic factors such as soil type, pH, soil temperature and weather. Mechanisms behind these biochemical processes are still not fully understood (Jansson and Hofmockel, 2020; Koritschoner et al., 2023; Y. Liu et al., 2023). Examples of unwanted interactions of soil nutrients include excess K causing a reduction in plant availability of Mg and Ca and excess P causing imbalances in Fe (YARA, 2022; YARA Technical Bulletin 2022). Plant Mg deficiency can result in leaf necrosis, reduced carbon metabolism and shorter roots and shoots (Guo et al., 2016). It is plausible that the build-up of Mg in field plots with high K could have reduced the ability of the plant to utilise the available Mg. Unfortunately, no measures of Mg were taken from the plant material harvested in 2018/19 but there was a significant reduction in yield in these years.

The regression analyses only tell half the story and without knowing nutrient densities, relative to treatment, from the harvested plant material it is possible to theorise, but not robustly conclude, that nutrient interactions affecting plant uptake of nutrients which were available in the field trial plots hindered crop establishment and growth in the later years of the trial. The results align with existing literature confirming that reductions in yield were driven by microbially managed soil nutrient conditions (Deyn, 2017). In the second half of the trial significantly more OM treated plots demonstrated a reduction in yield (compared to control: bare plot+plant) than earlier years of the trial (). A different plant was cultivated in most years of the trial making comparisons of yield between years non-viable. However, in 2008 and 2018 borage was cultivated, and demonstrated a significant reduction in yield ().

Quantifying microbial decomposition and nutrient availability from soil amendments in the trial has highlighted the importance of understanding the complexity of the effects caused by amending soils with organic matter. Each input is different, unlikely to be homogenous, potentially different in chemical structure year-to-year and will have varied lasting effects. Organic matter additions are not just carbon inputs but have significant feedbacks with soil biological and physiochemical processes.

3.5.4 Constraints to study and future work

One of the major challenges of the analysis presented here is the lack of data on biotic aspects of the trial. Previous to 2019 no analyses of soil biology were undertaken, and no analyses of plant uptake of essential nutrients were undertaken. It is therefore through comparison with existing research and regression analysis of the data available from the field trial that any reasoning for effects in the trial are attributed to wider biotic effects. Despite this there was significant build-up of soil nutrients, to levels which would be highly likely to have effects on plant health. Notes taken from discussion with RHS technical staff also highlight that the effects of plants were noticed in the trial, though not recorded as data for analysis. Other potential impacts of pests, pathogens and weed competition were not recorded but all may have contributed to issues with crop establishment in later years of the trial.

Further work is needed to assess the NUE of each of the cultivars from the trial and to quantify the differences of potential plant uptake of available N with the N availability of the organic amendment materials (Xu et al., 2020). Assessment of plant material to determine post-harvest levels of nutrient density would also be a useful addition. It was expected that the fertiliser treatment would increase available N however it is plausible that all N delivered by the application of fertiliser was used by the crop or was lost from the system due to run-off, leaching and volatilisation processes (Elrys et al., 2022). Analysis of the soils surrounding each of the plots (or the trial field) to assess nutrient losses from the system, including C and N labelling, would enable further understanding of the impacts of nutrients lost on the surrounding environment (Heijboer et al., 2016).

Organic matter was added to trial plots by volume. This was intended to mimic garden conditions and practice. It is unlikely that the majority of gardeners would correct for C or N content and apply organic matter to their gardens based on this. This decision means that the data collected from the trial is truly applicable to UK gardens and is representative of common practise. However, it also leaves the trial open to criticism in terms of comparability of the effects seen and large discrepancies in nutrient levels between treatments. A critical evaluation of the effects as representative of the treatments and garden practise has been possible and is valuable in relation to the original aim of understanding legacy effects of common garden organic matter input.

3.5.5 Concluding remarks

Objective: Critically evaluate the legacy effects of distinct organic matter additions in quantities relevant to gardens and determine how type of organic matter application influences changes to stoichiometry.

Hypothesis: Annual additions of the same organic material to garden soils will result in a build-up of available nutrients which will be dependent on the type of organic matter applied and concurrent with increases in SOM% as a direct result of OM applications.

Understanding how inputs drive changes to microbial community mediation of a dynamic soil system will help to improve guidance for gardeners considering SOM management decisions. The critical evaluation of a long-term field trial presented in this study confirms that organic matter addition has profound effects on soil physical and chemical parameters. If compost addition is an action by which our management steers the microbial community, it makes sense to know which way we want to steer. For example, the aim of adding organic matter in a garden system might be to improve soil texture, to provide sustained release of plant available nutrients or to suppress weeds when used as a mulch. All of these require a different form of input. These inputs may not be necessary, or may have negative impacts, if added annually without considering multitudinal effects of each addition on soil characteristics. Additionally, it was notable in this trial that the benefits of annual applications of the same material reduced, and nonbeneficial effects (excess nutrient status) increased in the later years of OM application.

Considering the C/N ratio of the inputs is a valuable parameter to consider when making garden specific decisions and has been shown to feedback with soil nutrient cycling (Dobson et al., 2021; Powlson et al., 2011). Adding the same material out of habit could have detrimental consequences to the overall aim of a garden, therefore alternating or mixing composts could a be a solution that more effectively supports multiple functions and guidance provided to gardeners should reflect this.

Implementing organic matter regimens based on multiple effects of specific materials instead of being based on a single end goal, such as increasing SOM%, can ensure that unwanted environmental impacts are kept to a minimum. Alternating different amendments or adding amendments biannually instead of annually could reduce some of these effects.

Based on findings in the existing literature and from this analysis of a long-term field trial it can be recommended that sales of organic matter material for application to soil include a basic outline of the nutrient status of the organic matter being supplied. Combining this information with plant nutrient requirements can assist gardeners in making the best decisions on applications to support plant health or other specific aims without contributing to pollution of water courses or hotspots of nutrient excess (Small et al., 2019). Though worthwhile, implementing this faces challenges due to the heterogeneity of composts/ organic matter produced on large commercial scales.

Applied research focusing on interactions between specific soil management regimens (UK gardens) and changes to soil characteristics was outlined as lacking in the existing literature on essential soil processes. Garden applications of organic matter increased SOM%, a process known to be mediated by soil micro-organisms, and resulted in significant changes to soil physiochemical parameters which have the potential to limit or enhance the benefits gardeners gain from their management decisions.

4 Organic matter additions to horticultural soils: Management for enhancing soil microbial function

Chapter 4 presents a series of experiments using soil and organic matter samples collected from the RHS Wisley field trial in 2019. The microbiological feedbacks with soil nutrient status legacy effects outlined in chapter 3 are explored. Conclusions regarding legacy and within-season effects of organic matter applications on microbial community structure and function are outlined.

4.1 Abstract

The type of organic amendment selected for addition to soil can have profound implications for carbon cycling processes. Understanding the link between this choice and its effect on the soil microbiome can improve our understanding of the capacity of these materials to improve carbon sequestration and cycling dynamics. This research focused on how organic matter amendment alters the indigenous soil microbial community composition and function to improve the capacity of the soil to cycle and store carbon in horticultural soils. Effects of annual application of various organic matter amendments (peat, bracken, bark, horse manure, garden compost) in a long-term (10year) field experiment were explored. The response of soil microorganisms was assessed as microbial biomass, community composition (phospholipid fatty acid profiles), arbuscular mycorrhizal fungi (neutral lipid biomarker 16:1ω5) and carbon functional cycling profiles (Multiple Substrate Induced Respiration: MSIR). Microbial biomass measurements in soils amended with compost and horse manure were significantly higher, and differed in composition, to all other treatments and the control. Peat provided the greatest shift of microbial community composition; bark increased the proportion of arbuscular mycorrhizal fungi. All treatments significantly differed to the control in an analysis of multiple substrate induced respiration. All treatments had a significant positive effect on total carbon. More targeted application of resources can improve efficiency and ensure the legacy

effects of organic matter applications are positive and support beneficial soil functioning.

4.1.1 Keywords

Soil organic matter, microbial respiration, soil quality, soil microorganisms, carbon

4.2 Introduction

Soil is an essential carbon sink and is a vital contributor to reaching targets for zero net carbon emissions in the UK (Sykes et al., 2019). Gardening is an increasingly sustainable practice and one in which the practitioners have a vested interest in making their land systems more sustainable for the benefit of themselves and future generations (Coisnon et al., 2019; Tresch et al., 2019)

Human land management has a significant impact on the soil microbial community (Lal, 2015). The loss of soil microbial bio- and functional diversity has negative implications for the provisioning of soil derived ecosystem services (Louca et al., 2018; Plaas et al., 2019) The dynamic and diverse network of soil biota is sensitive to both anthropogenic land management changes and climate influenced changes to soil physical and chemical properties (Bradford et al., 2016; Crowther et al., 2015b). Understanding the relationship between land management and the microbial community's functionality and composition is essential to restoring and preserving soils and the benefits derived from their microbial processes.

The addition of organic matter to garden soils is a common gardening practise and can provide benefits to garden ecosystems noted as; increased water holding capacity, reduced or improved bulk density, improved available nutrients, higher soil organic matter (SOM) and improved soil tilth and manageability (Dewaelheyns et al., 2013; Siedt et al., 2021). Repeated application of peat-based products can result in negative impacts such as acidification of soils (Taparia et al., 2021). Improper management of organic matter additions may also result in increased CO_2 and N_2O emissions from soils (Qasim et al., 2022).

Loss of organic matter has been shown to have detrimental consequences on soil functions which enhance plant health and provide essential ecosystem services (Tresch et al., 2019). These services include supply of nutrients, formation of soil aggregates, carbon sequestration and improvement of soil water holding capacity leading to increased flood prevention. Without microorganisms land managers could not maintain plant growth (Tresch et al., 2019). In addition, increased symptoms of poor land management such as the loss of nutrients into surrounding systems or losses of CO_2 from their systems would be incurred (Coisnon et al., 2019). The delivery of carbon sequestration services depends on having a functionally diverse community of microbes which work to break down organic matter added to the soil (Fan and Liang, 2015; Louca et al., 2018; Schloter et al., 2018). Inputs commonly added to garden systems are often locally sourced, even maintaining closed systems by composting internal garden and kitchen waste. Today, the awareness of the importance of soil organic matter has become a hot topic and both agriculturalists and horticulturalists are increasing efforts to return various forms of organic matter to their soils to improve soil organic matter and soil function (Chenu et al., 2019; Diacono and Montemurro, 2010; Schmidt et al., 2011).

4.2.1 Soil carbon dynamics and pools in UK gardens: a microbial responsibility

Effects of organic matter additions on garden soil ecosystems and the resulting functions from soil flora, fauna and fungi has seen a surge of interest in recent years (DeDeyn, 2017; Schloter *et al.*, 2018; Tresch *et al.*, 2019). The carbon cycle and the concept of a microbial "valve" through which all soil carbon sequestration processes are mediated is explained by Fan and Liang (2015). They suggest that the soil microbial population mediates the carbon released into the atmosphere from soil deposits and the carbon sequestered in stable pools in soil. Soil microbial lifestyle strategies dictate the efficiency of the carbon cycle (Fan and Liang, 2015; Kallenbach et al., 2019; Nottingham et al., 2019). Yet the complex and dynamic interactions that characterise belowground microbial community function are not fully understood (Fatichi et al., 2019; Kallenbach et al., 2019; Nottingham et al., 2019). It is thought that with increasing recalcitrance of SOM functional redundancy within the decomposer community decreases (Maron et al., 2018b). The microbial “valve” through which SOM passes during decomposition and sequestration becomes smaller with increasing recalcitrance of the material applied. The contribution of varying forms of carbon input results in availability of resources to different microbial domains (Hacquard et al., 2018). Different phyla can access different pools of carbon (Fatichi et al., 2019; Hacquard et al., 2018). All of this emphasises that carbon mineralisation is a microbially mediated process and that above- and below-ground litter inputs can manipulate the magnitude of mineralisation by limiting or increasing substrate availability to the microbial community (Zhang and Zhou, 2018). It is known that nitrogen fertiliser stimulates both mineralisation and competition for nitrogen within the microbial population (Hestrin et al., 2019). How nitrogen (N) input from organic matter and relation of N levels to carbon (C) in these additions changes microbial function is less robustly understood. Substrates with high C/N can provide resources that are more available to the decomposer community, thus changing dynamic competition for belowground resources (Qiao et al., 2019).

Drivers such as aboveground plant community composition and litter deposition can alter the below ground pH which can shift the composition of the belowground microbial community (Chandregowda et al., 2018). The addition of organic matter to soils has a similar effect, yearly addition of peat-based products results in a lower pH as seen in the 10year field trial used in this study (Duddigan et al., 2019). These measurable changes, such as a shift in pH or changes to the structure of the belowground microbial community can be used as indicators of how soil function is affected by external pressures or perturbation (Chandregowda et al., 2018).

Gardening practice gives us opportunity to examine the feedback between microorganisms and anthropogenic influences on the carbon cycle which dictate the rate at which greenhouse gases are regulated by the hugely diverse belowground microbial population (Cavicchioli et al., 2019; Fraser et al., 2016).

4.2.2 Soil organic matter cycling in UK gardens

Peat was included as a treatment in the field trial which was initiated in 2007. The scientific literature exploring the value of peatlands to carbon savings must be kept in mind when considering the results of the field trial, which began over a decade ago and included peat as a relevant substrate available to gardeners at the time (Alexander and Bragg, 2014). Now its inclusion enables a comparison with different inputs with the aim of finding an alternative to its use. The sustainability of peat extraction has become an important topic in recent years with Defra announcing a peatland restoration strategy with funding of £10million to restore 6,580ha of lowland peatland (Defra, 2018; Smith et al., 2018). Multiple studies reinforce the notion that peat is more valuable, economically and environmentally, when left in the ground. The Defra funded English Peatland project reference the projects' saving of 23,000tonnes of carbon per year (Defra, 2018; Smith et al., 2018). In addition the UK Government have proposed a ban on the sale of peat-based composts for amateur gardeners (Gov UK, 2021). By making these additions gardeners and farmers are having both short-term (within the growing season) and long-term effects on biochemical processes (Diacono and Montemurro, 2010; Schmidt et al., 2011; Sokol and Bradford, 2018; Yang et al., 2012).

4.2.3 Aims and hypotheses

The overall aims of this chapter are:

To examine the feedbacks between the soil microbial community and common types of carbon input, in the form of composts, to garden soil.

Establish the impact of changes to garden soil function (respiration) as influenced by feedbacks between repeated organic matter applications and soil microbial community structure following 10years of organic matter amendment.

There are a multitude of models and carbon fractionation studies which have debated ways to define different pools and forms of soil carbon (Jaconi et al., 2019; Keskin et al., 2019; Márquez et al., 2019; Zhang and Zhou, 2018). What has been highlighted as lacking in the body of literature on soil carbon dynamics is information linking this knowledge to the lifestyles of belowground bacterial and fungal populations (Kallenbach et al., 2019; Sokol and Bradford, 2018).

An assessment of a long-term field trial was completed in order to examine the effects of organic matter addition on soil microbial community structure and function (respiration). Different types of organic matter were applied to trial plots, annually for 12 years. On the twelfth year of the trial (2019) soils were sampled at two time-points, the first set of samples were taken prior to the year's application of organic matter (T1) and the second set taken 6weeks after the organic matter had been applied and incorporated (T2). This enabled a study of the legacy and within season effects of ten years (no organic matter was applied in the first year: 2007) of annual additions of each organic matter. Analyses of subsequent changes to SOM and resulting shifts in the structure of the soil microbial population were completed and compared to data collected from the different types of organic matter material (T0). Assessment was made of how the form of organic input directly alters the structure of the microbial community. The functional capacity of the soil microbial community was expected to change through the substitution of decomposer organisms depending on the substrate available. It was hypothesised that:

Organic matter applications will drive changes in soil microbial community structure and have legacy effects on community composition and function, with distinct differences in these effects being dependent on organic matter type applied.

If microbial biomass is maintained, then respiration will continue and respiration (implied decomposition) in high C/N or recalcitrant treatments (bark/ oxalic acid) will be dependent on increased abundance of fungal decomposers.

4.3 Materials and methods

4.3.1 Field sampling and sample preparation

Sampling was completed in March and April 2019 at RHS Wisley Gardens' organic matter field trial, details of which can be found in section 3.3. Briefly a randomised block design of 80 3x3m plots (6 treatments, 2 controls and 8xreplicates of each treatment). Only one control (plots with no organic matter addition applied but the same plant sown as the rest of the treatment groups. One treatment (compost applied at a half rate) was also not selected for study as no other treatments were applied at half rate, thus preventing any meaningful comparison, Using a gouge-auger 9 samples along a "W" transect of each plot were taken and amalgamated into separate bags for each treatment. Samples were transported from RHS Wisley to Cranfield University on ice in a cool box. Samples were split and sub-samples were either freeze dried, air dried in a drying cupboard with low humidity, or kept fresh and returned to the fridge (less than 4°C) for analysis of soil microbial biomass and PLFA community structures within 24hrs of sampling. One set of sub-samples was used for characterising each of the treatments for nutrient status, pH, organic matter, bulk density and total carbon % within the following 4weeks.

4.3.2 Soil nutrient status and pH

Measurements of macro and micro-nutrient status (total carbon and total nitrogen (ISO, 1995), elements soluble in aqua regia (ISO, 1998), Mg and K soluble in ammonium nitrate solution (ISO, 2002) and pH (ISO, 2005), organic matter % (loss on ignition, ISO, 1995) total carbon, estimated CEC and pH were analysed each year for the 10years of the trial. Following preliminary analysis of all years and findings of Chapter 3, organic matter treatments selected for use in the experiments in this chapter were: peat, bark, bracken, manure, garden compost, and a control with no organic matter applied. For soil characterisation analyses sub-samples were taken from the bulk field sample for each treatment before completing analysis of soil characteristics and nutrient status (Table 4-1). For organic matter analysis 6 samples were taken from different bags/ locations in the bulk organic matter material for each treatment material prior to it being applied to the soil (Table 4-2).

Table 4-1 Control soil and soil+organic matter treatment characteristics from field trial (2019). Numbers in brackets denote std. error of the mean.

	Soil		Manure		Compost		Bracken		Bark		Peat	
pH	6.5	(0.2)	7.6	(0.1)	7.8	(0.1)	6.4	(0.1)	5.9	(0.1)	4.9	(0.1)
OM%	6.4	(0.4)	14.3	(0.8)	13.5	(0.4)	13.6	(0.6)	20.5	(0.6)	31.1	(1.2)
C (%)	3.5	(0.4)	8.2	(1.3)	7.7	(0.7)	8.4	(0.7)	8.6	(0.4)	20.1	(0.9)
N (%)	0.3	(0.02)	0.6	(0.05)	0.5	(0.05)	0.5	(0.01)	0.4	(0.01)	0.7	(0.03)
C/N	11.8	(1.2)	14.1	(1)	14.4	(0.1)	15.7	(1.7)	21.6	(0.6)	30.0	(0.5)
CEC	13.5	(0.4)	17.6	(0.4)	20.0	(0.5)	19.3	(0.5)	18.1	(0.5)	20.2	(0.4)
Bulk density kg/m3 (n=3)	1113.3	(29.4)	777.7	(3.2)	806.3	(49.8)	601.7	(41.6)	471.0	(32.9)	422.7	(13.3)
(n=4)												
NO3- (mg/kg)	2.3	(0.3)	16.7	(1.9)	10.8	(1)	11.6	(1.9)	3.1	(0.8)	12.8	(2.7)
NH4+ (mg/kg)	0.7	(0.1)	2.5	(0.1)	1.9	(0.3)	2.1	(0.4)	3.1	(0.8)	17.3	(3.8)
Available N (kg/ha)	11.4	(1.3)	72.1	(7)	47.8	(4.2)	51.3	(8)	22.9	(5.3)	112.9	(22.8)
Soluble nutrients (total mg/kg)												
P	46.3	(8.6)	168.5	(6.0)	128.9	(4.2)	95.2	(11.1)	127.0	(13.7)	107.2	(10.1)
K	52.1	(3.6)	772.8	(38.2)	815.3	(24)	283.0	(25)	532.5	(38.8)	148.6	(14.9)
Ca	1327.1	(133.3)	2618.3	(85.1)	2823.8	(75.7)	3169.9	(163.9)	3549.0	(216.9)	3489.7	(240.1)
Mg	59.6	(6.2)	189.4	(6.0)	301.4	(8.7)	387.1	(12.9)	434.4	(11.8)	614.7	(8.9)
(n=6)												

Table 4-2 Mean values for organic matter (pre-application to trial plots) treatment material characteristics (2019). Numbers in brackets denote std. error of the mean.

	Manure		Compost		Bracken		Bark		Peat	
pH	8.0	(0.12)	8.1	(0.03)	5.5	(0.09)	6.5	(0.31)	4.3	(0.09)
OM%	35.1	(0.5)	52.1	(0.8)	23.3	(1.0)	42.1	(2.6)	37.0	(0.4)
C (%)	34.4	(3.7)	21.7	(0.8)	39.9	(1.6)	47.7	(0.4)	56.5	(2.3)
N (%)	1.6	(0.03)	1.2	(0.06)	1.9	(0.05)	0.8	(0.06)	1.4	(0.03)
C/N	21.0	(2.5)	17.8	(0.2)	21.0	(0.3)	61.3	(5)	39.5	(0.6)
Bulk density (kg/m3)	131.4	(5.4)	333.1	(11.8)	101.3	(5.8)	176.5	(6.3)	130.6	(4.8)
Total (available) N (mg/kg)	1767.1	(150.4)	305.8	(15)	654.5	(122.4)	39.8	(1.2)	132.2	(13.5)
Soluble P (mg/kg)	128.6	(11.4)	91.1	(6.8)	135.5	(27.7)	192.2	(22.5)	6.7	(0.3)
Soluble K (mg/kg)	17858.4	(675.4)	3173.5	(125.7)	1751.9	(223.3)	785.3	(130.8)	17.6	(3.5)

(n=3)

4.3.3 Microbial biomass

Microbial biomass was measured according to the fumigation-extraction method (Jenkinson, 1976; Andrzej Bieganowski et al., 2015). 10g sub-samples of fresh soil from T1 (plot samples prior to application of OM) and T2 (plot samples post-OM addition and incorporation) were processed separately and according to the same protocol. Each sample was weighed and placed in a clean, glass beaker. Fumigated samples were placed in a desiccator with alcohol-free chloroform and the non-fumigated samples were placed in a separate desiccator without chloroform. Desiccators were lined with damp paper and pressurised and evacuated before removing the fumigant and extracting samples with 0.5M K_2SO_4. Samples were shaken for 30minutes at 300/min. Each suspension was filtered through a Whatman no. 42 filter paper into a clean sample bottle. 5ml of sodium polyphosphate reagent was added to each sample bottle and analysed on a SFA-2000 segmented flow analyser with the coefficient of proportionality kEC – 0.45 (Joergensen, 1996).

4.3.4 Microbial community structure: PLFA and NLFA

Phospholipid Fatty Acid (PLFA) and Neutral Lipid Fatty Acid (NLFA) analyses were performed on sieved (2mm) and freeze-dried soil according to the method described by (Pawlett et al., 2013). Due to the high organic matter content of T0 samples 3g per sample were used for analysis as opposed to 7g for the soil/ compost samples in T1 and T2. In addition, an extra 10ml of Bligh and Dyer solvent (ratio of 1:2:0.8 methanol, chloroform, and citrate buffer) (1979) was used as the T0 samples adsorbed the full 20ml which was sufficient for use with T1 and T2. The solid phase extraction method was used for fractionation of the extracted lipids. The resulting fatty acid methyl esters (FAMEs), including those retained from NLFA fractionation, were processed using an Agilent Gas Chromatographer (6890 FID-FPD), with a HP-5 capillary column (Agilent technologies) and a temperature program starting at 50ºC (1min) to 160 ºC at 25 ºC per minute continuing to 2 ºC per min to 240 ºC and 25 ºC per min to 210 ºC. Injector temperature was set at 310 ºC and flame ionisation detector set at 320 ºC. FAMEs were recorded as relative abundance (mol%).

PLFA 18:2w6,9 was used as a biomarker for fungal estimation (Frostegård and Bååth, 1996). Neutral lipid (NLFA) 16:1w5 was used to quantify the arbuscular mycorrhizal fungi (AMF) biomass (Olsson, 1999; Pawlett et al., 2013). PLFA biomarkers from the literature by Frostegård, Tunlid and Bååth (2011) and Pawlett *et al,* (2013) were used with Gram positive (Gram+) biomarkers represented as i15:0, ai15:0, i16:0, i17:0, ai17:0 and Gram-negative biomarkers 16:1w7c, cyc17:0, 18:1w7, cyc19:0 with the addition of 15:0 used for total bacterial expression in the samples analysed (Frostegård et al., 2011a). Fungal PLFA biomarker 18:2w6,9 was used to estimate fungal populations and NLFA 16:1w5 for estimation of arbuscular mycorrhizal fungi (Frostegård et al., 2011a). A table of biomarkers and relative retention times can be found in the appendices section Table B-1 .

NLFA 16:1w5 is considered a more reliable signature of AMF community than the equivalent PLFA derived FAME and is found in storage lipids of spores and in the mechanism for carbon transportation between intraradical and extraradical mycelium. Although previously widely used for the estimation of AMF in soils the PLFA 16:1w5 was not selected for use as a biomarker of AMF due to its abundance in the lipids of other organisms, particularly soil borne bacteria (Frostegard and Booth, 1996; Frostegård, Tunlid and Bååth, 2011)

4.3.5 Microresp: Microbial community functional profiles

Multiple Substrate Induced Respiration (MSIR) functional profiles of the soil microbial communities were determined using the method of Microresp™ using the standard protocol (Chapman et al., 2007) with the only changes being selection of substrates, these were selected for representation of different levels of carbon availability (molecular weight) to microbial decomposition and relevance to availability in soil systems. Substrates were selected from a range of substrates provided in Campbell et al. (2003) outlined as being examples of acids, sugars and proteins found in soil systems. Samples were fresh soils, sieved (2mm) around 4hrs after sampling and incubated at 19ºC for 48hours prior to analysis. 4 replicates from each treatment were measured, and substrates; alanine, glucose, a~ketoglutaric, n~acetyl glucosamine, malic acid, citric acid, oxalic acid.

Prior to filling deep-well plates with individual replicates the moisture content of each treatment was recorded by drying a separate 5g subsample of each soil at 105°C for 24hours and determining the difference between the wet and dry weights of each soil (ISO, 2002). Peat and the control soil significantly differed to the rest of the treatments (which did not differ significantly from each other), as such 3 different substrate concentrations were prepared as per each of three different soil/water levels. Peat field samples on average had a soil moisture content of 20%, an average of 15% was recorded for the sum of the remaining treatments and 10% soil moisture for the control group.

Each substrate was prepared prior to filling the deep-well plates as 30mg of substrate per g^{-1} of soil water as outlined in (Creamer et al., 2009). Soils were incubated at 22-23°C for 6days in beakers inside containers with sealed containers with a beaker of water and a beaker of soda-lime prior to filling the deep-well plates. A design which incorporated four analytical replicates of each field replicate was used. Peat had an average weight of 0.16g soil per well, other treatments between 0.23-0.29g of soil per well and the control group 0.31g.

4.3.6 Total carbon

Samples from each treatment group and the control soil from T1 and T2 were oven dried at 105ºC for 24hours before being ground with a pestle and mortar to a fine powder. From each field replicate, four analytical replicates of 40mg were weighed and prepared as per BS 7755 Section 3.8:1995. Total carbon content of soil samples from both time points were analysed using Elementar Vario/// EL analyser.

4.3.7 Statistical analysis:

All analyses of data were completed using Statistica software version 13 (TIBCO™). Data were checked for normality (QQ-plots and Shapiro-Wilks' test). Levene's test was used to check homoscedasticity of data. NLFA data for T1 and T2 and bacterial data used for G+/G- bacterial ratios (sum of Gram+ biomarkers: i15:0, ai15:0, i16:0, i17:0, ai17:0/ sum of Gram-negative biomarkers: 16:1w7c, cyc17:0, 18:1w7, cyc19:0) were found to be non-Normally distributed, as such Kruskal-Wallis ANOVA and Friedman non-parametric testing were used. Both MSIR and PLFA data were analysed by subjecting principal component analyses (PCA) using a correlations matrix. Factor scores, total carbon, nutrient analyses, and microbial biomass analysis were analysed using one-way and repeated measures ANOVA with post-hoc testing carried out with Tukey's HSD with a significance threshold of 5%.

4.4 Results:

4.4.1 Carbon in field trial soils and organic matter amendments

Carbon to nitrogen (C/N) ratios were calculated for T0, T1 and T2. A table of C/N ratios for the organic matter inputs and soil samples can be found in section 4.3.2 Table 4-1 and Table 4-2. C/N for PE and BA treatments differed significantly from all other treatment groups.

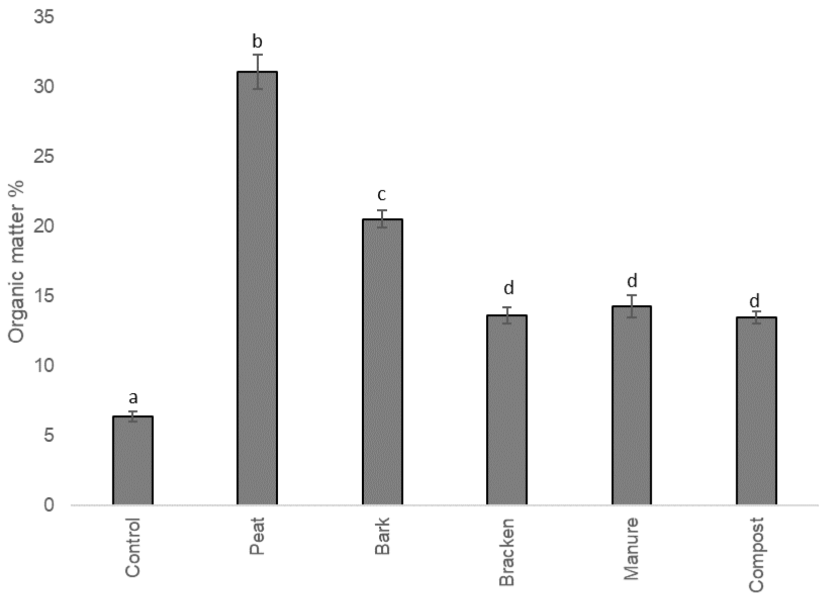


Figure 4-1 Organic matter % of soil samples taken from field trial at T1 (prior to the 2019 application of OM input material). Bars indicate standard error and letters above bars denote significant differences between homogenous groups from Tukey's HSD post-hoc test.

Table 4-3 Summary of one-way ANOVA for OM% of T1 (pre-OM addition in 2019) field trial soil samples

	df	F	P value
Treatment	5	132.871	0.000
Residual	42		

(n=6)

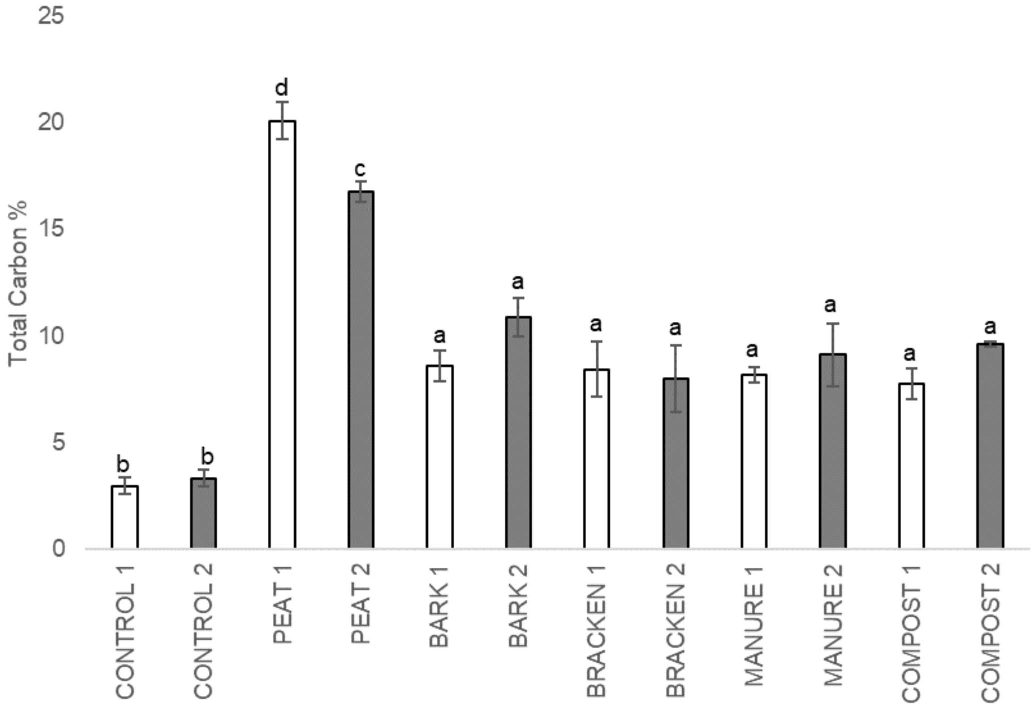


Figure 4-2 Soil total carbon (bars indicate SE). Data represent soils sampled before the 2019 addition of organic matter (T1) and after the 2019 (T2) OM addition and incorporation. Letters signifying homogenous groups from Tukey's HSD.

Table 4-4 Summary of one-way ANOVA of TC% at T1 and T2 sampling of field trial soils

	df	F	P value
Treatment	11	27.729	0.000
Residual	36		

(n=5)

All treatments had significantly higher total carbon content when compared to the control group (Figure 4-2). However, only peat saw a significant difference between sampling times. This effect was noted as a reduction in total carbon. None of the other treatment groups or the control saw within season effects on total carbon. All other treatments remained the same despite the addition of organic matter in the spring.

4.4.2 Microbial biomass and microbial community structure (PLFA/ NLFA) in field trial soils and organic matter amendment material

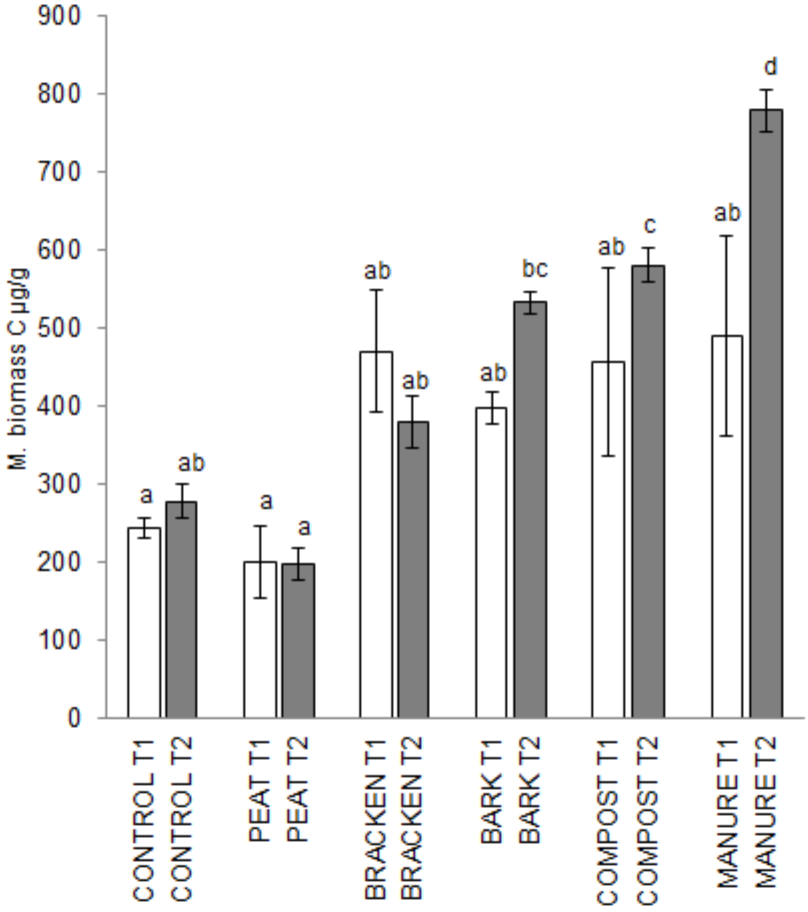


Figure 4-3 Soil Microbial biomass T1 and T2. Letters indicate homogenous groups from Tukey HSD. Unfilled bars are from early spring field samples prior to the 2019 application of organic matter (T1) and filled grey bars are 1month post application and incorporation of organic matter (T2).

Table 4-5 Summary of one-way ANOVA of microbial biomass C for T1 and T2 field trial soils.

	df	F	P value
Treatment	11	8.267	0.000
Residual	36		
(n=5)			

Peat had lower microbial biomass than the other treatments, but biomass was not significantly different between applications (Figure 4-3). The only significant differences when compared to the control were garden compost and manure at T2, demonstrating a within season effect of the addition of compost or manure on the soil microbial population.

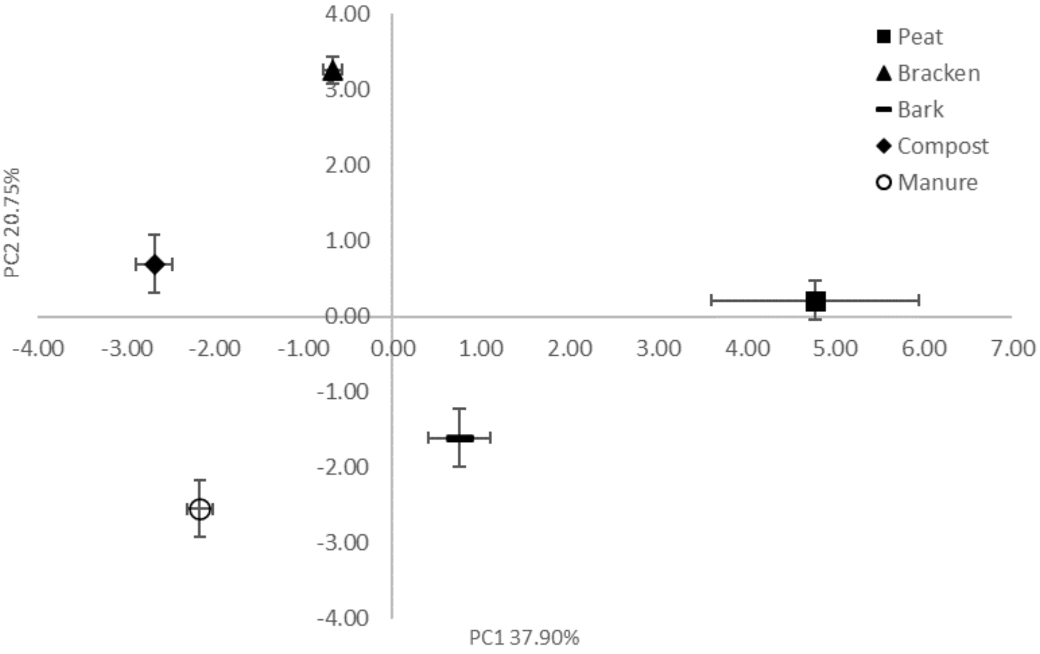
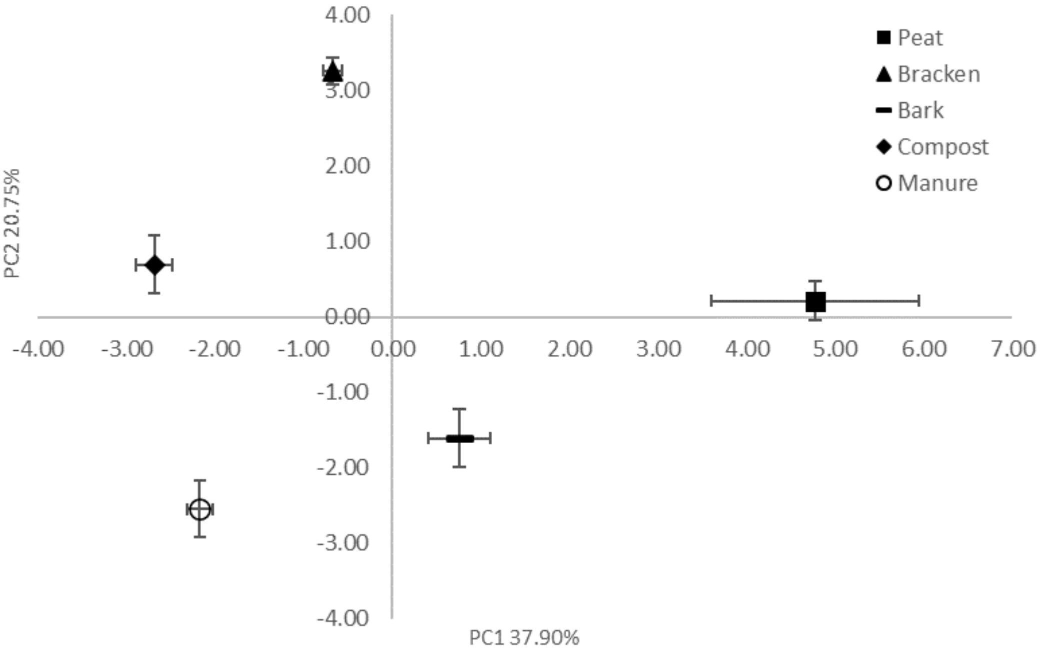


Figure 4-4 Phospholipid Fatty Acid profile of organic matter prior to addition to trial plots (T0) using principal component analysis. Error bars indicate standard error.

Table 4-6 Summary of one-way ANOVA of principle component analysis results of PLFA for OM samples (T0)

	df	F	P value
Treatment	8	43.051	0.000
Residual	18		



PLFA analysis of the organic matter amendment material prior to being added to trial plots (Figure 4-4 T0) identified that compost, bracken and manure were significantly different to peat and bark on PC1. On PC2 treatments significantly differed from each other, with the exception that peat was not different to compost ($P<0.05$). This demonstrates that all organic matter material (T0), to some degree, had an inherently different microbial community structure before application to the soil.

Fungal/ bacterial (F/B) ratios were significantly affected by organic matter input to field trial plots (Figure 4-7). Peat, bracken, garden compost and the control did not differ between T1 & T2. However, manure and bark saw a significant increase ($p = <0.05$) in F/B ratio following organic matter input. F/B in T0 samples were analysed, but no significant differences between organic matter types were found.

Gram+/ Gram- bacteria ratios were not found to be statistically significant between treatments or the control soil. There were no significant differences in any treatment between T1 & T2 in Gram- bacterial populations. However, bacterial PLFA biomarkers influenced both PC1 and PC2. Individual FAME biomarkers for Gram+ bacteria influenced PC1 weighting. 18:1w6,9 did not have a significant weighting on PC1 or 2 (

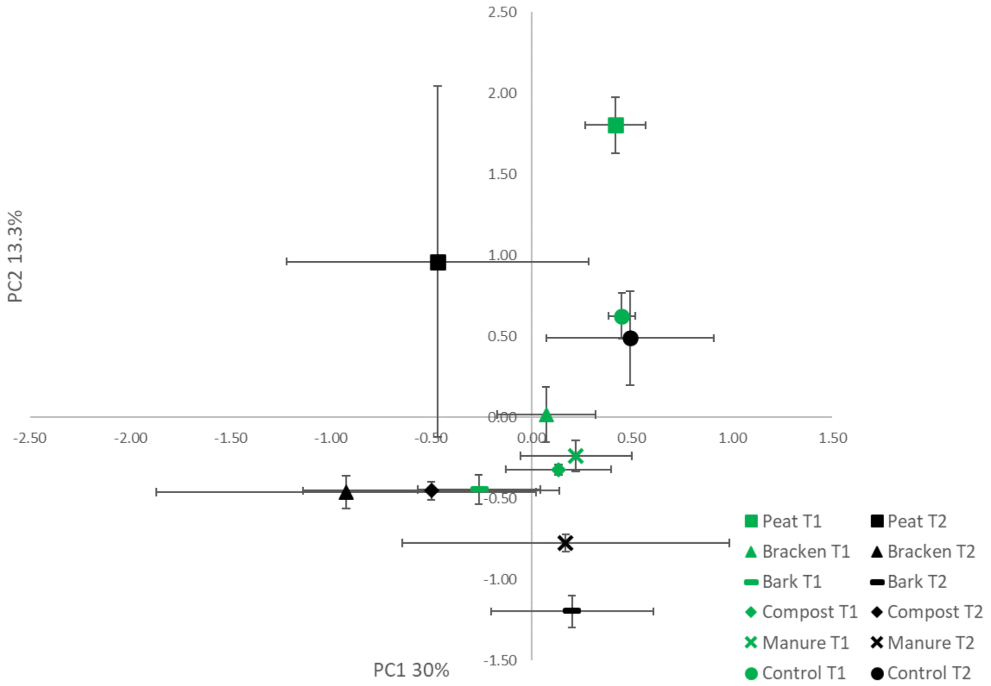


Figure 4-8).

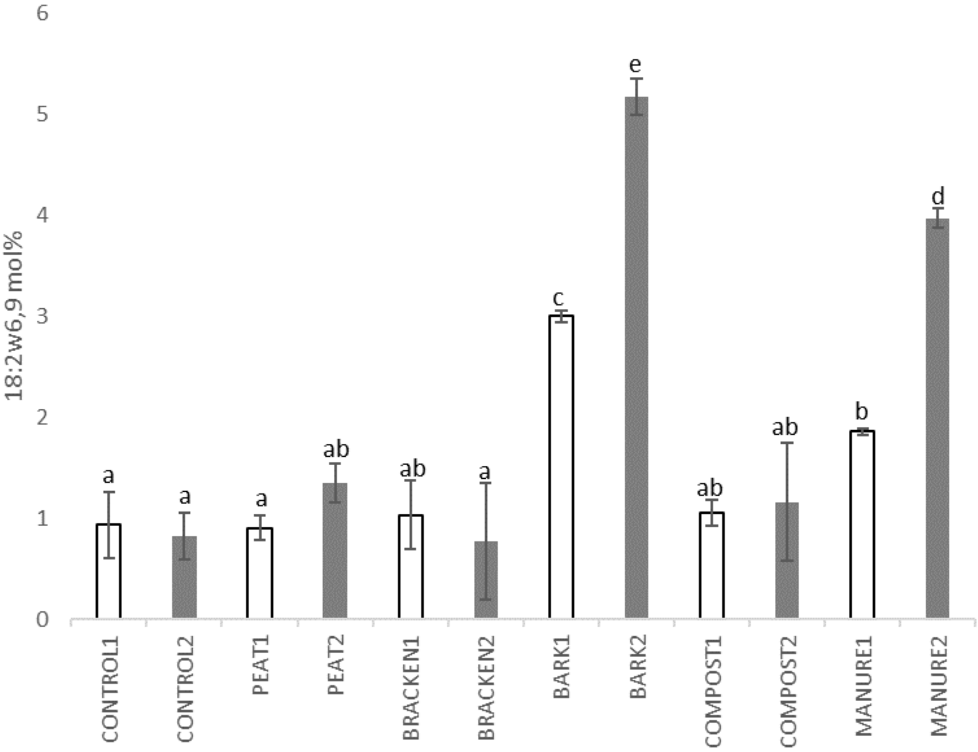


Figure 4-5 PLFA biomarker 18:2w6,9 in field trial plots before (1) and after organic matter application (2). Letters signify homogenous groups from Tukey's post-hoc test (p=0.000).

Table 4-7 Summary of one-way ANOVA for PLFA 18:2w6,9 in field trial soils (T1 & T2)

	df	F	P value
Treatment	11	23.506	0.000
Residual	36		

(n=5)

Neutral lipid fatty acid (NLFA) FAME 16:1w5 was used for the quantification of arbuscular mycorrhizal fungi (AMF) in field trial soil and organic matter samples. No significant differences (p = >0.05) were found for abundance of FAME 16:1w5 in OM samples (T0). T1 & T2 data were found to be non-Normally distributed. Levene's test of homogeneity of variance was used for population variances and variance was found to be significant (F= 3.973 and p = 0.000). Kruskal-Wallis ANOVA was applied to assess the data (Table 4-8).

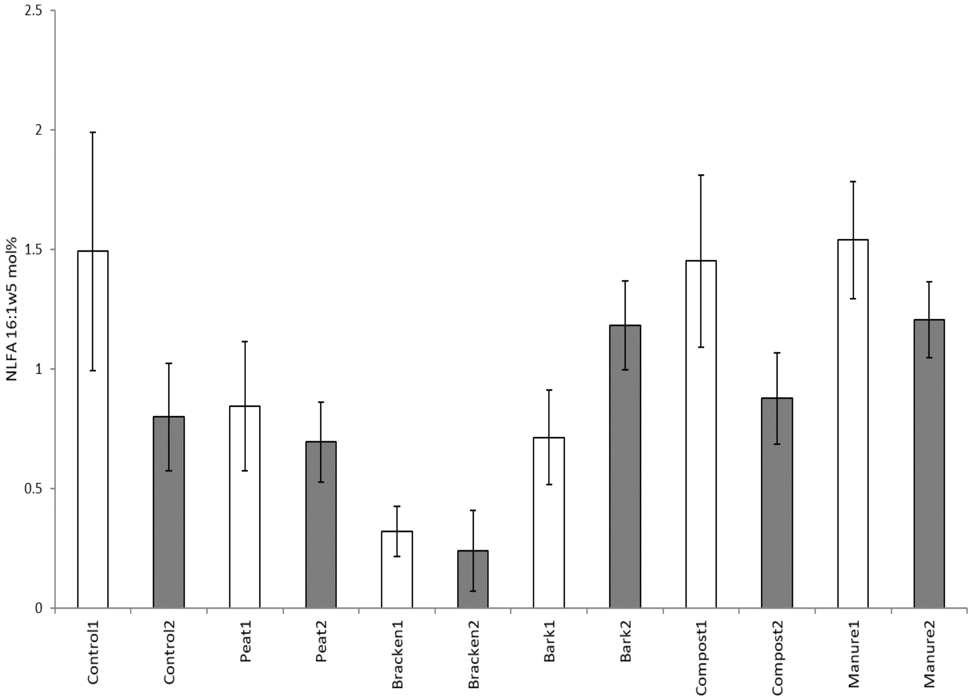


Figure 4-6 NLFA 16:1w5 mol% from soil samples at T1 and T2. Error bars indicate standard error of the mean.

Table 4-8 Summary of Kruskal-Wallis ANOVA and NLFA 16:1w5 in field trial samples T1 & T2

Kruskal-Wallis ANOVA	df	H	P Value
T1	5	10.712	0.054
T2	5	5.82	0.324

(n=24)

Sum of ranks	T1	T2
Control	65	36
Bracken	16	67
Bark	39	60
Manure	68.5	63
Compost	66.5	43
Peat	45	31

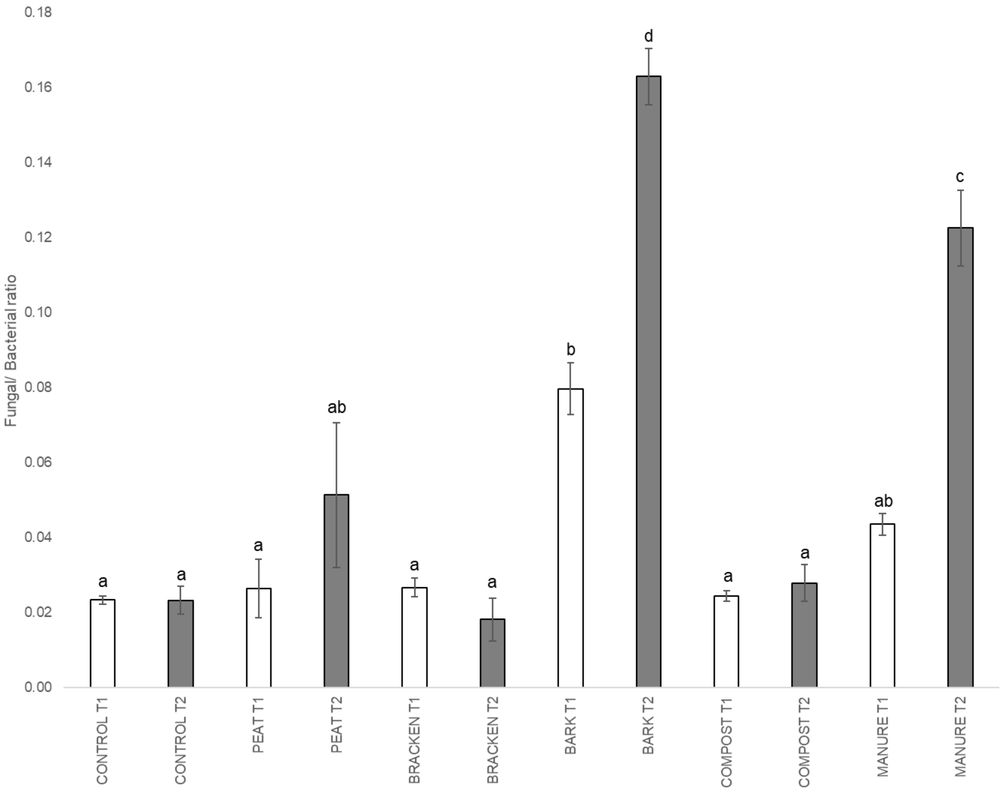


Figure 4-7 Fungal/ bacterial ratios for T1 (unfilled bars) and T2 (filled) samples. Error bars denote standard error and letters above bars indicate significant differences between treatments from Tukey's post-hoc test.

Table 4-9 Summary of one-way ANOVA of F/B ratios for field trial T1 & T2 samples

	df	F	P value
Treatment	11	19.139	0.000
Residual	36		

(n=5)

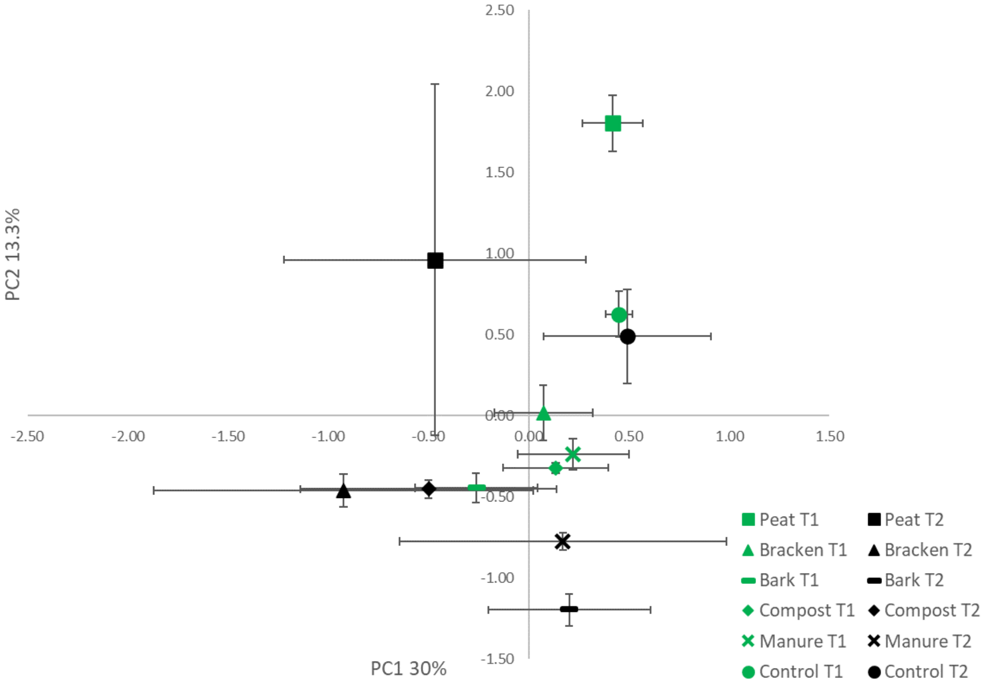


Figure 4-8 Figure 9 PCA of PLFA data from T1 and T2. Error bars denote standard error.

Table 4-10 Summary of one-way ANOVA and Levene's test of homogeneity on PCA (PLFA) scores of treatments (T1 & T2)

	df	F	P value
Treatment	22	3.977	0.000
Residual	70		

(n=5)

Levene's test	F	P value
PC1	3.524	0.002
PC2	4.766	0.000

Table 4-11 Weighting (>0.8 or <-0.8) of FAME (PLFA) biomarkers on principle components 1 & 2

	Positive weighting	Negative weighting
PC1	15:0i, 15:0ai, 16:1w7t, 16:1w7c, 16:0, Me17:0isomer, 17:0c, 18:1w9c	-
PC2	17:1w9t	-

4.4.3 Microbial respiration and MSIR functional profiles

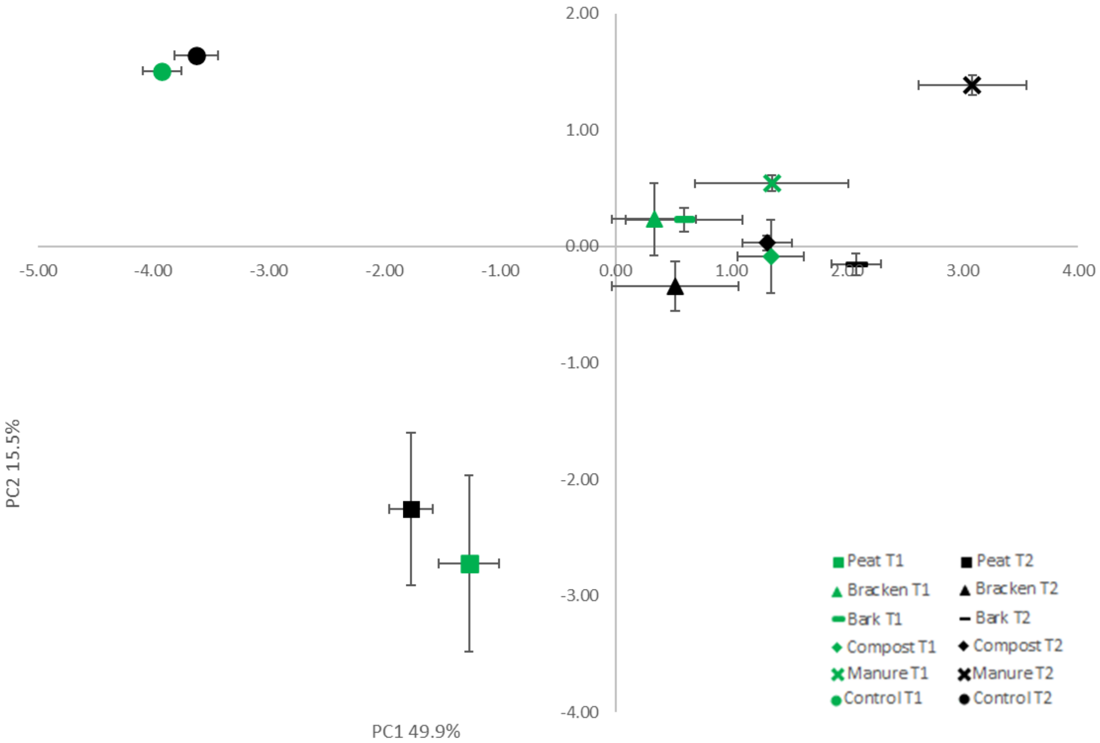


Figure 4-9 Microresp™ functional profile for field trial samples at time point 1 and 2 using PCA (p= <0.001).

Table 4-12 Summary of one-way ANOVA of treatment coordinates on PCA of MSIR for T1 & T2 field trial samples

	df	F	P value
Treatment	22	24.558	0.000
Residual	70		

(n=4)

MSIR differed (p = <0.001) in all treatments when compared to the control group at both time points). Peat differed significantly at both time points to all other treatments. Bark and manure differed significantly between T1 and T2 on PC1. In these treatments OM addition influenced microbial function. No within season effect was seen in the compost or bracken treatments though they did show a significant accumulative effect of OM application on microbial respiration profiles which can be seen from comparison with the control.

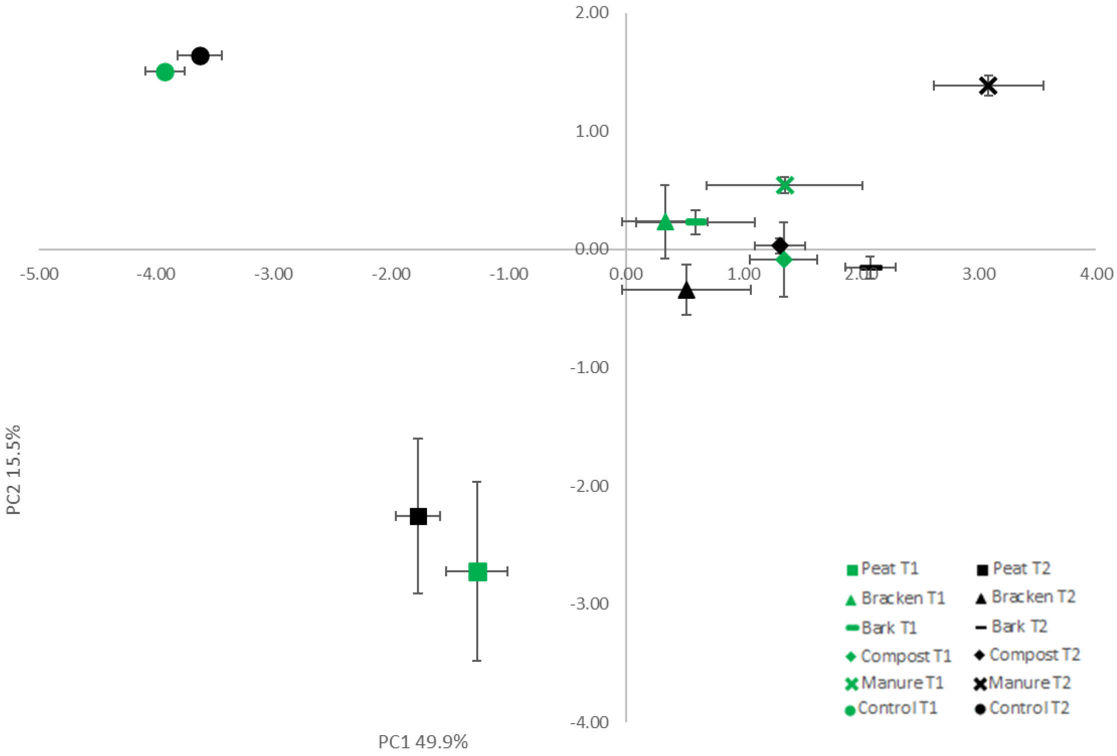


Figure 4-10 PLFA community profiles for T1 (green) & T2 (black) sampling times. Error bars indicate standard error.

All treatments significantly increased basal respiration, at both sampling times (Figure 4-11). The highest measure of basal respiration was recorded following the application of bracken compost at T2.

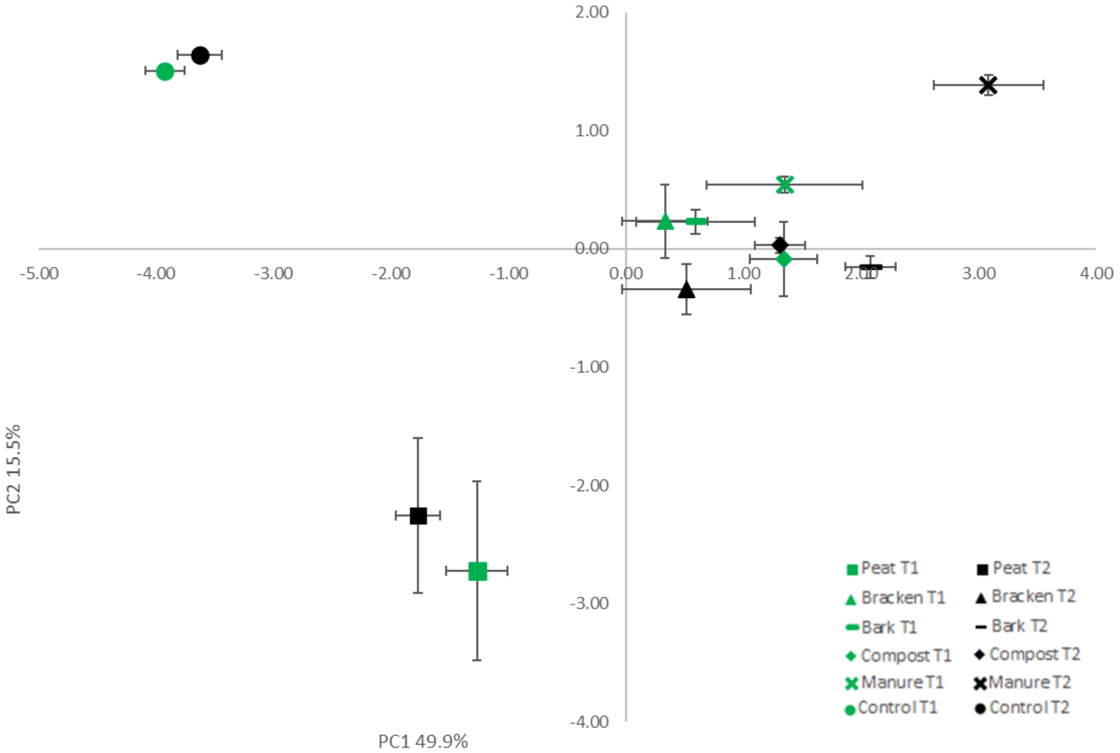


Figure 4-10 PLFA community profiles for T1 (green) & T2 (black) sampling times. Error bars indicate standard error.

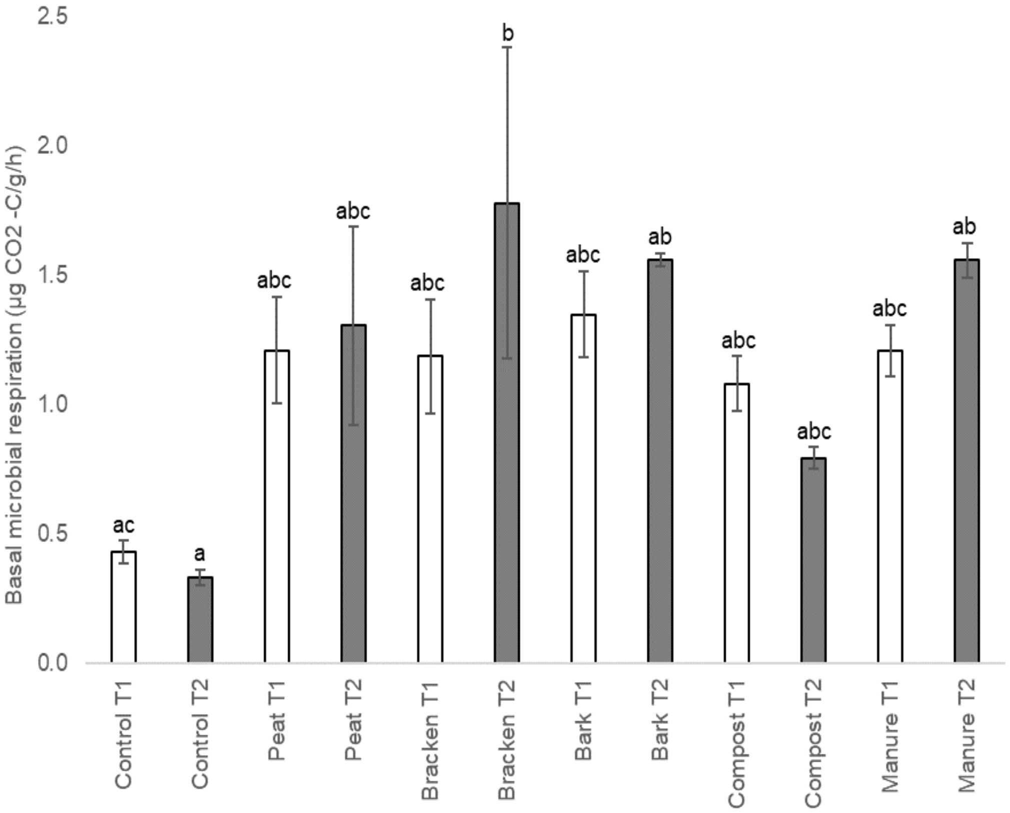


Figure 4-11 Basal microbial respiration in field trial samples. T1 samples (unfilled bars) taken prior to 2019 application of organic matter in early Spring. T2 (filled grey bars) samples were taken late Spring approximately 1month after the 2019 application and incorporation of 2019 organic matter. Error bars denote standard error and letters above bars indicate significant differences (p=0.002) between treatments.

Table 4-13 Summary of one-way ANOVA for basal respiration of T1 & T2 field trial samples

	df	F	P value
Treatment	11	3.508	0.002
Residual	36		

(n=4)

4.5 Discussion

4.5.1 Organic matter application and soil carbon

In the treatments that significantly increased the soil carbon to nitrogen (C/N) ratio a significant increase in fungal to bacterial (F/B) ratios was also demonstrated. The manure and bark treatments demonstrated the largest increases in F/B (Figure 4-7) and the highest increase in PLFA biomarker 18:1w6,9 (Figure 4-5). Bark, manure and compost treatments significantly increased microbial biomass measurements at T2 (Figure 4-3). Compost did not increase PLFA biomarker 18:2w6,9 (and related F/B ratio) which indicates that the increase to microbial biomass was not fungal and could be attributed to compost being a more labile resource (lowest C/N of any input Table 4-2) and more readily available to the bacterial population.

It is not surprising that after 10years of adding organic matter to soil that there was a significant quantifiable increase of soil organic matter measured in all treatments compared to the control group which had no inputs (Figure 4-1). Peat and bark had significantly higher OM% than the other treatments (Figure 4-1). In the bark treatment this may have been due to the high C/N ratio of the material added, and a build-up of C in the plots due to a smaller percentage of the microbial population being able to degrade the material. However, C/N of peat was lower than bark yet saw a similar build-up. This may be due to the decrease in pH of the peat treated plots and suggests N immobilisation in the field trial plots. It is well established that the fungal population and associated functions are tolerant to decreasing soil pH (Rousk et al., 2011; Xia et al., 2020). It is also accepted that substrate type can be a driver of microbial CUE (Carbon use efficiency: mineralisation/ immobilisation ratio). Davey et al. (2018) showed that the variety of carbon compounds available to the soil microbial community, which have the potential to be used in a range of catabolic processes, has significant effects on the community CUE. Not only are the organic matter amendments potentially introducing microbiota to the system but are adding distinct forms of carbon that can be utilised differently by diverse microbial community structures. Fungi have been shown to benefit from and/or by increasing the C/N ratio of soils however this interacts with the addition of more labile C on prokaryotes (De Boer et al., 2005).

Microbial biomass turnover is influenced by sensitivity to temperature changes and reduced availability of utilisable substrates, with these drivers being key controls of microbial biomass (Walker et al., 2018). Consensus on the level of influence of these drivers is lacking and highlights the need for more research which seeks to understand the relationship between substrate quantity, accessibility and microbial biomass (Walker et al., 2018).

It is also worth mentioning that rhizophereric inputs of carbon were not properly accounted for within the trial. Understanding the inputs of the different ornamental plant varieties cultivated each year of the trial would provide enhanced understanding of the interactions of belowground plant inputs (Zhou et al., 2021). The structure of bacterial rhizosphere populations and associated have been shown to be significantly affected by additions of glucose, which can also have subsequent effects on microbial functions involved in priming effects following C additions to soil (Zhou et al., 2021).

4.5.2 Microbial biomass

The paradigm that the stable fraction of soil organic carbon (SOC) is solely plant derived has shifted, with more recent research outlining that soil organic carbon in stable forms is predominantly a result of microbial processes (Crowther *et al.*, 2015). Effectively supporting the fungal population by increasing the C/N ratio of inputs or altering the composition of inputs can favour other soil organisms such as isopods which have been shown to switch consumption of materials such as leaf litter to fungi (Crowther et al., 2018). The grazing of fungi has been shown to alter levels of SOC, or enzymes involved in SOC formation, which would have resulted from fungal biomass decomposition (Crowther et al., 2015).

Microbial biomass analyses were completed for both sampling times (Figure 4-3) and for the organic matter amendments themselves. This enabled a consideration of the biomass of the organic matter material before application and incorporation to the soil in the trial plots. The results for microbial biomass demonstrate a positive impact of organic matter input on the living soil microbial biomass; an opportunity for further study would be to assess the soil microbial necromass. At the second sampling point (T2) compost and manure differed from the control group and from T1 (Figure 4-3). For manure this highlights the positive impact OM addition can have on soil microbial population without sacrificing available nitrogen which was highest in manure treatment material (Table 4-2).

Manure had significantly higher microbial biomass at T2 compared to all other treatments and was significantly different to all treatments and the control in MSIR profiles (). Despite the bark treatment increasing the level of fungal PLFA biomarker 18:2w6,9 and the F/B ratio (similar to the effect seen in the manure treatment) there was no significant increase noted in microbial biomass between T1 and T2 or compared to the other treatments (Figure 4-5, Figure 4-7, Figure 4-3). This

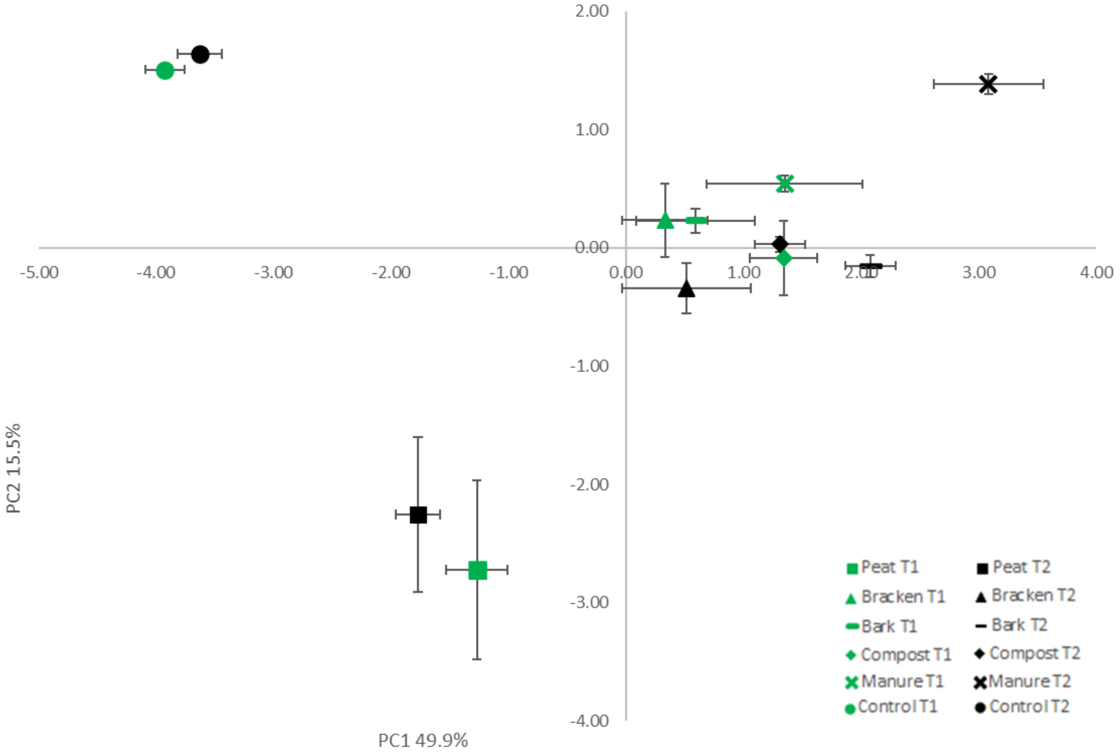


Figure 4-10 PLFA community profiles for T1 (green) & T2 (black) sampling times. Error bars indicate standard error.

may be the a legacy effect of long-term manure additions, resulting in a robust shift in the microbial community in favour of fungi, this effect is quantified in other work which quantifies strong correlations of fungal populations with organic matter (Lin et al., 2019). At T2 the bark treatment increased microbial biomass when compared to the control. García-Gil et al., (2000) highlight that some organic amendments, such as manures, not only add available carbon and nutrients which fuel the indigenous microbial population but also add a microbial population inherent to the substrate. Shanks et al, (2011) quantified high variability in cattle manure samples as a result of changes to feed, this may have had a significant effect on variability in the manure organic matter applied annually over the duration of the trial (2007-2019). However, no differences in F/B ratios were demonstrated in organic matter samples (T0) which would be expected if manure material imported higher fungal biomass than other OM materials. This goes some way in suggesting that changes to the fungal population happened after the OM material was added to soil.

Peat had the lowest microbial biomass of all the treatments (Figure 4-3) and the highest HWEC (Chapter 3 section 3.5.2) and total C (Figure 4-2) but no significant increase to PLFA 18:2w6,9 or to microbial biomass between T1 and T2 (Figure 4-5 & Figure 4-3). Criticisms of the use of PLFA analyses in short-term experimental designs highlight that rapid growth and/or turnover of the microbial population …. Hover the method is still largely considered useful for identifying a wide variety of signature fatty acids from whole soil samples and will likely continue to provide interesting, supporting data to more detailed DNA sequencing methods aiming to understand drivers of microbial community structure dynamics (Joergensen, 2022).

Findings in wider literature on carbon cycling in peatlands assist in explaining this effect. Peatlands are well established as important stocks of carbon. In Li et al, (2021) aerobic conditions we shown to increase microbial respiration and that this was significantly linked to soil temperature. Decomposition was reduced outside of optimum temperatures and O_2 levels, as was microbial respiration and survival (Li et al., 2021). Furthermore depth of soil/ peat, stage of decomposition, carbon content (and quantity of labile/ recalcitrant C) affected O_2 availability (Li et al., 2021). This can in part help to explain the results of this study, high total and labile (HWEC) carbon yet low measures of microbial biomass and significantly different respiration profiles to all other treatments (). The peat material had a significantly lower pH than other inputs which can affect microbial biomass and has been cited as a factor which is highly influential on the structure and function of the soil microbial population (Xia et al., 2020). Malik et al, (2018) have shown that pH changes exert significant control over microbially mediated carbon cycling processes. Microbial growth and decomposition rates were reduced in low pH soils (Malik et al., 2018).

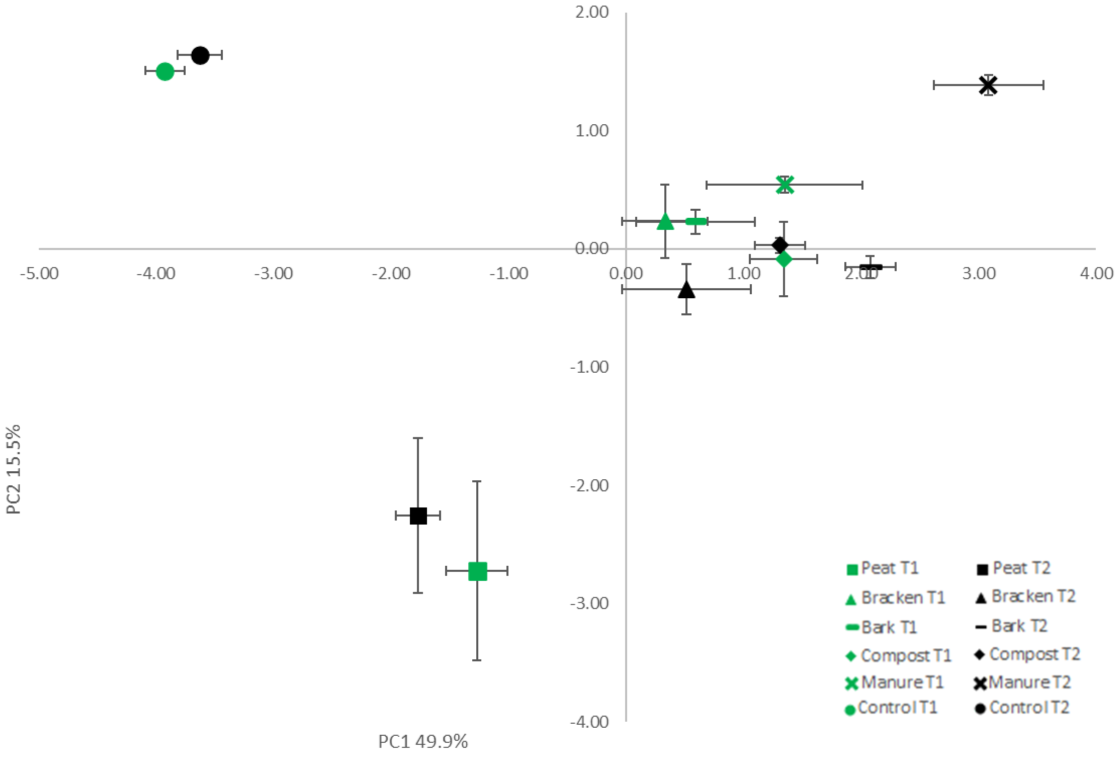


Figure 4-10 PLFA community profiles for T1 (green) & T2 (black) sampling times. Error bars indicate standard error.

4.5.3 Microbial respiration (MSIR)

Basal respiration significantly increased in all treatments compared to the control in both T1 and T2 (Figure 4-11), which aligns with work which has shown organic matter input as a driver of increased microbial respiration (Cleveland et al., 2007).

Malic acid, glucose and α-ketoglutaric acid had a significant positive weighting on PC1 which separated manure (T2) from all other treatments and all treatments from the control and peat (T1&T2). This may suggest that certain OM inputs promoted a stress response from the microbial community as increased utilisation of α-ketoglutaric acid and other carboxylic acids has been shown to be indicative of stress conditions (Bongiorno et al., 2020).

Between T1 and T2 only the manure treatment differed significantly (). The F/B ratio of the manure treatment differed significantly between T1 and T2 (Figure 4-7) and the result seen in the MSIR profile could have been due to a significant shift in the composition of the microbial community however we would expect this effect to have been noted in the bark treatment as well if this was the case as the F/B ratio of the bark treatment also significantly increased but no similar effect was seen in MSIR. The manure may have had more accessible dissolved organic matter than the bark, thus resulting in higher respiration than

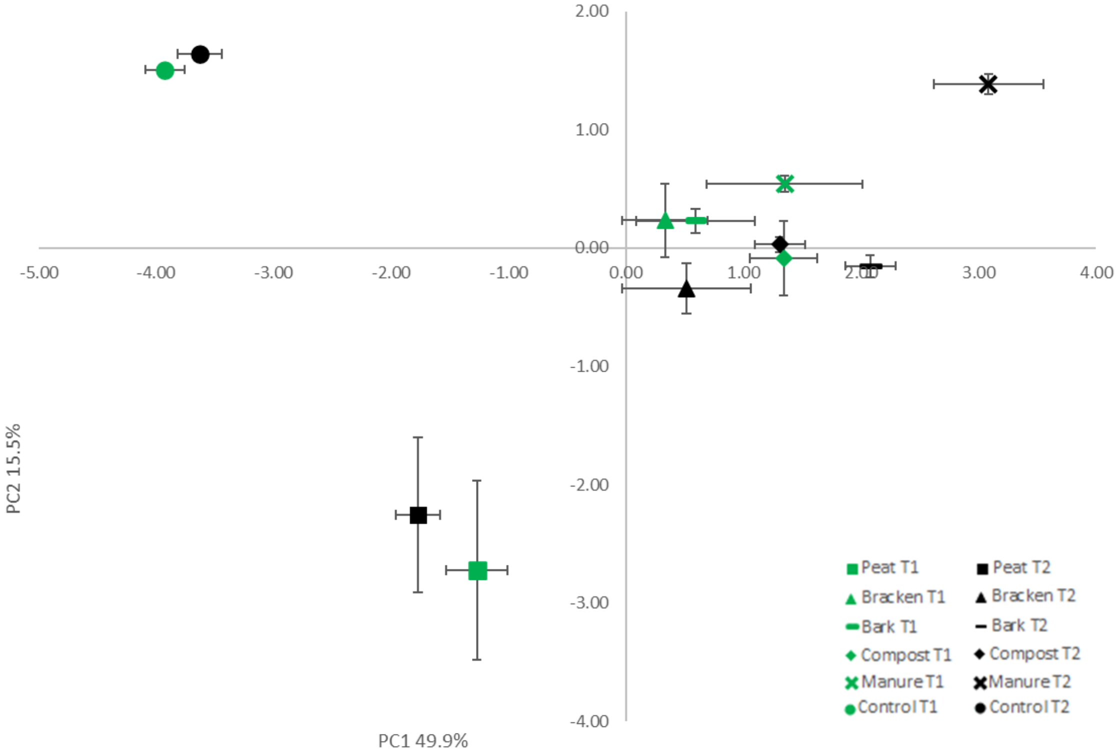
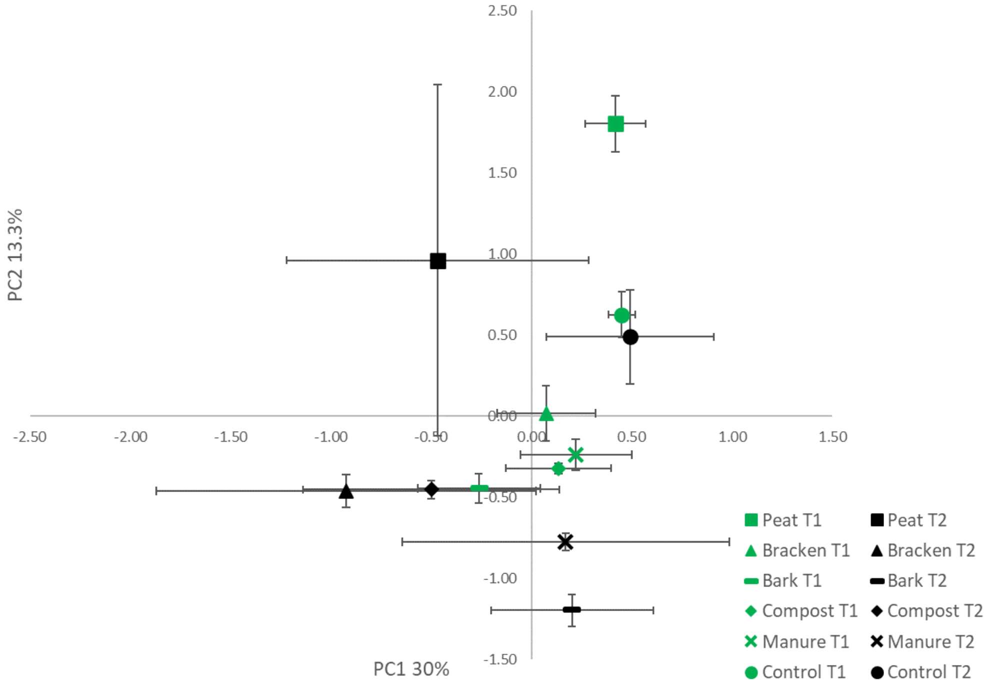


Figure 4-10 PLFA community profiles for T1 (green) & T2 (black) sampling times. Error bars indicate standard error.

the bark treatment. The bark treatment has a higher C/N ratio compared to manure and manure had significantly higher total available N and K (Table 4-2) than bark and all other organic matter treatment material. The availability of these nutrients could influence microbial respiration by "priming" the microbial population with an initial flush of available N as well as the additional substrates (oxalic acid/ citric acid/ alanine and water).

4.5.4 Microbial Community Composition

PLFA analyses demonstrated a highly significant (p= <0.001) effect of peat application on the microbial community (Figure 4-8). This may, in part, be due to the low pH of the peat OM material (Table 4-2) and acidification of the peat trial plots over the ten years of OM applications (Alexander and Bragg, 2014). No other treatment displayed such a decrease in pH. In general it is considered that a large and functionally diverse soil microbial population will facilitate mineralisation and increase plant available nutrients (Martínez-García et al., 2018a). Fungi are generally thought of as more capable of decomposition of plant derived C such as lignin and cellulose (Kaiser et al., 2014). The role of functional groups, and what drives competition between them, is integral to conceptual understanding of decomposition. C/N ratio of substrate



is highly cited as a driver of changes to functional community dynamics (Kaiser et al., 2014). This effect is increasingly seen as integral to soil organic matter dynamics and the availability of key nutrients for plant uptake (Hestrin et al., 2019; Martínez-García et al., 2018b). The findings of this study align with those of Kaiser et al, (2014) and others (Duddigan et al., 2019; Hoffland et al., 2020; Zhang et al., 2019) in that organic matter addition had significant feedbacks with microbial community composition. Further work to quantify microbial CUE, and interactions of CUE with different and distinct types of organic matter input, would increase understanding of the results presented here. Assessments of CUE from whole soils using a variety of methods, such as catabolic profiling e.g. Microresp, for the creation of Community Level Physiological Profiles (CLPPs) have increased our understanding of how community level response to different substrates and compounds changes soil conditions related metabolic products (Davey et al., 2018).

Bark and manure were the only treatments to differ significantly from the control and all other treatments in measures of PLFA 18:2w6,9. Both bark and manure differed significantly within season (Figure 4-5). These treatments had the highest measured total carbon content and the highest F/B ratios. Despite a significant increase in PLFA 18:2w6,9 in the bark treatment, there was not a significant increase in microbial biomass, which could indicate a shift in composition. An increase in the percentage of fungal biomass and reduction in bacterial biomass. Manure had the highest available P of all treatments at 128.3mg/l compared to the control at 50.9mg/l and significantly increased the available nitrogen in the field trial plots providing 64.7kg/ha compared to the control treatments 11.3kg/ha. Bark showed a much lower level of available nitrogen however it was still more than double that of the control treatment. The fungal component in provisioning of nutrients through decomposition of organic matter and mineralisation processes cannot be discounted when considering the role of the soil microbiome in supporting plant health and regulating carbon cycling processes (Crowther et al., 2014).

Comparatively there were no significant differences in the manure treatment when analysing the abundance of 16:1w5 NLFA, used as a biomarker for arbuscular mycorrhizal fungi (AMF) populations (Figure 4-6). The bark treatment did influence NLFA 16:1w5 and was the only treatment to demonstrate an increase in this biomarker (Figure 4-6). All other treatments saw no significant increases in NLFA 16:1w5 (Figure 4-6). Senescence of AMF hyphae contributes to the soil necromass and in order to consider their role in the extended network of soil fungi more in depth study of the soil necromass would improve the understanding of the findings of this study (Martínez-García *et al.*, 2018).

Though important contributors of carbon deposits to soils through senescence of hyphae and glomalin production and glomalin related soil proteins (GRSPs), AMF are not key decomposers (Holatko et al., 2021; Martínez-García et al., 2018b; Wright et al., 2006). Furthermore, AMF colonisation of plant roots is depressed in soils which are phosphorus rich due to decreased plant investment (Nouri et al., 2014). High P levels were noted in organic matter treated soils, though if P was the limiting factor for AMF abundance it would be expected to see higher levels of AMF biomarker 16:1w5 in the control soil. Variance in FAME (NLFA) biomarker 16:1w5 was high, as such no conclusively significant differences were noted. However, there is the potential that rotavation and the use of annual plant species contributed to low levels of AMF in all trial plots. Tillage regime has been shown to be impact AMF hyphal development and be a major influence on AMF community structure (Wilson et al., 2009; Wright et al., 2007).

F/B ratios were wide ranging between the treatments showing that OM input had a significant effect on soil microbial community composition (Figure 4-7). The highest F/B was seen in bark treated soil. This could be due to the high C/N of the bark amendment and high levels of carbohydrates (lignin and cellulose) material resulting in a reduction in bacterial species in the early stages of decomposition. A reduction in availability of easily digestible carbon forms may have impacted bacteria, being unable to thrive on resources which fungal populations can utilise thus allowing fungi to dominate. To confirm this further analysis of the composition of the organic matter amendment materials would be necessary.

Gram- populations have been shown to be more sensitive to stress, particularly water/ drought stress than Gram+ bacteria. This has been attributed to Gram+ bacteria having a thicker cell wall and slower growth rate. Species within the Gram+ phylum have been shown to be able to utilise more recalcitrant forms of carbon and inorganic N while Gram- are more able to utilise more labile sources of C and only organic N (Fanin et al., 2019). The increased utilisation of carboxylic acid in MSIR analysis and changes in PLFA profiles in this study suggests that effects of limitations on microbial function under stress conditions caused by organic matter inputs be evidence of the interactions such as those of Fanin et al. (2019). Hacquard *et al.*, (2018) suggest that drought environments are more oligotrophic, and that metabolic ability and efficiency may also be the reasoning behind the differences between Gram- and Gram- populations.

Using PCA analysis it is possible to highlight changes to microbial community composition. Despite the different PLFA profiles of organic matter material (T0) no significant differences were found between F/B ratios in treatment material (T0). Once applied to the soil significant changes occurred to the F/B of soil samples. This suggests that the microbial community present in the soil is adapting to the amendments once applied and incorporated. All additions to soil significantly altered the soil microbial community structure. Over time the diverse belowground community altered and continued delivering decomposition functions tailored to the OM applied.

4.5.5 Legacy effects in field trial plots

Although this study focuses on the 11th and 12th year of the field trial, the effects cannot be considered as isolated from the legacy effects of more than ten years of annual organic matter input and crop growth, including rhizosphere interactions and C deposits. A build-up of soil carbon and the quantity of available nutrients accrued over the ten years of the trial are interesting to consider in relation to within season effects (T1 Vs. T2). Short-term beneficial effects such as increases in plant-available nutrients can result in cumulative detrimental impacts on soil biology, plant health, and the wider environment.

Martínez-García et al*,* (2018) highlighted legacy effects of “soil health treatments” (e.g., compost, green manures) on F/B ratios that lasted 6years after the final application. They defined legacy affects as the “still measurable effects of an application after a certain timespan (Martínez-García et al., 2018)” and highlight the long-lasting effect of soil management on soil properties and soil-derived ecosystem services. In wider literature it is well understood that changes to abiotic conditions, often influenced by land management, such as the addition of organic matter to soils, can have a lasting effect on soil microbial community structure and function (Li et al., 2019; Preece et al., 2019). Being able to characterise and reliably predict what effects long-term management has on the soil microbiome and why is a subject of continuing scientific study. The findings of this study demonstrate significant feedbacks between organic matter management (type of organic matter treatment compared to control soil) and microbial community structure (significant differences between PLFA community profiles) and function (MSIR community profiles of OM treatment compared to control). With effects differing between short-term and long-term.

Often named the “master variable”, pH was significantly changed over the ten years, with the peat treatment plots reaching approximately pH4 and being significantly different to other treatments and the control. This is widely acknowledged to affect nutrient availability. Soil organic matter (%) is increasingly considered to be as much of a “master variable” as pH when trying to understand the microbial role in carbon cycling (Neina, 2019). Increasing SOM or preserving existing SOM is essential for land managers aiming to sustainably manage productive ecosystems (Feller et al., 2012). Gardeners, through habit, commonly add organic matter to soil. The effects of OM commonly added to soils on soil carbon dynamics which are mediated by the soil microbial community have been explored in this study and areas for future work identified as inclusion of quantification of microbial necromass for comparison to microbial biomass C and measurement of microbial CUE. Further analysis of the composition of organic matter inputs, particularly C lability and fractionation, and quantification of lignin and cellulose would improve understanding of the findings presented here. Conclusions are of value to UK gardeners looking to adapt practice, such as the move away from peat-based inputs. The findings of this study are also of value in terms of understanding the relationships between repeated land management and the legacy that is left behind in our soils.

4.6 Conclusion

When discussing the results in this study it is important to consider that the trial plots have had yearly applications of each of the organic matter amendments studied for a total of 10 years. All effects are not isolated annual effects but cumulative effects from a decade of applications. Separating out the legacy effects of ten years of organic matter addition to the trial plots is a challenge and determining the difference between long-term effects and within season effects is possible only by comparison with the control group.

The original aim of this chapter was to examine the feedbacks between C input to soil in the form of common garden organic matter additions on soil community structure and function. An assessment was made of microbial respiration and community structure, changes to which were distinct to different organic matter treatments confirming the original hypothesis. The secondary hypothesis was only partly confirmed, higher fungal biomass was measured in the bark treated plots, and respiration was higher than the control. Without more detailed analysis of the bark material, it is not possible to conclude that “recalcitrance” of the bark material was the driver for these changes. In addition, further analysis of fungal diversity, using methods which provide finer detail on microbial community diversity such as high throughput sequences (Nilsson et al., 2019), would aid in understanding the effects of high C/N ratio treatments on the fungal domain and related functions.

The composition of these amendments and the transformation of labile, stable, and inert C fractions is managed by an adaptive microbial community able to maintain function through substitution. Understanding this functionality and how soil microbial life reacts to anthropogenic management of soils will increase our understanding of the terrestrial carbon cycle. The intricate structure of belowground communities and the effects of land management decisions on their functioning is a growing field with more studies citing the importance of the soil microbiome in carbon cycling and thus climate change mitigation and adaptation (Bradford et al., 2016; Cavicchioli et al., 2019; Crowther et al., 2015b).

The microbial populations' ability to release or sequester carbon is sensitive to land management changes such as organic matter applications seen in the results of the RHS field trial analysed in this study. Although additions were added on a volume per plot basis it would be advantageous to make the additions according to the carbon content of each material. However, we can consider that the manner of adding them by volume is representative of gardeners' practise and as such it is still valuable to assess the effect of the additions as they were added in the trial.

It is possible to conclude from this experiment that ten years of organic matter application had a statistically significant effect on soil carbon and losses of carbon (CO_2) from soils treated with different types of organic matter (OM). Significant treatment effects on the structure of the microbial community were quantified confirming the original hypothesis. The changes to microbial function and community composition following OM addition bolster the view that the soil microbiome mediates carbon cycling and adaptively reacts to inputs. To robustly confirm the hypothesis that decomposition of high C/N or recalcitrant soil additions is dependent of fungal decomposers would require further analyses of the fate of C added to soils through C labelling, further measures of PLFA or DNA/ RNA sequencing and estimation of fungal contribution to microbial necromass C. However, the increase in fungal biomass (PLFA 18:2w6,9) coupled with high basal respiration confirms maintenance of function of fungal decomposers in the bark treatment. High variance in NLFA analysis prevented robust conclusion of treatment effects on relative abundance of AMF species in different treatments. Increasing replication and frequency of sampling times could help to provide more detail on the significance of AMF within the microbial community structure and the influence of OM types.

Understanding the carbon cycle means we must understand the complex nature of the worlds' smallest and arguably most important organisms. Tailoring improvements to land management practise with the soil microbial community in mind can provide advancements in our ability to adapt to anthropogenic climate change effects. This experiment has evaluated the cumulative effects of long-term additions of distinct types of organic matter on soil biology and highlights the need for repeatable experiments which focus on microbial function.

5 Allocation of soil respiration to soil fungi and bacteria using selective inhibition

Chapter 5 presents a novel experimental design for the assessment of microbial respiration following organic matter addition to soil and maintenance of function under inhibitory effects. The aim was to quantify the role of bacteria and fungi domains in CO_2 output from soils after organic matter application. Two organic matter types were selected from the analyses presented in previous chapters and soil type was a clay-loam. Inhibition pressure was applied using a bacteria or fungi – specific biocide.

5.1 Abstract

Soil organic matter contains a vast proportion of global carbon stocks. Improving soil organic matter is a key goal for land managers and is recommended as a beneficial action for mitigating climate change. Traditional understanding of the implications of adding organic matter to soil have focused on the chemical and physical structure of the input. This study focuses on the role of biological community composition and influences of input on its structure and function (respiration). A selective inhibition microcosm experiment was conducted to examine management specific to gardens. Distinct differences were identified in the influence of organic matter input (manure/ compost) on microbial respiration and microbial respiration was significantly higher under inhibitory effects of applied biocide in the manure treated soils. Differences in basal respiration and changes to respiration in response to the availability of resources (glucose and cyclodextrin) were also higher in manure treated soils. A bactericide (bronopol) and a fungicide (cycloheximide) were applied to explore specific roles of fungi and bacteria and how their inhibition would influence the delivery of function.

PLFA community profiles for all treatments (+/- organic matter and +/- biocide) significantly differed to the control soil. Addition of biocide to both manure and compost treatments increased the ratio of Gram-positive/ Gram-negative PLFA biomarkers. Organic matter additions resulted in retained function even under inhibition effects. Organic matter additions are recommended as beneficial to soil biodiversity and the functions this diversity delivers. Contribution of specific domains to these functions is a subject of continuing research. Robust understanding of the effects of organic matter inputs on the microbial population improves best-practise recommendations for gardens, resulting in wider environmental and ecological benefit.

Keywords: Selective inhibition; PLFA; microbial respiration; organic matter; manure; compost

5.2 Introduction

Common practise in gardens, such as the addition of composts, provides a valuable area of study to increase understanding of how soil food-webs respond to inputs of organic matter, which although can be nutrient dense are aimed at improving soil quality parameters such as increasing soil organic matter (SOM), improving tilth and texture, and supporting primary production (Duong et al., 2012; Houot et al., 2007). A functionally and biologically diverse ecosystem is essential for maintaining key functions and processes (Barrios, 2007; Adhikari and Hartemink, 2016; Schloter et al., 2018).

Despite the context specific practise and decision-making found in garden settings, research aimed specifically at gardeners is less abundant than that focused on agricultural systems. Gardeners have a different set of aims and objectives when considering management options. Gardens are often comprised of areas with diverse end goals, for example raised beds, recreational areas or patios, lawns, shrub and perennial boarders (Tresch et al., 2019). This results in heterogenous spaces with high above- and belowground diversity, typically smaller in scale than agricultural systems (Tresch et al., 2019). Gardens can increase ecosystem connectivity in urban areas and collectively are a substantial component of regional land-use (Dewaelheyns et al., 2013). Exploring and quantifying the effects of gardening decisions is an area which would benefit gardeners and non-agricultural land managers across the UK.

Land management, such as the addition of organic matter, changes the chemical and physical parameters of the soil system. This can increase or decrease resource availability to microbes as well as limiting or providing habitat. Re-focusing attention on the accessibility of the input to microbial life enables a more holistic view of abiotic parameters which influence biological functions (Dungait et al., 2012). Investigating the mechanisms with which the microbial community adapts to input and how resilient it is to the changes, or the scale of the changes, is where more research into context specific changes resulting from land management decisions is required. These land management decisions affect how we preserve the natural resources that we rely on for primary productivity, cultural heritage, and the future provisioning of all ecosystem services (Louca et al., 2018).

The soil microbiome delivers a range of services all as essential as the provision of nutrients for plants, particularly in systems such as gardens. Gardens provide an interesting context as they comprise a large area of green land, particularly in urban areas (Loram et al., 2007). Land used as gardens, parks and allotments in UK is 7.45% of total land areas (Defra 2016). Gardeners choose management strategies with more than plant productivity as an end-goal (Loram et al., 2007; Barthel, Folke and Colding, 2010; Power, 2010).

The role of the soil microbial population in the carbon cycle is well researched with multiple studies highlighting the importance of a broad range of belowground microbial life (Maron et al., 2018; Moscatelli et al., 2018). Soil microorganisms are known to have a key role in soil organic matter (SOM) turnover (Schmidt et al., 2011). However, the chemical, biological and physical structure of SOM is complex. Many studies have focused on the effect of nitrogen fertiliser on the microbial decomposer population (Brussaard, Clarholm and Andre, 1999; Barrios, 2007; Hestrin et al., 2019). Garden specific studies which focus on the use of different organic matter amendments and effects of these additions on microbial functions (such as respiration) are scarcer. There is a gap in existing research for studies which highlight specific contexts, such as UK gardens. By doing this the accuracy of knowledge on how microbes respond to specific management will be increased and made transferable to other systems such as urban green spaces, recreational areas and other non-intensively managed landscapes.

Soil organic matter contains a vast amount of global carbon, estimated at 3500-4800 Pg C (Lehmann and Kleber, 2015). Soil organic matter and soil carbon cycling are vital parameters of soil quality assessment (Bünemann et al., 2018). Additions of compost change soil chemical and physical parameters which creates feedbacks with the microbial population, such as changes to soil pH which can influence fungal/bacterial (F/B) ratios (Pastorelli et al., 2018). Adding organic matter to soil changes the percentage of soil organic matter measurable in soil over time if applied annually (Duong et al., 2012). These additions affect other soil chemical, physical and biological parameters (such as microbial biomass, respiration, and fungal/bacterial ratios) and result in feedbacks with microbial function such as decomposition and respiration (Huang et al., 2021; Liang & Zhu, 2021; Louca et al., 2018).

The ecosystem may only be as great as the sum of all parts. Decomposition may be reliant on a functionally and biologically diverse and balanced soil community (Wagg et al., 2014, 2019). Studies which assess the impact of inhibitory effects on fungi and bacteria and subsequent effects on remaining microbiota, in relation to functions such as respiration, furthers our understanding of how to manage soils in a changing climate. Selective inhibition experiments can help us examine how resilient the microbial community is to changes, such as limitation of resources or a disturbance event which effects or limits subsections of the community.

It is well established that bacteria and fungi are crucial components of soil carbon cycling (Kallenbach et al., 2019; Li et al., 2019; Tully and McAskill, 2019). Measuring microbial respiration using methods such as multiple substrate induced respiration (MSIR) provides information on the functional role of the soil microbial community in carbon cycling processes (Creamer et al., 2016; Pawlett et al., 2013). Selective inhibition studies aim to quantify the role and function of particular domains (eukaryote and prokaryote) to these processes (Bailey et al., 2003; Sassi et al., 2012). Combining the use of antibiotics within Microresp© MSIR profiles is a novel method to quantify changes in microbial respiration when provided with different resources (glucose and cyclodextrin).

To investigate respiration (and by proxy, decomposition) functionality of soil treated with organic matter a laboratory-based experiment was conducted which applied inhibition pressure to soil. The aim was to quantify the effects of changes in microbial, bacterial, and fungal biomass to CO_2 output of soils treated with organic matter. To analyse whether there are benefits of OM application to maintaining function when abiotic conditions reduce diversity, inhibition effects were applied using biocides (bronopol/ cycloheximide). It was hypothesised that:

Compared to soil without OM addition, OM input would result in maintained microbial respiration following addition of a complex carbon source (cyclodextrin), even under inhibition pressure.

Changes to the structure of the microbial community as a result of biocide application will be significantly reduced in soils treated with OM compared to a control soil.

It is generally assumed that bacterial groups are less able to capitalise on resources with a higher molecular weight than fungal groups which produce extracellular enzymes for plant material (lignin and cellulose) decomposition (Kaiser et al., 2014; Siuda and Chróst, 2002). If fungal groups are inhibited it is possible that decomposition functions will be impaired, or that bacterial groups will capitalise on reduced competition and provide a substitute for the fungal decomposition niche. Microbial function could be maintained when a high molecular weight substrate is available if the fungal decomposer population is uninhibited. The two OM inputs in this study had different nutrient characteristics and C/N ratios (Table 5-1) prior to addition to the soil. Effects of each OM input were expected to be distinct and unique.

5.3 Materials and methods

A 3x2 factorial design comprised of 3 soil types: control soil, soil+manure, soil+compost and 2 biocides (inhibitors) bronopol, cycloheximide and 3x replicated of each treatment was used to test the hypotheses. Garden compost (compost) was made at RHS Wisley from plant matter collected from Wisley gardens with the composting processes continuing for approximately 1year prior to use. Compost was screened for weed seed and other material such as stone before use. The Wisley gardens composting facility is monitored by in house staff with regular temperature checks completed by RHS Wisley technicians. Material from the gardens is divided up, stacked, covered and turned regularly. Manure was an organic, peat-free stable manure purchased from Country Natural. Soil was a standard Kettering loam which was screened for weed seed and contaminants and sieved to 5mm (purchased from Boughton Loam UK) with approximately 40%sand, 40%silt, 20%clay, further characteristics can be seen in appendix Figure B-1.

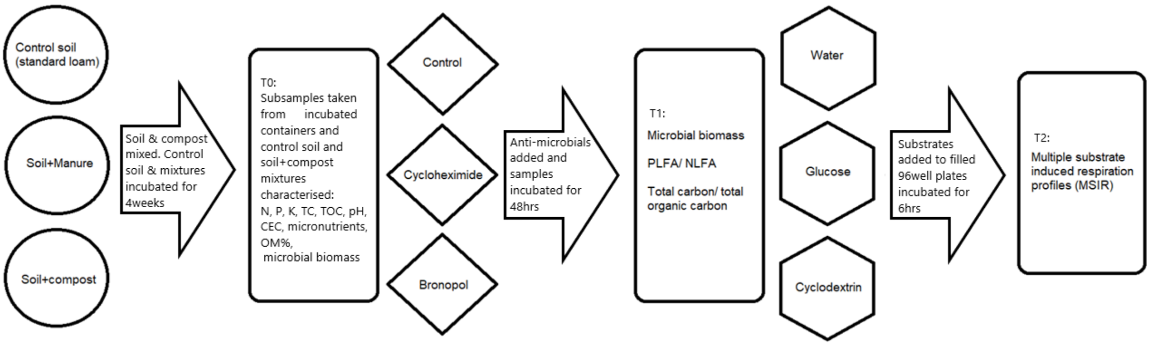


Figure 5-1 Schematic diagram of experimental design and running order. Soil and soil compost mixtures were characterised before the addition of biocide. Key analyses are listed in rectangles.

Table 5-1 Characteristics of organic matter material prior to addition to soil. Numbers in brackets indicate standard error.

	Composted manure		Garden compost	
pH	8	(0.12)	8.13	(0.03)
Total N (% of W/W)	1.67	(0.03)	1.23	(0.06)
Total C (% of W/W)	32.5	(3.72)	21.7	(0.76)
Carbon:Nitrogen	19.5	(2.48)	17.8	(0.19)
Total soluble (mg/l)				
N	232.2	(19.75)	101.87	(5.01)
P	16.9	(1.5)	30.3	(2.26)
K	2346.6	(56.17)	1057.1	(39.36)
Mg	101.9	(5.75)	9.8	(1.13)
Fe	1.7	(0.29)	8.89	(4.6)
Dry matter (%)	35.1	(0.47)	52.1	(0.76)
Bulk density (kg/m3)	373.7	(10.58)	639.3	(13.67)
(n=3)				

The control used for the experimental design was the standard Kettering loam soil minus the addition of any organic matter. Manure and garden compost were added to soil in separate containers and mixed thoroughly. 4parts soil and 1 part compost were mixed resulting in soil/ compost mixture of 20% compost and 80% soil. This rate is widely recommended in gardening grey literature and by the Royal Horticultural Society for incorporation of soil conditioners (Royal Horticultural Society, 2022).

Soil and organic matter+soil mixes (OM+S) were stored in 50L containers with loose lids in a polytunnel at Cranfield University and incubated for 4weeks at 16°C-20°C. After the incubation period control soil and OM+S were characterised, and the data presented Table 5-2. Methods used for characterisation were based on British Standards; total N and total C were determined using the dry combustion method (elementary analysis) on oven-dried soil. Total organic C a 4mol/l hydrochloric acid was added gradually to removed organic C before completing the oxidisation process and measurement (BS 7755-3.8:1995), macro and micro-nutrients status were quantified using (ISO, 1995, 1993). Elements soluble in aqua regia used a method based on US EPA Method 3051 (BS 7755: 3.13:1998 ISO, 1998) involving extraction with HCl and HNO_3. The extract is then clarified and DI water added before determining trace metals atomic absorption/ emission abd O contect with spectrometry of the solution. Mg and K soluble in ammonium nitrate solution as per (BS 3882:1994/ ISO, 2002) using air dried soil and Mg determined using atomic absorption and K in the extract determined by atomic emission. The evaluation of pH was completed as per BS ISO 10390:2005, using a ratio of 1:5 dried soil to water in a solution of 1mol/l KCl using a glass electrode. Organic matter % (loss on ignition) was determined using methods developed from McDonald (1984). The mass of oven dried samples was recorded before heating to 450°C and the difference in weight following combustion used as the measure of organic matter % of the original sample (BS EN 13040:2000 and ISO, 1995).

Table 5-2 Characteristics of control soil (-organic matter) and soil+manure treatment and soil+compost treatment. Numbers in brackets indicate standard error.

	Control		Soil+ manure		Soil+ garden compost	
Total N (%)	0.113	(0.02)	0.291	(0.05)	0.325	(0.007)
Available phosphorous (mg/l)	19.8	(0.71)	46.4	(0.92)	54.8	(0.49)
Available potassium (mg/l)	152	(4.91)	1424	(5.78)	791	(10.93)
Total Carbon (%)	1.243	(0.03)	3.7	(0.04)	4.5	(0.15)
Total organic carbon (%)	1.002	(0.05)	3.108	(0.22)	3.917	(0.08)
Carbon:Nitrogen	10.99	(0.14)	12.71	(0.25)	13.88	(0.34)
pH	7.6	(0.06)	7.8	(0.06)	7.8	(0.02)
CEC	13.8	(0.03)	19.4	(1.12)	15.8	(0.6)
Organic matter % (LOI)	3.1	(0.12)	5.9	(0.03)	9.8	(0.03)
(n=3)						

Following the 4week incubation period subsamples were taken from the soil and compost mixtures, which were sieved to 10mm, rather than the standard 2mm, to avoid separating out the loam soil and composts. Following the initial 4week incubation period after the soil and OM+S were mixed, a bacterial inhibitor: bronopol, applied at 10mg per 1g soil or a fungal inhibitor: cycloheximide 10mg per 1g soil were applied to subsamples taken from the main sample containers. Concentrations of the antimicrobials were based on work by Pan et al, (2019) who explored the efficacy of both bronopol and cycloheximide at two different concentrations and over a sequence of time points following application of both biocides to whole soil samples. Concentrations of the solution added to each microcosm were adjusted according to the moisture content the control soil and the OM+S treatments to ensure all microcosms had comparable moisture content (Pan et al., 2019). The treated sample microcosms and the control microcosms were randomised and left to incubate for a further 48hours at 18-20ºC.

5.3.1 Microbial biomass

Microbial biomass was determined using the fumigation-extraction method as detailed in Jenkinson (1976) and BS 7755: 4.4.5:1997 / ISO 14240-2:1997, with KEC of 0.45 (Joergensen, 1996). 12g of soil or soil+organic matter treatment was used for the analysis. Resultant carbon was analysed using a SFA-2000 segmented flow analyser and adjusted with the proportionality coefficient kEC = 0.45.

5.3.2 Phospholipid fatty acid (PLFA) analysis

Extraction of lipids and analysis of fatty acid methyl esters (FAMEs) was completed according to (Frostegard et al., 1993). 6g of fresh soil was used to extract lipids using Bligh and Dyer (1959). In order to keep the time-frame between application of anti-microbial and completion of PLFA analysis soils were used fresh and not freeze dried, which has been shown to be effective in existing studies (Pawlett et al., 2009; Wu et al., 2009). This enabled the extraction of PLFAs at the same time as respiration profiles were analysed and ensured the highest concentrations of individual PLFAs were quantified (Wu et al., 2009). Sub-samples were taken from the same containers as the ones from which Microresp™ plates were filled to ensure community profiles represented were identical to those which were analysed in MSIR analysis.

The quantity of water used to create the citrate buffer was adjusted according to the moisture content of each soil to ensure the correct concentration of the buffer (Wu et al., 2009). Lipids were then subjected to solid phase extraction and derivatised using gas chromatography (Agilent 6890 FID-FPD). The temperature program was set to start at 50ºC and raise to 160ºC at 25ºC per minute to 210ºC. Injector temperature was 310ºC and flame ionisation detector set at 320ºC. FAME results were calculated as relative abundance (mol%) and identified by comparison of retention times to standards provided by Supelco.

PLFA biomarkers selected to represent bacteria and fungi were selected following a review of the literature by; Ehlers et al., 2008; Frostegard et al., 1993; Lindahl et al., 1997; Pawlett et al., 2013; Tunlid and White, 1992; Zelles, 1999. Gram-positive biomarkers are represented as mol% of i15:0, ai15:0, i16:0, i17:0, ai17:0 and Gram-negative biomarkers 16:1w7c, cyc17:0, 18:1w7, cyc19:0 with the addition of 15:0 used for total bacterial expression in the samples analysed.

Due to levels of fungal PLFA biomarker 18:2w6,9 being above zero fungal PLFA biomarker 18:1w9c was used in addition to 18:1w6,9 after a positive correlation between the two biomarkers was found to be significant (p= 0.015) as described in Frostegård et al, (2011). The combination of 18:2w6,9 and 18:1w9c has been reliably used to estimate non-mycorrhizal fungal populations in non-agricultural soils (Frostegård et al., 2011; Joergensen, 2021; Myers et al., 2001; Zhao et al., 2017).

5.3.3 Microbial respiration (Microresp)

From the treated and control microcosms subsamples were taken and used to fill the Microresp™ plates in a randomised design. Microresp™ was performed in accordance with Chapman et al 2007. Substrates (cyclodextrin and glucose) were added to each of the plates as the equivalent of 30mg of substrate per gram of water in each of the soils, this was added to each of the wells in 25ul aliquots. 25ul of deionised water was added to each control group well. Plates were left at 20ºC for a 6hour incubation period. Methods were adapted from (Chapman et al., 2007). One plate was prepared with control soil, GC and HM and dI H2O applied for quantification of basal respiration. Soil filled deep-well plates were sealed with corresponding plates filled with cresol red indicator dye and agar and sealed for 6hours. Analysis of the plates was completed immediately after unsealing each plate using a SoftmaxPro 384 spectrophotometer plate reader.

Glucose was selected to represent a labile and cyclodextrin for its recalcitrance. Glucose is readily available to microbes, with 6 carbon atoms, produced by plants during photosynthesis and used in the production of cellulose. Naturally available in soils both in the rhizosphere through root exudates and in leaf litter it was considered to be a good representation of a readily available resource during the process of carbon decomposition in soil systems (Zhou et al., 2021). Cyclodextrin was used as a representation of a resource less available to microbial decomposition. Due to its more complex, cyclical structure it is less freely decomposed. Cyclodextrin is a stable sugar polymer which is water soluble and as such was able to be used within the Microresp method and represent resources that are less easily decomposed by sections of the microbial population, such as cellulose and lignin (Poulson et al., 2022).

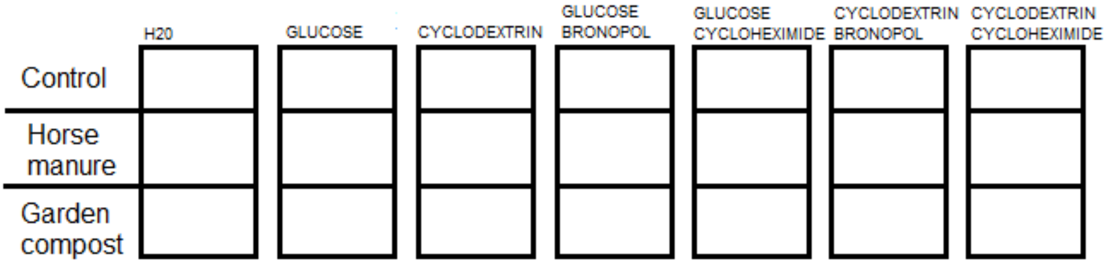
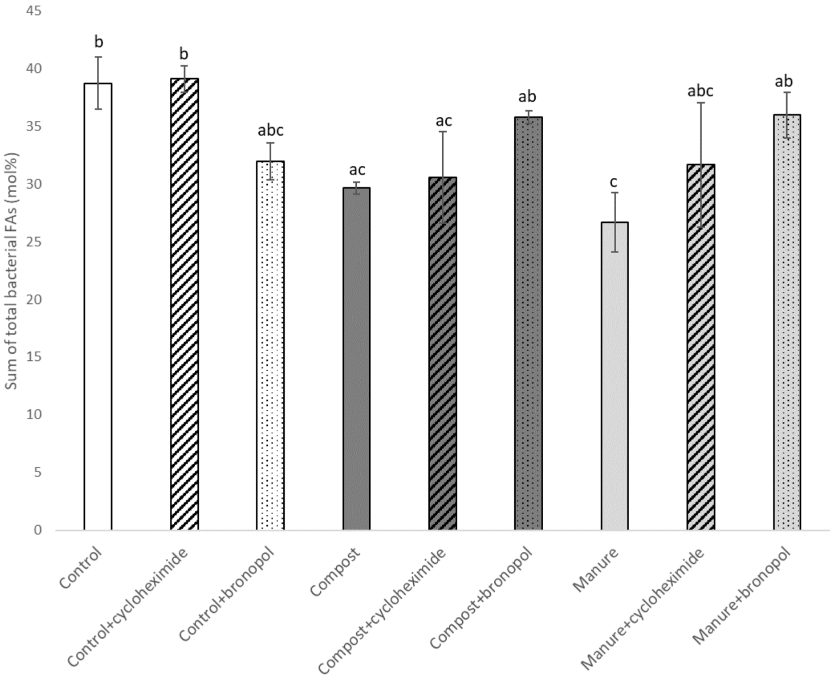


Figure 5-2 Schematic diagram of 96well plate treatment set-up for the analysis of microbial respiration using the Microresp method. Rectangles indicate 96 deep-well plates, labelled with different organic matter/ soil/ substrate and biocide additions.

5.3.4 Statistical analysis

All analysis was completed using Statistica software version 13 (TIBCO™) and R software (version 2022.07,2+576). Univariate data (pH, C and N contents, CEC, N, P, K) and data resulting from analysis of microbial respiration and community structure were subject to a QQ-plot test of normality. All data was found to be normally distributed, with the exception of microbial respiration data for (manure treatment group). As such results from this data was analysed using the non-parametric Friedman test and Bonferroni post-hoc testing. All other data were subject to parametric testing. Regression analysis for PLFA biomarkers (18:2w6,9 and 18:1w9c) was completed using R linear modelling and packages GGplot, GGally. Treatment effects of the compost and the anti-microbial treatments on composition of PLFAs were assessed by principal component analysis (PCA) and factor scores were assessed using ANOVA and Tukey's honest significant difference (HSD) post-hoc test with a significance of threshold of 5%.

Total bacteria PLFA biomarkers and total Gram+ FAs were analysed by factorial ANOVA of 9 treatments (+/- OM and +/- biocide and 3xreplication) with results considered significant if p = <0.05. Total bacteria was found to have a significant p value (p= 0.048) and Gram+ ANOVA result was significant at p= 0.023. At a significance level of 5% it was possible to reject the null hypothesis that all population means were the same however the ANOVA result for total bacteria PLFA/ FAMEs (p = 0.048) was only just significant. Tukey's HSD post-hoc test, comparing all 36 pairwise comparisons did not reveal significant pairwise differences between treatment mean values for total bacteria FA % or Gram+. Homogeneity of variance for total bacterial FAs was tested using Levene's post-hoc test and variability was found to be high (p=0.003). The reason for this variability is likely due to the replicate number (n=3) for each treatment combined with high variability within treatments due to biocide application. Due to the high variance and the significance of one-way ANOVA results in total bacterial FA data and the conservative nature of Tukey's post-hoc testing, Fisher's LSD test was applied and revealed the location of differences between population means. The results of this are included in the results section in Figure 5-11 and Figure 5-7.



5.4 Results

5.4.1 Microbial biomass

No significant differences of total microbial biomass (C) between manure+soil, compost+soil or control soil were noted (Figure 5-3). Addition of cycloheximide significantly ($p= \leq 0.05$) increased the microbial biomass of the control soil and the addition of bronopol increased the biomass of the compost treated soils (Figure 5-3).

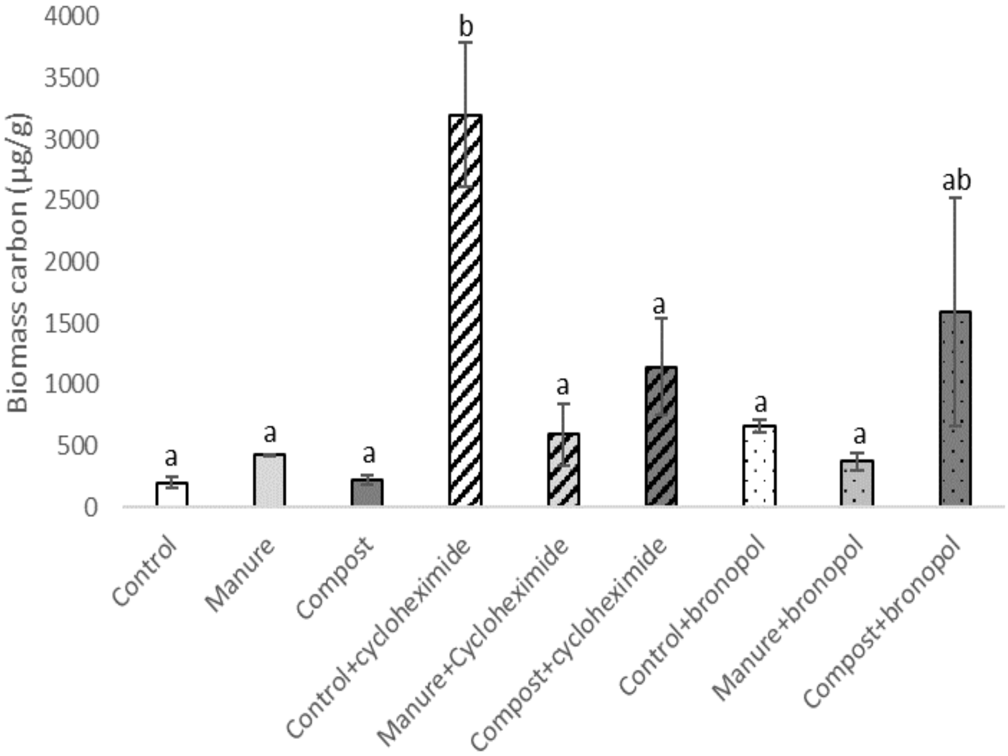


Figure 5-3 Microbial biomass carbon (mean and SE) for control soil and treatments (compost/ manure), with or without addition of biocides bronopol or cycloheximide. White bars with or without pattern represent control soil, light grey bars represent manure treated soil and dark grey bars represent garden compost treated soil. Striped bars indicate soils/ soil+ organic matter were treated with cycloheximide and dots indicate soils/ soil+ organic matter were treated with bronopol. Letters denote significant differences from Tukey's HSD with a significance level of 0.05.

Table 5-3 Summary of factorial ANOVA of microbial biomass C

Source of variation	df	F	P value
OM treatment	2	3.775	0.042
Biocide	2	8.75	0.002
OM*Biocide	4		0.005
Residual	18		

(n=3)

5.4.2 PLFA

PCA analysis of PLFA data revealed significant differences in microbial community population structure between treatments (+/- organic matter addition) and as a result of biocide application (Figure 5-4). Eigenvalues for PC1 and PC2 were 35% and 18%. Overall treatment effect on PLFA profiles was assessed using ANOVA and found to be significant (p=0.000). Significant differences between pair-wise comparisons of treatments and control on PC1 and PC2 are presented in Figure 5-5.

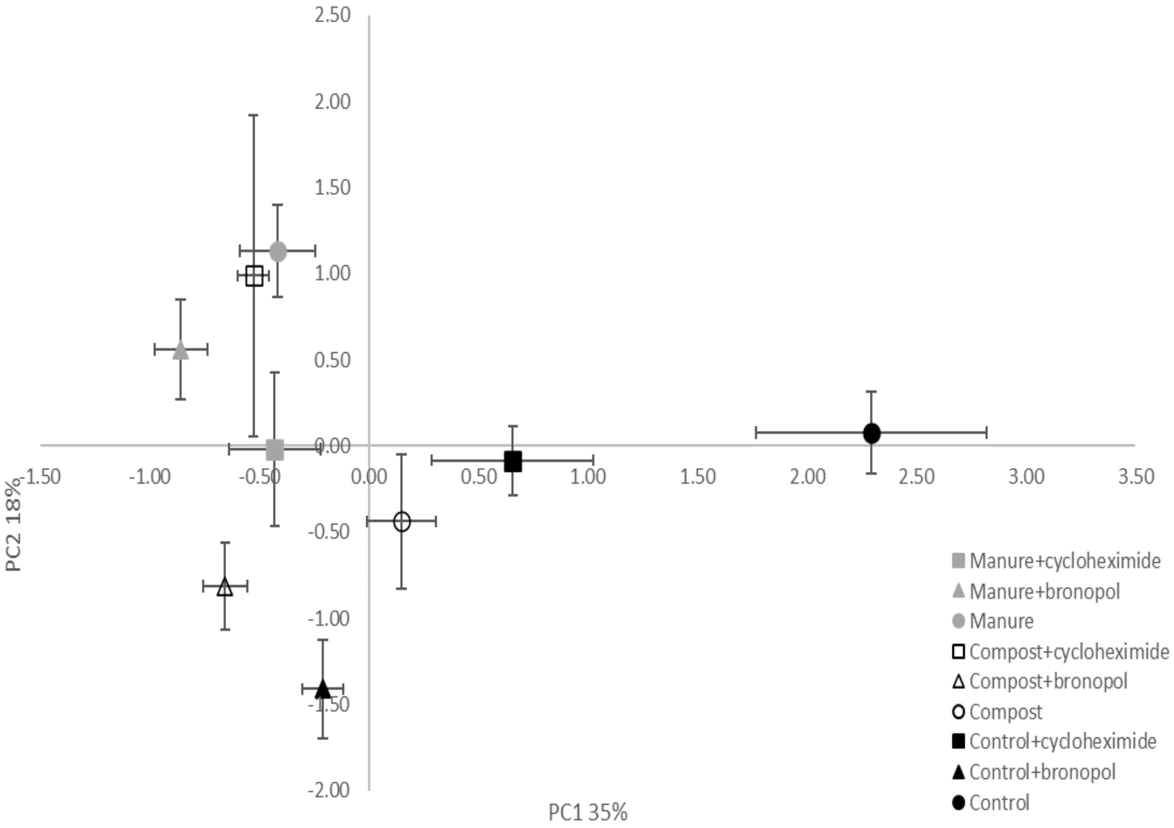


Figure 5-4 PCA depicting PLFA community profiles for control soil and treatments compost or manure addition, +/- cycloheximide or bronopol.

Table 5-4 Summary of factorial ANOVA for PLFA scores of all treatments

Source of variation	df	F	P value
OM treatment	4	13.436	0.000
Biocide	4	8.059	0.000
OM*Biocide	8	4.353	0.001
Residual	18		

(n=3)

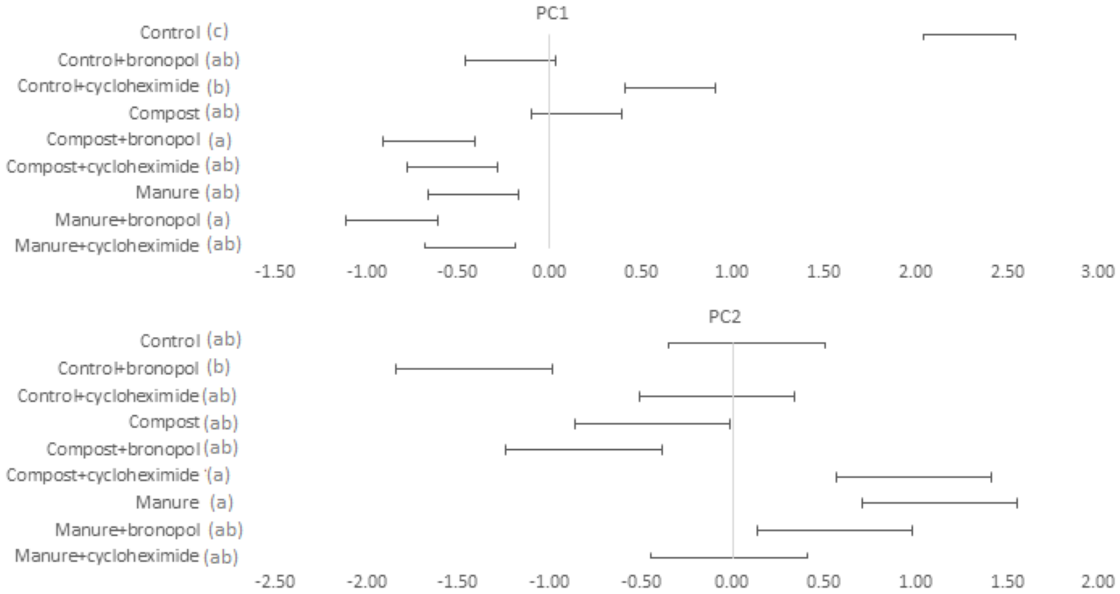


Figure 5-5 Significant differences between pairwise comparisons of principle component scores for each treatment. Bars indicate standard error and letters in brackets indicate homogenous groups.

Fatty acids (FAs) with a positive (>0.8) loading on PC1 were 15:0i, 16:1w7c, 16:1w5, Me17:0isomer, Me17:0isomer2, 18:1w9c, 19:1w6 and cyc19:0, no biomarkers were found to have a significant negative weighting on PC1. On PC2 no biomarkers had a positive weighting, however 17:1w8c, UK29:68 had a negative weighting (Table 5-5).

Table 5-5 Indication of significant positive (>0.8) or negative (<-0.8) loading on PC1 and PC2 from microbial PLFA.

	PC1	PC2
Positive loading (>0.8)	15:0i, 16:1w7c, 161w5 Me17:0isomer, Me17:0isomer2, 18:1w9c, 19:1w6, cyc19:0	-
Negative loading (<-0.8)	14:0	17:1w8c, UK29.68

Fungal biomarker 18:2w6,9 was not found to have a significant weighting on either principal component, fungal biomarker 18:1w9c had a positive weighting on PC1 (Table 5-5). A positive correlation Bacterial biomarkers 15:0i (Gram+), 16:1w7c and 19:0cyc (Gram-) had the largest positive effect on PC1 highlighting that the change in microbial community structure as driven by OM input was more significantly affected by shifts in the bacterial population than changes to in the fungal population.

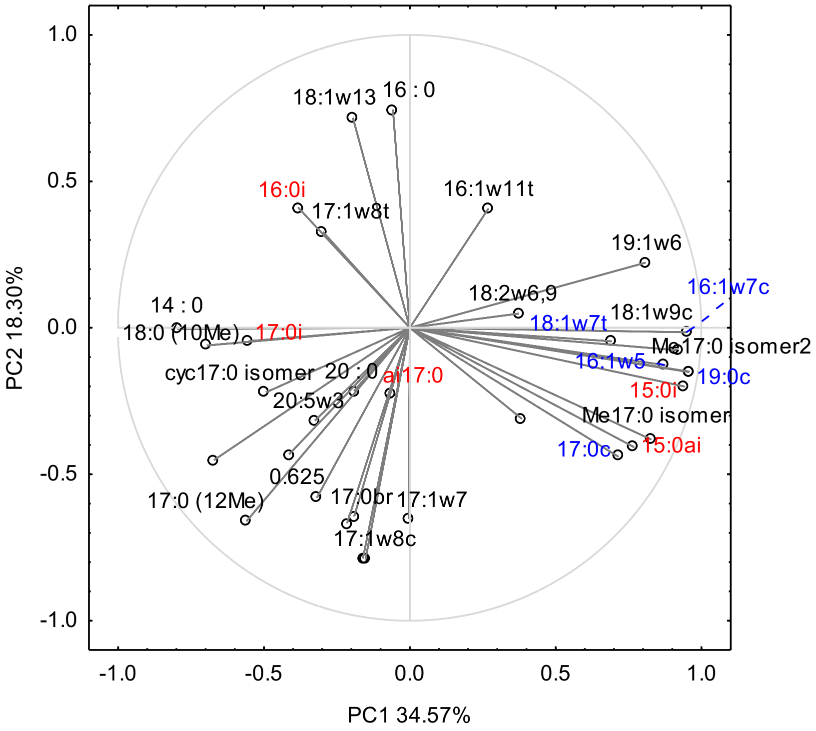
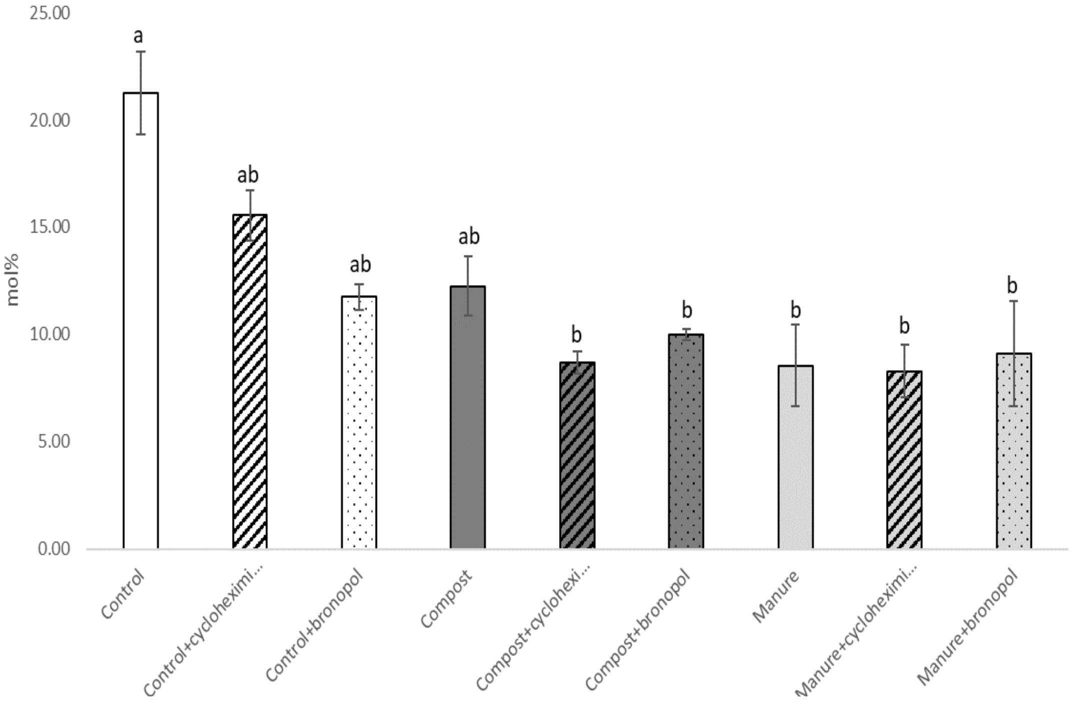


Figure 5-6 Weighting of individual PLFA biomarkers on the factor plane. Gram+ are highlighted in red and Gram- in blue. Weighting was considered significant on each factor if the score was >0.8 or <-0.8.

No significant changes of total Gram+ bacterial FAs were recorded between any treatment (Figure 5-7). Control soil had the highest proportion of Gram- negative bacterial FAs, this was significantly reduced by biocide application (Figure 5-8). No significant difference in Gram- FAs was noted in the manure treatment following biocide application and total Gram- bacterial FAs were lower than the control +/- biocide. In the garden compost treatment a reduction in Gram- FAs was recorded following application of either biocide (



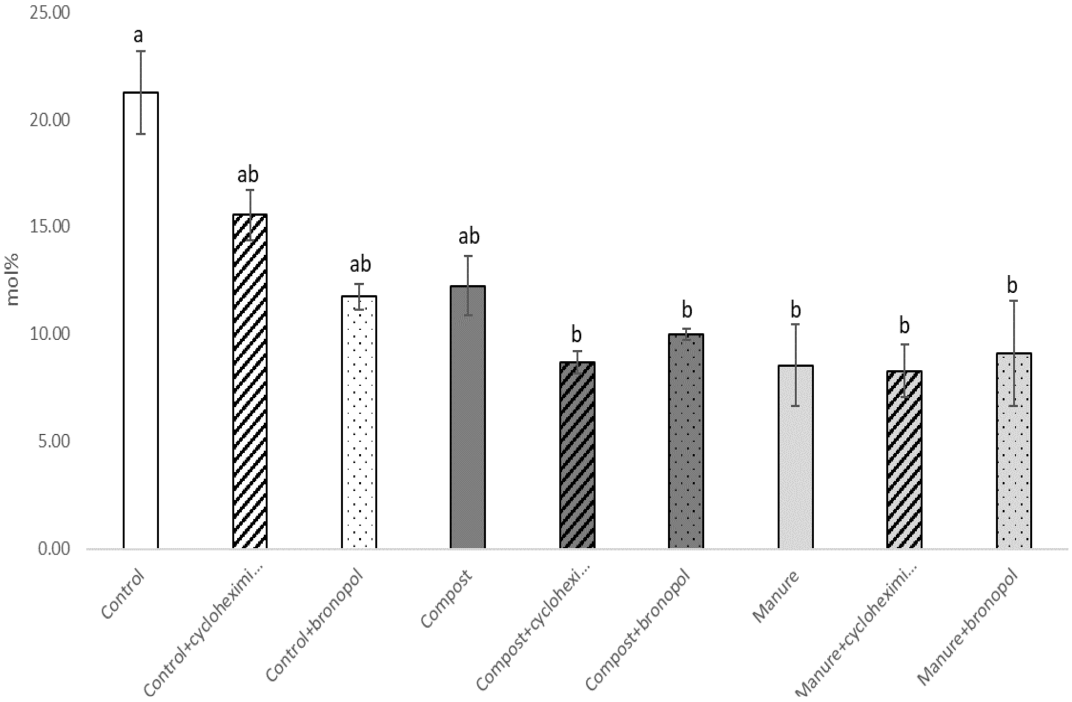


Figure 5-8).

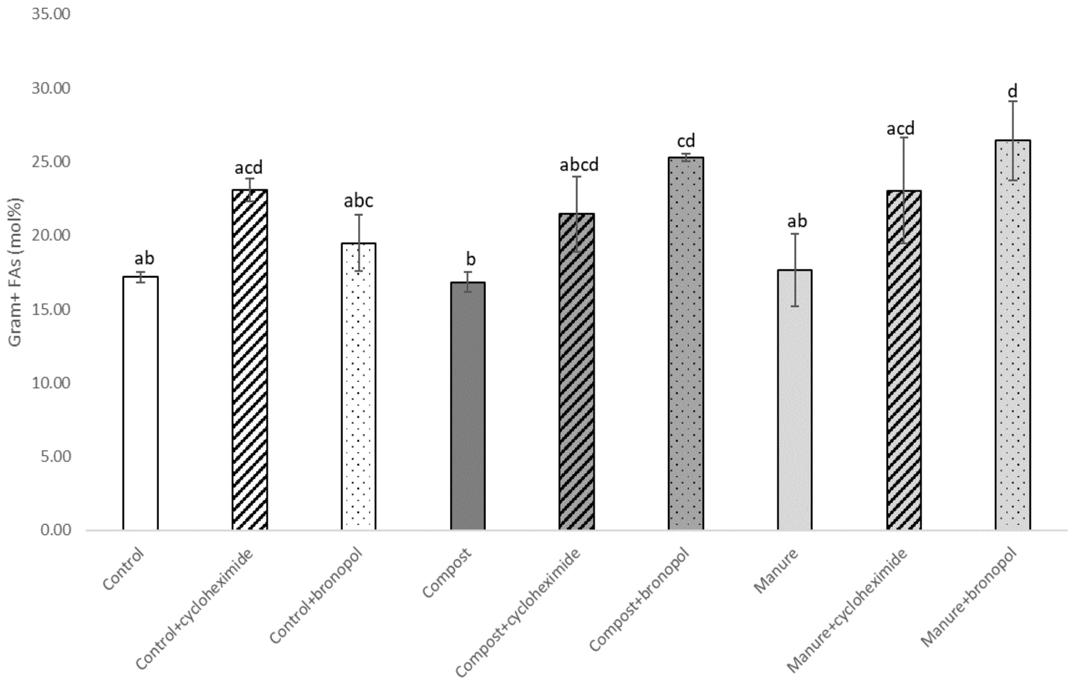


Figure 5-7 Relative abundance of Gram-positive (Gram+) FAs (mol%). Error bars depict standard error. One-way ANOVA revealed p= 0.023, letters denote LSD from Fisher's post-hoc.

Table 5-6 Summary of factorial ANOVA for total Gram+ FAME (PLFA) biomarkers

Source of variation	df	F	P value
OM treatment	2	1.087	0.358
Biocide	2	8.680	0.002
OM*Biocide	4	1.248	0.326
Residual	18		
(n=3)			
Levene		3.378	0.015

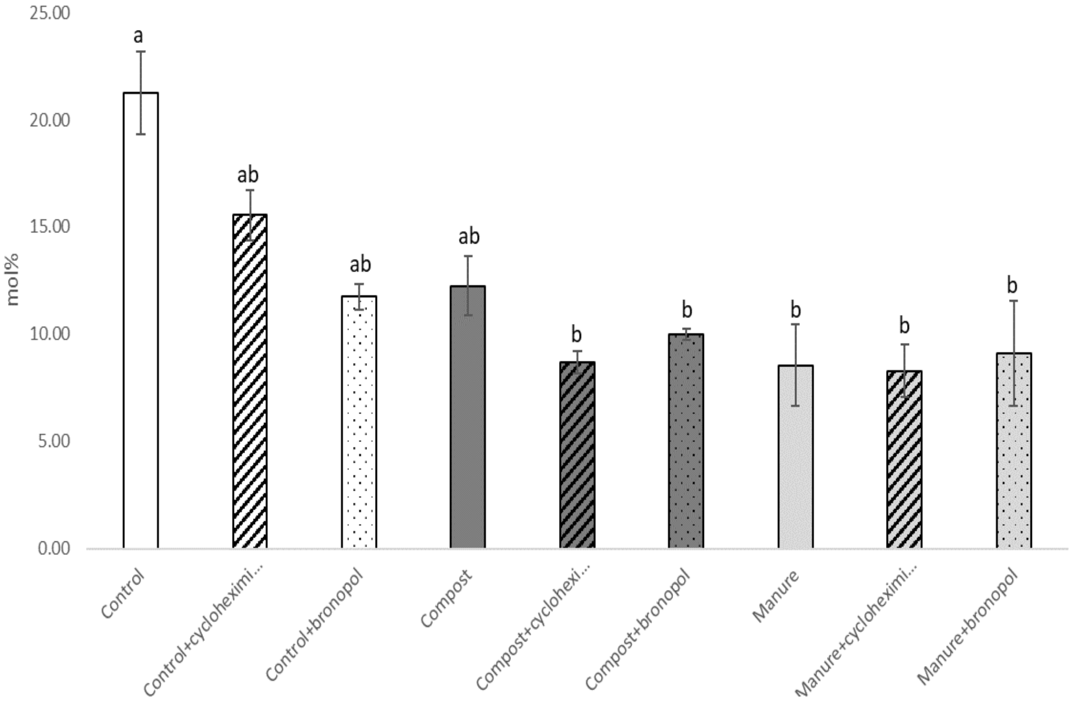


Figure 5-8 Relative abundance of Gram-negative bacteria FAs. Control soil is represented with white bars, compost treatment with dark grey and manure treatment with light grey bars, addition of cycloheximide is indicated with striped bars and bronopol treatment with dotted bars. Error bars denote std error. Letters above bars denote significant differences as per Tukey's HSD.

Table 5-7 Summary of factorial ANOVA for Gram- FAME (PLFA) biomarkers

Source of variation	df	F	P value
OM treatment	2	22.496	0.000
Biocide	2	5.83	0.011
OM*Biocide	4	3.376	0.031
Residual	18		

(n=3)

Gram+/Gram- bacteria FA ratios (G+/G-) were increased by biocide application (Figure 5-9*).* The control soil (-biocide) had the lowest G+/G- ratio and manure, with the addition of either biocide, had the highest. No significant effects on G+/G- ratios within organic matter treatments between cycloheximide or bronopol application were determined, suggesting that both biocides had a similar effect on G+ and G- populations. Significant differences noted between the relative abundance of FAs in the control soil treated with biocide and the effects of garden compost or manure application +/- biocide addition demonstrates that both organic matter application and antimicrobial application significantly altered the soil microbial community structure. Cycloheximide application significantly changed the composition of the microbial community, notably the addition of cycloheximide also increased microbial respiration in both manure and compost treatments (Figure 5-13).

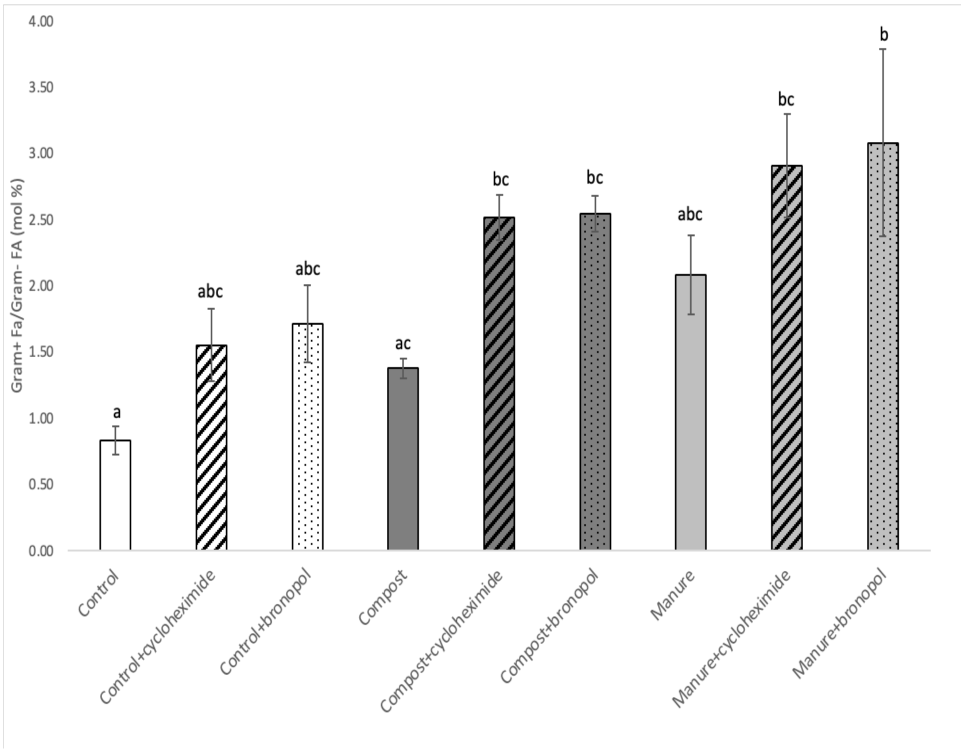


Figure 5-9 Ratio of Gram+ bacteria FAs / Gram- bacteria FAs. Control soil is represented with white bars, compost treatment with dark grey and manure treatment with light grey bars, addition of cycloheximide is indicated with striped bars and bronopol treatment with dotted bars. Error bars denote std error. Letters above bars denote groups significantly (p = 0.001) different from each other using Tukey's post-hoc test with a significance level of 0.05.

Table 5-8 Summary of factorial ANOVA for Gram+/Gram- ratios of FAME (PLFA) biomarkers

Source of variation	df	F	P value
OM treatment	2	12.400	0.000
Biocide	2	8.618	0.002
OM*Biocide	4	0.115	0.975
Residual	18		

(n=3)

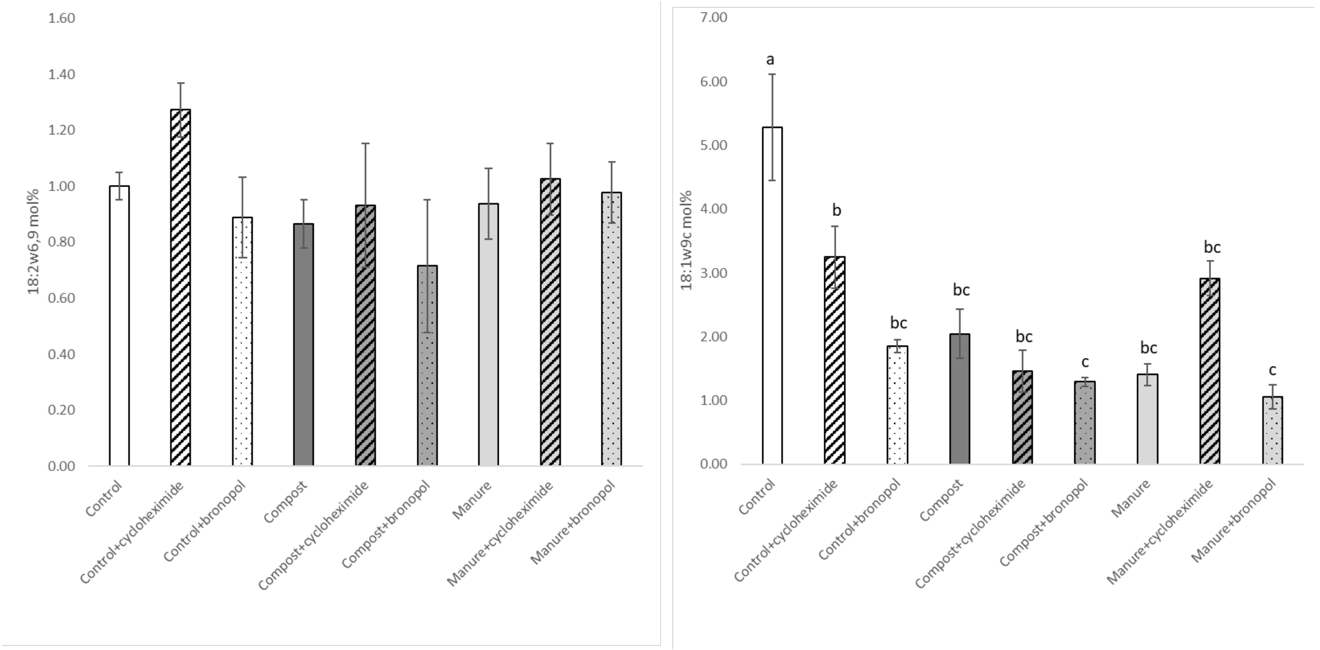


Figure 5-10 Relative abundance (mol%) of fungal PLFA biomarker 18:2w6,9 (non-significant p = 0.425) and 18:1w9c (p = 0.000). Error bars indicate standard error. Control soil bars are white, compost dark grey and manure light grey. Addition of cycloheximide indicated by striped bars, and bronopol addition indicated by dotted bars. Letters above bars indicate significant difference between homogenous groups from Tukey's HSD post-hoc testing.

Table 5-9 Summary of factorial ANOVA for FAME (PLFA) biomarker 18:1w9c

Source of variation	df	F	P value
OM treatment	2	21.139	0.000
Biocide	2	12.492	0.000
OM*Biocide	4	7.543	0.000
Residual	18		

(n=3)

Relative abundance of fungal PLFA 18:2w6,9 was analysed using one-way ANOVA and no significant differences were found between treatment means. FA 18:1w9c was also analysed and suitability of its use is discussed in section 5.3.2 and in 5.5.2. Significant differences (p = 0.000) were found in levels of 18:1w9c between OM and biocide treatments. Both have been included for comparison in Figure 5-10.

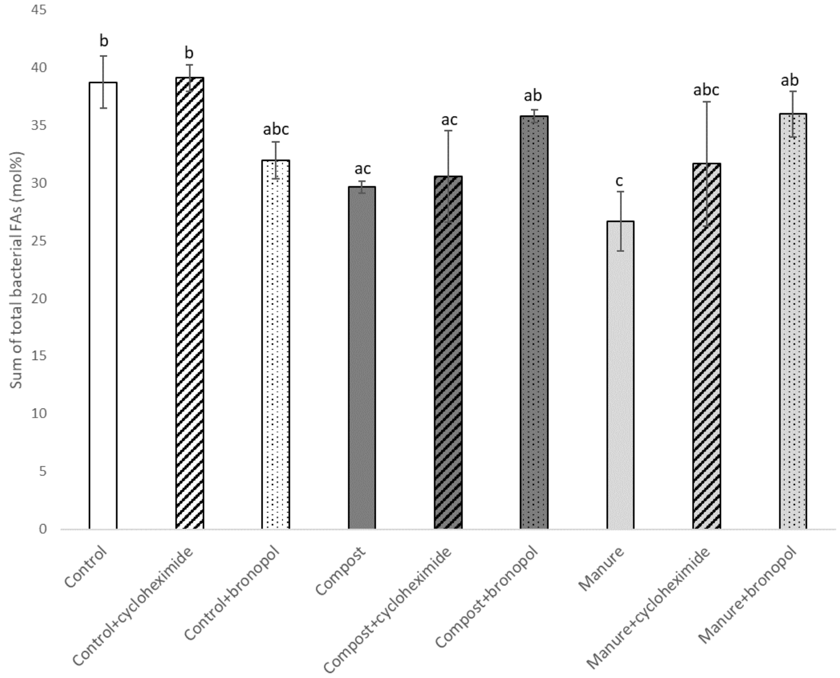


Figure 5-11 Total bacterial PLFA biomarkers (mol%). Significant difference between treatment means was identified using one-way ANOVA (p = 0.0482) with letters indicating results of Fishers LSD.

Table 5-10 Summary of factorial ANOVA for total bacterial (PLFA) biomarkers

Source of variation	df	F	P value
OM treatment	2	3.342	0.058
Biocide	2	0.927	0.413
OM*Biocide	4	2.936	0.049
Residual	18		
(n=3)			
Levene's test			0.003

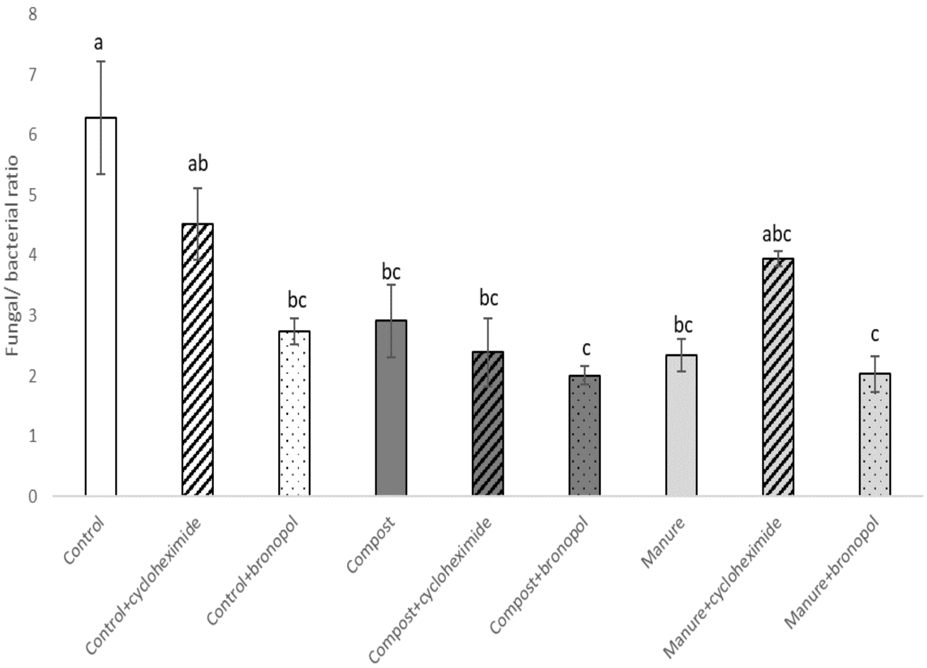


Figure 5-12 Ratio of fungal FA to total bacterial FAs. Control soil is represented with white bars, compost treatment with dark grey and manure treatment with light grey bars, addition of cycloheximide is indicated with striped bars and bronopol treatment with dotted bars. Error bars denote std error and letters above bars indicate significant differences between treatments identified through post-hoc Tukey's HSD.

Table 5-11 Summary of factorial ANOVA for Fungal/ Bacterial (F/B) ratio of FAME (PLFA) fungal and bacterial population biomarkers

Source of variation	df	F	P value
OM treatment	2	15.52	0.000
Biocide	2	9.151	0.001
OM*Biocide	4	4.539	0.010
Residual	18		

(n=3)

The control soil had the highest fungal to bacterial (F/B) ratio (Figure 5-12) compared to compost or manure treatments or soils and soil+organic matter treatments with the addition of either biocide. Both biocides significantly reduced the F/B ratio in the control soil. No significant difference between F/B of compost and manure treatments was identified. Bronopol application to either compost or manure treatments reduced F/B. Application of cycloheximide to the manure treatment increased F/B ratio compared to manure without biocide or manure+bronopol.

5.4.3 Microbial respiration

Basal respiration was significantly higher in both manure and compost treatments compared to basal respiration in the control soil (Figure 5-13). Results for measured respiration demonstrated that substrate addition (glucose or cyclodextrin), in combination with either biocide significantly altered microbial respiration in both compost and manure treated soils compared to basal respiration (Figure 5-13). Addition of glucose to the control or manure treatment increased respiration, unexpectedly the addition of glucose to the compost treatment decreased measured respiration compared to basal respiration (Figure 5-13).

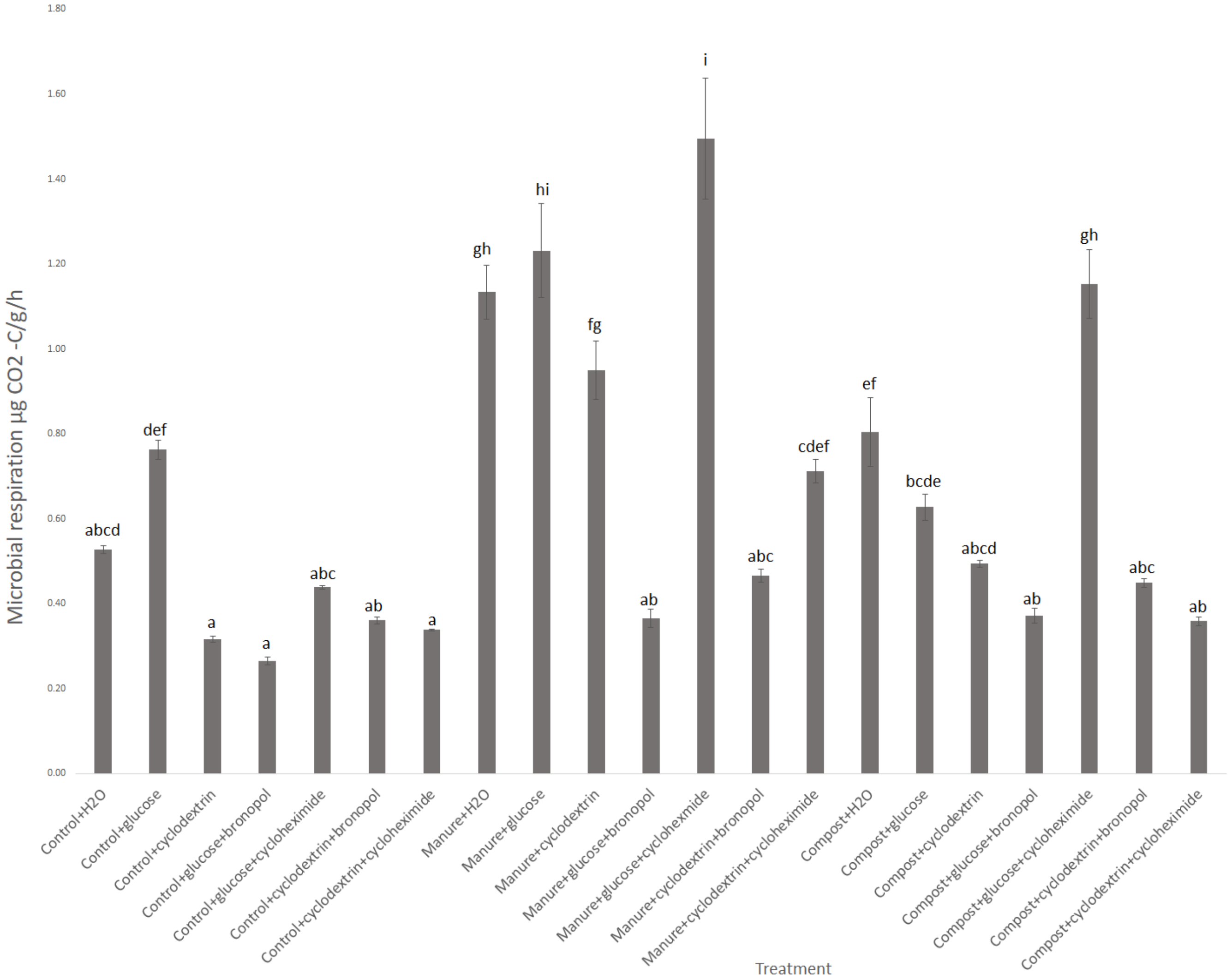


Figure 5-13 Respiration profiles for control soil and +/- OM treatments (compost or manure), +/- biocide treatments (cycloheximide or bronopol), +/- carbon substrate (glucose or cyclodextrin) on X axis. Results of Bonferroni method post-hoc testing is represented with letters above bars. Error bars indicate standard error.

Table 5-12 Summary of Friedman test of microbial respiration results for all treatments. Summary of Bonferroni post-hoc test

	df	Chi-Sq	P value
Friedman	20	556.248	0.000

(n=32)

	df	MS	Alpha
Dunn-Bonferroni	651	0.0881	0.05

The addition of cyclodextrin decreased respiration (compared to basal respiration) in the control and both treatments. However, respiration was significantly higher than the control+cyclodextrin in the manure+cyclodextrin treatment. This was also shown in the compost+cyclodextrin treatment (Figure 5-13).

The combined addition of bronopol and glucose to either treatment or control resulted in a significant decrease in respiration compared to glucose addition without bronopol (Figure 5-13). When cycloheximide was added in combination with glucose microbial respiration in both manure and compost treatments respiration significantly increased, this increase was not recorded in the control soil.

The combined application of cyclodextrin and cycloheximide significantly reduced microbial respiration in both manure and compost treatments when compared to basal respiration, though respiration remained higher than the control+cyclodextrin+cycloheximide in manure+cyclodextrin+cycloheximide. There were no significant differences in respiration between control+cyclodextrin and control+cyclodextrin+bronopol or control+cyclodextrin+cycloheximide.

When glucose was added in combination with cycloheximide to either manure or compost treatments respiration was significantly increased compared to the control+glucose+cycloheximide and to basal respiration of the relative treatments. Inversely, when cycloheximide was applied without glucose respiration significantly decreased. Addition of bronopol significantly lowered respiration compared to basal respiration when applied to either manure or compost despite addition of either substrate.

With the addition of both glucose and cycloheximide the most significant increase in respiration was seen in the manure treatment. A significant increase was also noted in the compost treatment though the increase was less than in the manure treatment (Figure 5-13).

In the control group (no compost addition) the highest recorded microbial respiration rate was measured when glucose was added. The addition of cyclodextrin or any substrate in combination with anti-microbial product resulted in significantly lower respiration rates compared to the addition of glucose.

5.5 Discussion

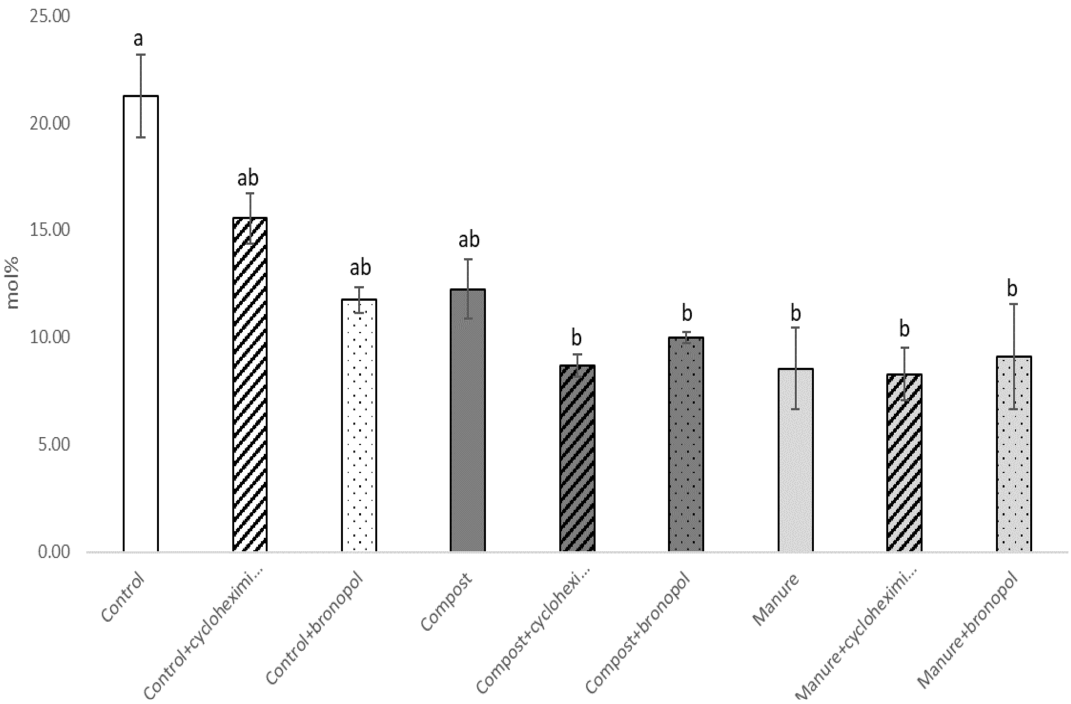
The potential for soil ecosystems to maintain function through stress events or changes in abiotic parameters, which increase competition or the availability of resources, is an important framework in which to consider the soil microbiome (Griffiths and Philippot, 2013; Maron et al., 2018, Stolz, 2017). Selective inhibition has been used in various studies since the early 1970's (Anderson and Domsch, 1973, Ingham and Coleman, 1984). Microbial mechanisms behind C cycling and how these are affected by changes as a result of organic matter inputs to soil are not robustly understood, with many soil carbon models using linear predictions of microbial biomass as a result of C inputs to soils (Craig et al., 2021). In this study significant changes between soil and soil+organic matter treatments in PLFA community structure and respiration profiles were identified. Additional shifts in community structure were recorded following application of biocides to soil. These changes were distinct depending on the type of organic matter applied.

5.5.1 Microbial biomass and community structure

No significant increases in microbial biomass (C) were noted for either organic matter treatment when compared to the control soil (Figure 5-3). The time between application of organic matter and measurement of microbial biomass may not have been sufficient for any changes to soil stoichiometry to influence the total microbial biomass. Ng et al, (2014) proposed that the “size” of the microbial biomass (using total PLFA as described in Frostegård and Bååth, 1996) does not depend on the chemical structure of input to the soil system and that timing (of microbial biomass analysis) following organic matter addition affected the results considerably. Moorhead et al, (2013) also found microbial biomass (fumigation-extraction method) to vary between sampling times and depending on soil type. They recorded differences between sand and loam soils in microbial biomass measurements immediately after litter-material addition that reduced to zero difference by day 3. Microbial biomass can be considered a small but dynamic portion of soil C requiring multiple sampling times if it is to be considered in relation to organic matter input and decomposition (Moorhead et al., 2013).

No correlations between soil/ soil+compost characteristics (Table 5-2) and microbial biomass C were found to be significant. With a longer timeframe (e.g. 8+ weeks) it would be expected to see an increase in microbial biomass C, as was noted in Griffiths et al, (2004) and Heijboer et al, (2016). A significant increase in microbial biomass was measured when cycloheximide was added to the control soil. Cycloheximide was selected for use in this study according to existing literature which noted the effective use of cycloheximide as a fungal inhibitor (Boyle et al., 2008; Pan et al., 2019; Rousk et al., 2011). However Ladan and Jacinthe (2016) found that cycloheximide demonstrated low efficacy as a biocide. If not sufficiently effective in inhibiting the fungal population in this study, it is possible that the biocide was utilised by the microbial population as a C source in the control soil which had a significantly lower total and organic C compared to the OM treatments (Table 5-2). It is also possible that cycloheximide interfered with the fumigation-extraction method, if this were the case it could be expected to see a similar increase in the other treatments to which cycloheximide was applied.

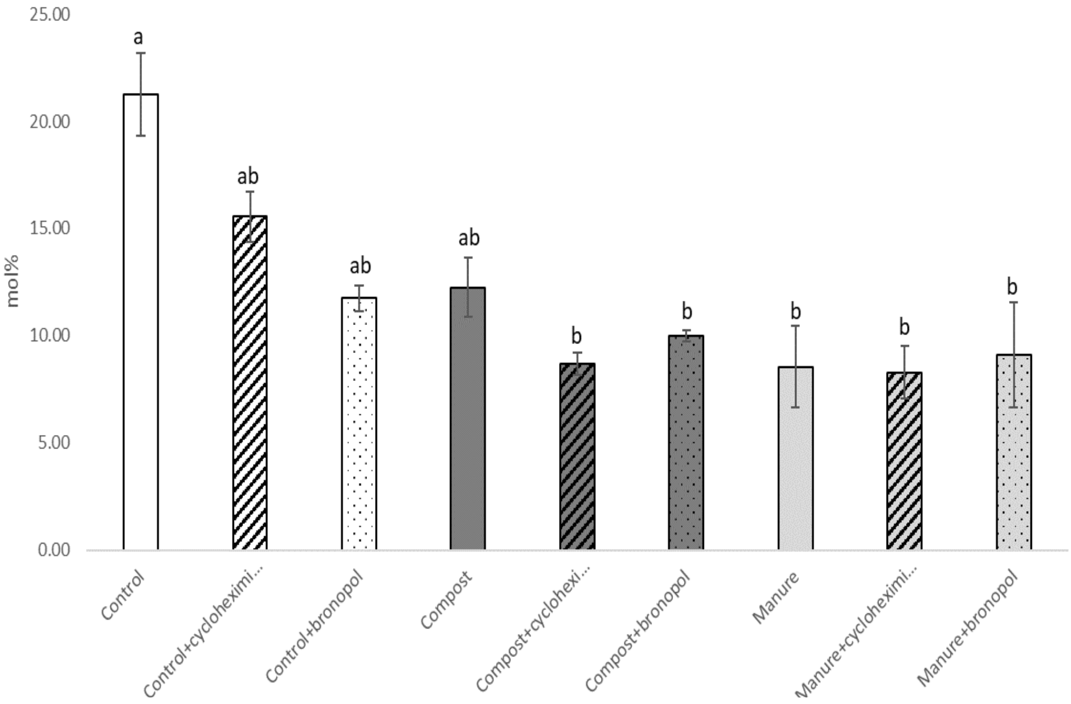
Despite the lack of increase in microbial biomass, organic amendments altered microbial community composition (Figure 5-3 and Figure 5-4*)*. Gram- bacteria FAs decreased with addition of organic matter but in manure treatment replicates no further decrease was noted with the application of either biocide (Figure 5-8). Gram- bacteria have been shown to be dependent on plant-derived labile C as a resource, with Gram+ able to capitalise on more recalcitrant soil organic matter inputs (Fanin et al., 2019). The compost material had a C/N of 17.8 and the manure 19.5 (Table 5-1) which may have altered the resource availability for Gram- bacteria in favour of Gram+ bacteria. The increase in G+/G- ratios in organic matter treated soils is a characteristic reflected in existing literature on changes to the structure of soil community PLFA profiles driven by input to the soil system (Fanin et al., 2019; Zheng et al., 2021).



Recalcitrant material has been shown to be less easily transformed into microbial biomass than biomass produced from labile resources (Crowther et al., 2015a), and from microbial necromass (Kaiser et al., 2014). This is an important aspect to consider when interpreting the results of this experiment post-application of antibiotics, which can prevent or reduce how remaining C sources are utilised by non-target organisms or provide an increase of microbial necromass to be used a resource by non-target organisms. It is also possible that the diversity and composition of the microbial population in manure (shown to be significantly different to compost in Figure 5-4) increased resilience to the biocides.

Zheng et al, (2021) traced turnover of microbial biomass using C^{13} labelled carbon and found that the structure of PLFA marker composition shifts over time. Transformation of labelled C inputs was associated with different microorganisms at different stages of the decomposition process (Zheng et al., 2021). Fungi utilised the input first followed by Gram+ bacteria. Results presented here show a strong influence of bacterial biomarkers on community structure as a result of treatments. This demonstrates a similar snapshot of secondary decomposition process as highlighted by Zheng et al, (2021). Establishing how much of the total organic C (TOC) was microbial necromass would be an interesting development on the work presented here.

It was expected that although bronopol may have some inhibitory effect on the fungal population, the bacterial community would be more significantly affected (Bailey et al., 2003). No significant reduction of fungal FA (18:2w6,9) was found in this study as a result of bronopol application but a significant reduction in Gram- FAs was noted in the control and treatments with the application of either biocide (Figure 5-10 and Figure 5-8). Fungal and bacterial species occupy different niches and transformation of organic matter is not carried out by a single species (Oldfield et



al., 2018a). Though fungi may initiate the decomposition of material with, for example, a high C/N ratio the process is continued by Gram+, arbuscular mycorrhizal fungi (AMF), actinomycetes and Gran- bacteria (Zheng et al., 2021). Without this sequence, decomposition of carbon inputs is altered and there may be a reduction in the assimilation of C input into microbial biomass and resulting necromass (Zheng et al., 2021).

5.5.2 Microbial community structure

Principal component analysis of community structure revealed the FAs that had the most influence on community structure were bacterial and predominantly Gram+ (Figure 5-7). Gram+ bacteria are predominantly aerobic and have been associated with species rich and woody sites (Zhang and Chu, 2012). Though the overall C/N ratio of the compost material was lower than the manure, woody material such as brash cuttings and stems and stalks from a large variety of species from the RHS Wisley gardens site e.g., rose and fruit tree pruning, were used to produce the RHS compost and may be a reason for this shift.

The significant influence of changes in bacterial community structure on overall community composition are reflected in wider literature such as Liu et al, (2022) who concluded, from long-term analysis of compost applications to soil, that bacteria were more sensitive to changes caused by compost addition. They also propose that the cause of changes community diversity and composition are due to changes in resource C resource provisioning as a result of input (Liu et al., 2022). The results found within this shorter tern experiment align with the findings of Liu et al, (2022) as both manure and compost community PLFA profiles were significantly different, despite the short time-frame of the experiment. This is in contrast to de Sosa et al, (2021) who did not find type of compost influence on microbial community structure to be as significant as time of sampling following organic matter addition to soil. Organic matter types vary widely and it is valuable to consider this variation in future experimental design by including distinct types of organic matter as well as analysis of the effects of sampling at different stages post-application and along the decomposition gradient of the organic matter itself (Ho et al., 2022; Larney et al., 2006; Yang et al., 2022).

Demoling et al, (2008) suggest that 18:2w6,9 has low response sensitivity to recent stress, disturbance events and fertiliser input. They also outline similar effects as found in this study (increase in G+/G- bacteria ratio and decrease of F/B ratio Figure 5-9 and Figure 5-12) when they quantified the response of bacterial and fungal populations to N fertilisation in forest systems (Demoling et al., 2008). No significant changes to fungal biomarker 18:2w6,9 were noted in the manure or compost treatment following the addition of bronopol or cycloheximide (Figure 5-10). 18:2w6,9 did not have a significant impact on PLFA community profiles between organic matter treatments (Figure 5-6). However, 18:1w9c had a significant positive weighting on PC1 and was significantly reduced by both compost treatment and biocide application (Figure 5-10). Issues with the use of 18:1w9c as a fungal biomarker are extensively discussed in the existing literature on PLFA classification (Frostegård et al., 2011b; Joergensen, 2021; Rousk et al., 2011; Zhu et al., 2012). Confounding effects due to the potential of 18:1w9c to be found in living plant material or in bacterial species are outlined as profound only when levels of 18:2w6,9 are zero (zero fungal biomass) as is noted in some agricultural soils, or when living plant material is not removed from the sample before PLFA extraction (Frostegård et al., 2011b). Kaiser et al, (2010) provide a more detailed evaluation of the use of 18:1w9c and 18:2w6,9 and emphasise the negligible contribution of plant roots to measures of either biomarker, concluding that both are suitable as biomarkers of fungal populations in soil. As no living plant was used in this study and levels of 18:2w6,9 were above zero it was considered appropriate to use as a fungal biomarker.

In addition Joergensen (2021) propose that 18:1w9c is indicative of Mucoromycotina spp. and Francioli et al, (2016) quantified significant increases of Zygomycota (at time of publication Mucoromycotina was included in the phylum Zygomycota) in soils following applications of manure. The combined use of 18:1w6,9 and 18:1w9c has been used as an indication of soil fungal populations by Zhao et al, (2017). Classified as copiotrophic saprophytes Zygomycota populations have been shown to increase in systems which are managed and have an abundance of organic material with a low C/N ratio (T. Liu et al., 2022). Considering this it is somewhat surprising that the control soil had the highest level of 18:1w9c.

Though unexpected this is an effect that has been noted in existing studies which have quantified the abundance of Mucoromycotina spp. at different stages of SOM decomposition and in different systems (grasslands, forests, alpine and agricultural). Štursová et al, (2020) posit that there is limited reliable information on substrate use of Mucoromycotina but that they are generally considered to be involved early on in the decomposition of carbohydrates, can survive in a wide-range of nutrient deficient conditions and have been shown to degrade granite rock (Brunner et al., 2011). *Mortierella spp.* (phylum Mucoromycotina) have been shown to be widespread in bulk soil samples analysed through DNA and RNA-analyses (Ozimek and Hanaka, 2021). If a plant had been grown in the control soil there would be some risk that the high levels of 18:1w9c could be a product of plant roots, but as no plant was grown in the control soil prior to or during the experiment it is worth considering the control soil as having abundant levels of a common pioneer decomposer microorganism in a low nutrient and low organic matter soil (Schneider et al., 2012). The population of which was decreased by biocide application and by OM application, at a late stage of decomposition, favouring other phylum.

The PLFA community profile of the manure treatment +/- biocide revealed significant differences on PC1 (Figure 5-4) compared to control soil +/- relative biocide treatments. Control soil treated with biocide differed significantly to control soil+biocides. These changes were primarily due to changes in bacterial community structure (Figure 5-6). There was no significant difference between manure and manure+biocides on PC1 which suggests that organic matter addition (specifically manure) supports the second hypothesis; that OM addition would provide community structural resilience to inhibitory effects from biocide application. Griffiths and Philippot (2013) demonstrated that organic matter addition increased resilience of the soil system by provision of resources for the microbial population.

Manure had the highest basal respiration and following addition of cyclodextrin and respiration was higher than either compost or control. Studies comparing manure and garden or “green” compost are limited in the existing literature, one such study completed by Ros et al, (2006) compared basal respiration using continuous flow, infrared gas analysis from soils treated with a variety of organic matter amendments. They found that the application of organic matter increased basal respiration (Ros et al., 2006). The same effect was found using the Microresp method in this study. When bronopol was added respiration significantly decreased (Figure 5-13). If bacterial groups were the most affected by biocide application, then it is possible that the fungal population, shown to be essential in the initial stages of organic matter decomposition were less affected by biocide treatments and were able to maintain decomposition functions.

Repeating the analyses with an alternative fungal specific biocide would enable a more thorough determination of whether the organic matter enhanced resilience of the fungal population or whether cycloheximide had very low efficacy as an anti-fungal. Repeating this experiment with a composted bark addition or equivalent with a higher C/N ratio would provide further evidence of whether the fungal community has more of a significant role to play in respiration when only high carbon inputs are available (Kallenbach and Grandy, 2011).

The structure of the microbial population (PLFA profile - Figure 5-4) was changed by both compost addition and by anti-microbial application. This correlated with significant differences in measured respiration (Figure 5-13), and supports studies which stipulate changes to soil organic matter quality influence soil microbial function (Liang et al., 2017; Liang and Zhu, 2021; Tully and McAskill, 2019).

5.5.3 Respiration and biocide effects influence on function

Rapid decomposition of easily utilised C substrates (such as glucose) was expected to cause a measurable increase in respiration (Degens and Harris, 1997; Zhou et al., 2021). In this study the control+glucose and manure+glucose increased respiration (Figure 5-13). When glucose was added in combination with bronopol, respiration in both OM treatments and the control, significantly decreased. This effect goes some way in confirming that bronopol successfully inhibited the bacterial population. In addition, respiration of the control group was lower than both OM treatments when bronopol was added. This highlights the potential that organic matter addition to soil can increase microbial functional resilience (de Sosa et al., 2021; Griffiths and Philippot, 2013).

Manure addition increased basal respiration and respiration following glucose or cyclodextrin addition when compared to the control (Figure 5-13). When a more utilisable resource (glucose) was added in combination with cycloheximide respiration significantly increased in manure and compost treatments compared to respective basal respiration and to respiration measured in the control soil, the effect being the most significant in the manure treatment. Surprisingly, when glucose was added in combination with cycloheximide respiration increased significantly in both manure and compost treatments but not the control in which respiration significantly decreased (Figure 5-13). Cycloheximide was effective at inhibiting microbial function in the control soil even when an easily utilisable resource (glucose) was available. The opposite was true for the application of cycloheximide+glucose in OM treated soils in which it had a stimulatory effect. Manure+cyclohexmide and compost+cycloheximide both had significantly different PLFA community profiles compared to control+cyclohexmmide (Figure 5-4). While some of the results were expected and align with existing literature (increase in respiration as a result of glucose application to soils), other results infer potential interference of cycloheximide within the experimental design.

Swallow and Quideau (2020) noted that the use of bronopol in their selective inhibition work severely restricted the ability of soil microbes to utilise glucose inputs. The same effect was seen in this study, when glucose was added in combination with bronopol respiration was significantly lower than respiration measured after the addition of glucose without bronopol (Figure 5-13). Respiration was marginally higher in organic matter treatment replicates treated with bronopol compared to control replicates treated with bronopol which supports the original hypothesis that organic matter treatment would help to maintain respiration function under inhibition pressure. It is not possible to draw any similar or robust conclusion regarding the replicates treated with cyclohexmide. Potential explanations of the results can be related to the recalcitrance of cyclodextrin (high molecular weight) and that manure had the highest C/N ratio. It is possible that the manure microbial community accelerated OM decomposition to increase N supply from the OM input (Cui et al., 2020). Further work is needed to robustly confirm the effects of cycloheximide and to separate out whether the organic matter application to soil ensured maintenance of respiration under inhibition pressure or if non-target effects of cycloheximide stimulated microbial respiration when combined with glucose. The latter seems more likely, considering the increase in Gram+ FAs in OM treated soils and that bacteria are noted to quickly capitalise on labile substrates such as glucose. Stamatiadis et al, (1990) also found that cycloheximide application significantly stimulated short-term response of bacterial respiration following glucose.

Control soils treated with cyclodextrin had lower respiration than basal respiration or respiration measured following glucose addition. A reduction was measured when cyclodextrin was added to OM treatments though measured respiration remained higher than in the control+cyclodextrin. This reduction in respiration was expected due to the higher molecular weight of cyclodextrin comparative to glucose. Respiration of both OM treatments with addition of cyclodextrin decreased further when either biocide was applied, again remaining higher than the control+cyclodextrin+biocides. This demonstrates that inhibitory effects on the microbial population result in loss of function (respiration) when available C is less metabolically accessible. However, as respiration of either OM+cyclodextrin+biocide treatment remained higher than the control treated with the same substrate and biocides it can be concluded that inhibitory effects on soil function are reduced with the application of OM.

The effects of a biocide on specific microbes and not others may cause remaining facultative anaerobes, capable of completing their life cycles, to capitalise on resources that would have been utilised by the section of the population on which the antibiotics were effective (Schmidt et al., 2011). To limit the potential of microbial life capitalising on necromass created by the antibiotic addition the timeline of this study aimed to reduce the amount of time that lapsed between application of the anti-microbial product and assessment of PLFA and microbial biomass. However, there is a requirement of a sufficient period of incubation to ensure the anti-microbials have had time to take effect. The experimental design intended to provide sufficient incubation time whilst limiting the opportunity for microbes to capitalise on the cell death of other microbes. This short time period may have resulted in a similar effect on PLFA analysis as outlined by Frostegård et al., 2011b). Frostegård et al, proposed that degradation of PLFAs may be reduced when a “toxic substance” that can inhibit microbial life simultaneously reduces the degradation of the PLFAs. Reduction in the turnover rate of fungal FAs in the biocide treated replicates may have required a longer time-period prior to PLFA analysis to provide an accurate estimate of reductions in living soil fungi (Frostegård et al., 2011b).

The lack of significant differences in respiration between control+cyclodextrin/ control+cyclodextrin+bronopol/ control+cyclodextrin+cycloheximide can be attributed to differences between the PLFA community profile of the control soil and the OM treatment PLFA profiles (Figure 5-4). It is possible that the sections of the community responsible for decomposition (and measured respiration) in the manure/ compost treatments were not present in the control soils and therefore no measurable effect of biocide on respiration after substrate application were noted (Abujabhah et al., 2016; Martínez-García et al., 2018b).

Adding anti-microbial products or attempting to inhibit soil microbial diversity or adding organic matter addition did not, in this study, adversely affect microbial biomass, but it did significantly change the structure of the soil microbial community. Whether the composition of the soil microbiome dictates its functional ability, and how management of soils influences microbiome structure, is an ongoing topic of research in the wider literature (Dungait et al., 2012; Li et al., 2019; Louca et al., 2018; Oldfield et al., 2018b). It was hypothesised that addition of organic matter would support resilience of respiration functionality to inhibitory effects. Which was somewhat confirmed by the manure treatment which maintained higher measured respiration following biocide application when compared to the control soil without organic matter.

5.5.4 Challenges, limitations and future work

There are challenges and constraints that have been considered within the interpretation of the results presented. Issues that are worth highlighting in relation to this trial are echoed in other papers and include overlap in specificity of biocides (Bailey et al., 2003). Non-target toxicity of anti-microbials (bronopol and cycloheximide) has been cited as being a major factor in some studies and non-significant in other trials (Pan et al., 2019; Swallow and Quideau, 2020). Despite some studies outlining non-target effects of cycloheximide the majority outline successful inhibition of the fungal population with limited or no non-target effects on bacteria (DeCrappeo et al., 2017; Maeda et al., 2017; Pan et al., 2019). The increase in respiration quantified as result of cycloheximide applied in combination with glucose, as noted in Stamatiadis et al, (1990), and the lack of significant effects of cycloheximide on bacterial biomarkers can support the conclusion that cycloheximide did not have non-target effects on bacteria. The level of efficacy on targeted fungi requires further work to robustly determine.

It is difficult to separate a single domain but inhibiting a section of the community can help identify the role of competition between domains in processes such as decomposition and substrate utilisation (Rousk et al., 2008). Land management can have a deleterious or inhibitory effect on either fungi or bacteria, by limiting resource availability or increasing competition effects within the community (Demoling et al., 2008; Lehmann et al., 2011; Rousk et al., 2011, 2008; Wu et al., 2022). In horticultural soils the microbiome is manipulated by different sets of management decisions made by individual gardeners. Despite not increasing soil microbial biomass, compost additions may help soils recover from events that inhibit sections of the community and prevent dominance of one phylum due to an abundance or restriction of resources. Retaining this diversity is a key benefit of soil organic matter additions.

Quantifying and separating the effect of increasing microbial necromass which can provide a resource for remaining microbial life would improve the understanding of the effects outlined in the results presented from this study (Ladan and Jacinthe, 2016). Quantifying this effect, and the time-period required to ensure measurement of PLFAs following potential enzymatic interactions with biocides would be an improvement to this study. A second sampling time between organic matter addition, incorporation and biomass analysis may help to account for potential for increased microbial biomass following application of organic matter that was not accounted for in the time frame of this experiment.

C and N labelling use to differentiate whether the biocides themselves were being used as a substrate by the microbial population would be of benefit to the analysis of organic matter and/or substrate assimilation by the microbial population. This may help to explain the increase of microbial biomass in some soils when biocides were applied. In addition to this use of DNA/ RNA analysis of soil samples to confirm phylum level identity behind changes in PLFA biomarkers would increase the robustness of reasons proposed in the discussion for the influence of OM application on community structure.

Comparison of garden systems with either agricultural or forest systems clearly has disadvantages but considering the scarcity of literature on garden compost use specifically there is a necessity to draw comparison from relatable studies despite different management. In general it can be assumed that forest systems provide higher organic matter input (due to litter deposition) and decreased tillage than agricultural (Heinz et al., 2015; Sterkenburg et al., 2015). This potentially increases transferability of data from forest systems to garden systems which incorporate long-term and regular organic matter inputs without mechanical tillage.

Increasing understanding of the “diversity-function” relationship between SOM cycling and drivers of change to the soil microbiome can improve functional resilience of managed soil systems (Wagg et al., 2019). This work is positioned within literature which explores the microbial role in SOM is cycling and what happens to the microbial community structure and function under different regimens and aligns with wider literature aimed at improving management of SOM.

5.5.5 Concluding statements

Increased understanding of the effects of soil management, such as input of organic matter, on biological processes is critical to understanding the essential nature of soil biodiversity to essential soil functions (Wagg et al., 2019, 2014). Diversity increases functional resilience in soil ecosystems (Maron et al., 2018b; Wagg et al., 2014). Anthropogenic influences on soil systems result in changes to microbial diversity and thus function (Soliveres et al., 2016). Functions and processes delivered by the soil microbiome are essential to the success of garden systems and as such should be considered when making management decisions.

The aim of the experimental process presented in this chapter was to examine whether fungal and/or bacterial inhibition would reduce respiration as a result of reduced capacity for decomposition of recalcitrant/labile substrate inputs and whether the addition of organic matter would provide resilience to respiration functionality.

Manure maintained higher respiration levels than the control soil when cycloheximide induced inhibition effects were applied. Despite no immediate increase in microbial biomass there was a significant effect of manure application on microbial community structure which resulted in higher respiration than the control, when either a recalcitrant or labile C resource (cyclodextrin or glucose) were applied. If respiration can be used to imply decomposition (Krishna and Mohan, 2017), then it is possible to conclude that decomposition and associated release and availability of nutrients was increased by manure addition. Similar effects, but to a lesser extent, were quantified as a result of compost addition, implying that the benefit of OM application is input dependent. “Organic matter” as a category is not a sufficiently detailed nomenclature for the production, classification, sale, and study of composts and soil improvers. More research which compares and contrasts different types of organic matter is needed to fully understand the relationship between input and output of SOM cycling processes as mediated by soil organisms.

Manure retained function compared to control soils when subjected to inhibition effects. Linking knowledge of the soil microbiome and how it changes in response to additions of organic matter can improve advice for gardeners who typically repeat practice based on tradition, culture, personal experience, or availability of resources. Being equipped with knowledge on the effects of specific resources can help to ensure that gardens are effectively managing potential benefits and ecosystem services within the UK's gardens.

This comparison between inputs contributes to research which increases understanding of gardens and the specific management practices which influence the garden ecosystem.

6 Synthesis and Conclusions

Chapter synopsis

This chapter synthesises key findings from the research on organic matter additions to garden soils presented in the previous chapters. Key findings and their relevance to the original aim and objectives are summarised. Following this is a statement of the contribution to knowledge gained from the work completed over the course of this project and routes for further work are outlined.

6.1.1 Key findings: Chapter 2

Objective addressed:

1. Complete a critical review of existing research into organic matter additions commonly made to UK garden systems and studies that link input to microbial mechanisms of decomposition functions.

A critical review of existing literature exploring organic matter applications to garden soils and the influence of these impacts on carbon cycling processes was presented in Chapter 2. It addressed a prominent research knowledge gap regarding the effects of land-management common in non-agricultural contexts, specifically UK gardens. Particularly lacking are repeatable, long-term experimental designs which can contribute field level data and information to improve accurate modelling of the biological processes as effected by a common practise, organic matter or compost addition to soil.

The essentiality of the soil microbiome for, arguably all, of the terrestrial ecosystem functions that are necessary for human life on Earth has been underestimated (Cavicchioli et al., 2019). The literature review highlighted that an expanding body of scientific research is working to address this. The review also outlined that despite an increasing number of studies linking vital ecosystem services to microbially mediated processes there is still a lack of understanding of fundamental mechanisms behind these processes.

The review also highlighted that improved management in UK gardens can support surrounding systems by reducing or offsetting negative environmental effects and can increase provision of ecosystem services if targeted research efforts ensure gardens are managed efficiently and accurately.

6.1.2 Key findings: Chapter 3

Objective addressed:

2. Synthesise the key fluctuations of soil stoichiometry as influenced by organic matter addition in a long-term field trial.
3. Evaluate the legacy effects of annual organic matter input to a garden specific field trial and select treatments for further analysis based on distinct features of input relevant to UK garden contexts.

The analysis of legacy effects presented in Chapter 3 using data collected between 2007-2019 was completed with permission of RHS Wisley. The synthesis of long-term trends within the data provided quantitative evaluation of different organic matter addition effects on available nutrient levels in soil. Statistical analysis revealed a variety of changes to trial soils that were characteristic of the type of organic matter.

Findings highlighted the necessity of having measures of soil biology considered in combination with measures of soil chemical processes. Without the measures of soil microbial community structure and function it cannot be said with certainty the extent to which field trial treatment effects (nutrient build-up) were biologically governed. However, it was considered feasible that the effects seen were due to microbial adaptation and changes of community structure through comparison with existing literature. On this premise the hypothesis for the series of experiments in chapter 4 were designed and the hypothesis for Chapter 3 confirmed.

6.1.3 Key findings: Chapter 4

Objective addressed:

4. Establish the impact of changes to garden soil functions (MSIR) as influenced by organic matter input and changes to microbial community structure after 10years of organic matter amendments.

Chapter 4 presented the findings from a series of experiments designed to quantify changes to a microbiological function (respiration) and link changes in functional output to changes in community structure (PLFA/ NLFA/ microbial biomass C). It was hypothesised that if microbial biomass was maintained then respiration would continue, even when treatments only included recalcitrant materials (high C/N material such as bark and substrates such as oxalic acid).

The hypothesis was confirmed as basal respiration was not significantly lower in bark amended soils, highlighting functional resilience of the microbiological community subjected to high C inputs. Fungal biomass (PLFA 18:2w6,9) increased following OM input in bark treated plots (the treatment with the highest C/N ratio). As only small changes were recorded in total microbial biomass this suggests changes to the structure of the population in response to the input, whilst respiration was maintained.

6.1.4 Key findings: Chapter 5

Objective addressed:

> Quantify the benefit of organic matter addition on soil microbiological function by applying inhibitory pressures to the microbial population and quantifying resilience of soil function provided by organic matter addition

Developing on results and analysis presented in Chapter 4, the findings in Chapter 4 a more in-depth study of microbial function in relation to community structure. The hypothesis was designed to test how changes in the structure of the community, domain-specific inhibitory effects (biocide application), alter the ability of the microbial community to maintain function and cycle inputs that range from readily available to more recalcitrant.

The experiment was deigned to explore the idea that a diverse microbial population is key to maintaining function and that the relative abundance of different portions of community (for example bacteria/ fungi or antagonist/mutualists) can be influenced by effects of organic matter addition on the availability of nutrients. In the experiment an attempt was made to inhibit specific domains of the microbiome following a review of existing research which confirmed the efficacy of the biocides. The design incorporated the novel use of biocides in combination with existing methods (MSIR – Microresp).

As there were no significant differences in microbial biomass between either organic matter treatments or the control but significant differences between PLFA community profiles it was concluded that the structure of the microbial community was different for each treatment. Following biocide application the microbial community structure did not change significantly in the manure treated soils but did in the control. This implies that manure treated soil community structure was more resilient to inhibition. Overall manure also maintained respiration functions despite inhibition pressure. Manure has been cited as a microbially rich input and its application beneficial for supporting robust soil ecosystems. This is view is supported by the findings of Chapter 5. Though without careful management it must also be emphasised that manure applications can lead to nutrient losses from systems.

The original aim of the experimental design for Chapter 5 was to quantify the effects of organic matter addition on soil community structure and function. This was not without issues. Using biocides was a complex process which may have supplied sections of the microbial population with a carbon resource not accounted for in the experimental design. Even so, the findings can still be considered of use for linking mechanisms behind carbon cycling processes to soil microbial domains. Further work using labelled C would be an interesting development to the study presented in Chapter 5.

6.1.5 Objective 6: Recommendations for soil organic matter management in UK gardens

The accumulative findings of this research have enabled several recommendations for organic matter application management in UK gardens.

I. A recommendation for compost producers and suppliers to include an outline of the macro-nutrient, organic matter% and total C content of compost produced and sold in UK outlets. It is a requirement of compost and soil conditioner suppliers to meet essential packaging requirements, however as soil conditioners are not included in a sector with special requirements for details on the contents of packaging (e.g. food and drink, products for children) and it is not a legal requirement to provide detailed information on composition. The product must meet minimum standards (e.g. tested for pathogens, heavy metals), be as described and suitable for the purpose of purchase (e.g. weight of contents), and be traceable back to the original source of production materials (Gov UK, 2022a). Though soil conditioners and composts are not homogenous materials inclusion of approximate figures on the characteristics of the material would enable more accurate application and reduce build-up of excess nutrients such as those identified in this thesis.

II. Gardeners producing their own inputs should seek to understand the characteristics of the material they are producing to increase resource use efficiency and to reduce the unintended negative impacts of regular organic matter application such as build-up of nutrients to levels which limit plant growth or reduction in pH. Home soil testing kits vary in accuracy and require some interpretation of results (Faber et al., 2007). Furthermore demand for a low-cost way of approximating nutrient status of soils and water has increased in recent years (Golicz et al., 2021). Technological advancements such as the use of smartphones in combination with test kits to assist in the interpretation of results have been explored with success in Golicz et al, (2021) and offer opportunities to the gardening community. Further work is needed to understand at what intervals this testing should be completed and outlining of any barriers to uptake.

III. In order to mitigate build-up of available nutrients and potential leaching as a result of annual organic matter additions gardeners could be advised to alternate inputs e.g.
    - A high-carbon input alternated biannually with nitrogen rich input to reduce potential for nitrogen losses following continued input of excess nitrogen from organic matter. This would be facilitated by recommendation I. & II.
    - Organic matter input alternating with a year of no organic matter input would reduce the build-up of nutrients and allow a "lag" period in which microorganisms can adapt to the input applied prior to further input which may induce a "priming" effect or alter decomposition processes continuing on from the previous input.

IV. Gardeners using high carbon inputs as soil conditioners whilst simultaneously wanting to support plant growth through a supply of nutrients from organic matter, could be advised to apply biannually instead of annually to account for decreased decomposition rates caused by the build-up of high C material.

6.2 Discussion

The discussion in this chapter brings together the findings of the project and explores the limitations of the study and the opportunities for future research and industry developments. The contribution of this research to wider knowledge is outlined and the relevance of the work in a wider context of garden management are revisited.

The aim of the research was to evaluate the within season and legacy effects of different types of organic matter addition on UK garden soils. In particular, the effect of distinct types of addition on microbial community carbon and nutrient cycling functions. To do this it was important to understand what makes one type of organic matter distinct from another and which inputs are most commonly applied to UK garden soil.

New knowledge was generated about the long-term effects of organic matter application on soil chemical and physical properties through the analysis and interpretation of field trial data from 2007-2019. Further knowledge on the biological role in the effects seen in long-term data from the field trial was gained through thorough experimental analysis focused on microbiological community structure and respiration function. There is an urgent need to understand biological factors which govern persistence or decomposition of soil organic matter (SOM) in order to better predict how soils will respond to future global change (Schmidt et al., 2011). Studies of soil C and SOM dynamics continue to increase emphasis on the central role of environmental and biological factors in SOM and soil C processes. This study aligns with this emphasis demonstrating significant impacts of microbial community structure on soil respiration as a result of changes to C availability in the form of OM inputs. Furthermore, the significant difference between community structure immediately following input and legacy effects of 10years of OM input is evidence that microbial regulation of decomposition is not a short-term or isolated process and supports wider literature which shows microbial community adaptation to a wide range of inputs (Hoffland et al., 2020; Schneider et al., 2012).

The field trial enabled the assessment of whether long-term efforts to improve soil quality parameters by adding organic matter achieve the intended goals (improvement to soil structure, provision of plant available nutrients/ support of plant growth, increase in SOM%) and/ or have unintended localised and wider environmental impacts. In addition, analyses concluded whether benefits or unintended impacts of OM addition were due to effects of input on changes to soil microbial population structure and function.

Addition of organic matter to soil is widely considered a beneficial practise for the purpose of improving soil structure, C storage, water-retention/ permeability, and nutrient availability (de Sosa et al., 2021). This study evaluated a selection of these potential benefits and how different inputs interact with soil biology to deliver them. Evidence of the beneficial effects of organic matter input included increased resilience of microbial community function to inhibition effects in organic matter treated soils in Chapter 5. Chapter 4 provided evidence of the profound effect that type of organic matter input can have on microbial community structure, with changes in structure related to changes in function. These findings highlight the importance for consideration of the biological role in organic matter and carbon cycling when making management decisions regarding type of compost of soil conditioner and frequency of application.

This research also highlighted other non-intended effects of specific types of organic matter input, evidenced by significant build-up of soluble nutrients (N, P, Mg, K) dependent on the type of OM applied in Chapter 3 and lower pH of peat treated soils in Chapter 4. It was possible to confirm that organic matter inputs significantly decrease bulk density, though how low a gardener requires bulk density to be may be a matter of personal preference or the intended purpose of garden area (e.g. vegetable crop or perennial borders). This emphasises that consideration of the aim before repeated application of organic matter is advisable.

CO_2 output from soils was significantly different in different organic matter treatments confirming the original hypothesis that distinct types of organic matter would result in variable CO_2 output. Evidence of SOM build-up over the course of the field trial coupled with significant differences in microbial community structure between treatments in the final years of the trial confirmed the hypothesis that long-term application of distinct types of organic matter would influence microbial community structure. Chapter 4 also provided evidence of significant within season and legacy effects of organic matter input type on the relative abundance of fungal and bacterial populations. Existing literature has established negative implications of peat-use on microbial activity and abundance (Taparia et al., 2021). The findings of this study align with this as evidenced by peat treated soils having significantly lower microbial biomass than all other treatments.

The research presented in this thesis indicates that organic matter additions in UK gardens have profound effects on microbial carbon cycling and associated nutrient availability, and that this is dependent on type of organic matter applied. The effects of organic matter application are dynamic and fluctuate within a growing season and accumulate over time. Short-term benefits may evolve into long-term detrimental impacts on the wider environment and that the influence of soil additions on soil biology is a central component of this shift.

6.2.1 Garden management of organic matter material and applications

Management of organic matter additions is integral to ensuring benefit of soil derived ecosystem services, such as improving SOM%, supporting soil biota, supplying plant available nutrients through decomposition of applied material. Whether impacts of organic matter application are positive for gardeners and the wider environment is reliant on how they support or limit different guilds, domains, or keystone taxa within the biological component of soil (Banerjee et al., 2018).

Composted manure applications repeatedly imported excess K and increased F/B ratios following application in results from all experimental chapters, regardless of soil type. Whilst this may be a good thing for decomposition due to the association of fungal decomposer species with C decomposition processes (Štursová et al., 2020), in ornamental plant systems the higher proportion of fungi may result in increased plant pathogens (Taparia et al., 2021). Theories like this may form barriers to uptake of peat alternatives or adjustments to garden management. Management decisions are nuanced and highly dependent on the goals of the gardener, location, and the nature of heterogenous garden systems. Recent studies show that gardeners are ecologically minded and have an emotional attachment to the land they garden (Lee and Matarrita-Cascante, 2019a). Gardeners have been shown to increase sustainable practise the more “trust” and understanding they have in ecological and environmental processes (Coisnon et al., 2019). Enabling gardeners to make informed decisions by providing relevant and accessible information will be key in ensuring provision of ecosystem services from organic matter applications to garden soil.

6.2.2 Limitations of the study

The original hypothesis was, in part, confirmed as organic matter did significantly influence microbial community functionality, though correlation of this with carbon and nutrient availability does not provide complete evidence that this is the only cause of changes to respiration. Inclusion of plots which were not rotavated or a comparison with other methods of incorporation of organic matter to the field trial would enable evaluation of whether the effects of incorporation method would influence the results presented in this study.

Most field trials have limitations that cannot be avoided. In some respects, this makes field trial data truer to actual practice. Issues with continuity are particularly relevant in long-term trials and examples of issues related to the duration of the field trial include potential variation in the materials or suppliers of materials, differences in data collection or sampling accuracy or consistency between years, adverse weather conditions, confounding results of variables such as plant yield. An experiment was designed to test the impact of water-logging and drought conditions on microbial function under organic matter treatments selected from the RHS trial, unfortunately the 2020 COVID-19 pandemic lockdowns and lab closures resulted in the loss of the experimental data.

Opportunities for modelling the CO_2 output of different soil types treated with a variety of materials would be an interesting development on the findings presented here. An effort was made in Chapter 5 to address this by using a clay-loam rather than a sandy-loam as in the RHS field trial site. Further improvement would be several field-trial sites, such as at RHS Wisley, on different soil types. Biological analyses from the beginning of the trial would also have enabled long-term comparisons of biological community structure. In the research presented all biological measures were compared to the control (no OM input) but the control soil will have been subject to the same rotavation and weather events as the rest of the treatment plots. Comparison is still possible by proxy, but this highlights the need for biological indicators of soil quality to be included and emphasised in analyses of soil C and SOM dynamics.

Inclusion of more measures of plant uptake of nutrients, or sampling of the areas surrounding the trial and/ or replicated plots would provide more information on nutrient cycling and losses from the system. An additional experiment was designed to analyse nutrient pathways and plant uptake of specific nutrients that significantly correlated with plant yield in the RHS trial, but due to extreme weather conditions in 2020 the initial stages of the experiment were lost.

6.2.3 Opportunity for industry

Practical recommendations for industry and for best-practise advice on garden applications of organic matter delivered by organisations such as the RHS were generated and are detailed in section 6.1.5.

Targeted and precise applications of organic matter to soils, as is demonstrated in precision applications of fertiliser in agricultural systems, requires industry (producers of organic matter inputs) to provide more detailed information to gardeners. This presents incentive and opportunity to develop user-friendly technology, methods and products for soil/ compost/ organic matter testing, monitoring and application.

There is increasing pressure on industry to make operations more sustainable. An relevant and current example of this is the UK government proposal to ban peat based compost sales for non-commercial growers (Gov UK, 2022b). This demonstrates industry trends in acknowledging the collective impact of amateur gardeners on environmental systems with reasons for the ban referencing the carbon stocks of peat-lands, transitions to net zero carbon emissions and ecosystem services (Gov UK, 2022b). Transfer of garden specific, scientific information through organisations such as the RHS to the gardening community will enable gardeners to adapt current management and mitigate negative environmental impacts of organic matter management.

6.2.4 Research contribution

This research has presented analytical, critical and theoretical interpretation of the effects of a long-term field trial on garden context specific organic matter addition management. The novel contributions of this research to the subject area include:

Findings of a literature review identifying areas where research is lacking (such as UK gardens, allotments and green spaces) and their role in contributing to or mitigating against effects and drivers of climate change. In addition, the role that research can play in ensuring gardens are managed in a way that ensures their longevity in a changing climate.

The hypothesis and structure of the analytical design in Chapter 3 resulted in identification of significant accumulation of nutrients, relevant to organic matter addition, which had not been identified in previous analyses of the RHS trial.

Generation of a repeatable assessment of in-situ soil biology and associated functions, emphasising the importance of including evaluation of soil biology in future field-trial design.

Creation of a novel method to combine selective inhibition, MSIR and PLFA analyses resulting in contribution to the understanding of domain specific soil microbiome function.

Development of recommendations for garden management which include consideration of the impacts of management on soil biological function.

6.2.5 Future work

Following completion of this research project several areas of interest for future work have become apparent.

6.2.5.1 Long-term field trials and garden settings

Long-term field trials are a valuable tool to improve understanding of how terrestrial systems are affected by a specific management in-situ. Availability of long-term data can facilitate quantification of permanent and stable trends in soil chemical, physical and biological parameters (Francioli et al., 2016). Short-term studies provide valuable information on short-term and dynamic responses of soil systems to change. Long-term data allows for evaluation of the accuracy of predictions from soil C and SOM models (Smith et al., 1997). Long-term studies also inform management decisions in the present which influence soil processes in the future.

Citizen science projects involving gardeners and data collection from databases (such as the 600,000 official RHS members) help to ensure dissemination of research to inform best-guidance recommendations, in addition these studies can improve researchers understanding of how wide-spread practise and trends are (Chalmin-Pui et al., 2021; Coisnon et al., 2019). Data collection from gardens for comparison with data in controlled environments increases understanding of how organic matter cycling is regulated in applied settings. Trends and changes in garden practise are linked to socio-economic and cultural influences on garden decisions, which should be considered in the context of this work but were deemed beyond the remit for study. Coisnon et al. (2019) outline drivers of the uptake of sustainable gardening as institutional or individual. They also evaluate differences between urban and rural gardeners and the influence of neighbours and horticultural organisations (Coisnon et al., 2019). If recommendations are made from research further work is needed to understand barriers to uptake of these recommendations.

6.2.5.2 Exploring large-scale impacts of changes to microbial function

In Chapter 4 basal microbial respiration (measured CO_2) approximately doubled when any of the organic matter additions were added to a sandy loam, this effect was not the same on the clay loam used in Chapter 5. Managing this effect on a national scale, and tailoring recommendations for organic matter management to soil type, could result in significant reductions of CO_2 losses from soil whilst retaining decomposition functions that support nutrient provision for plant growth.

Accurate life cycle analyses accounting for the accumulative impact of organic matter management on a regional, national or global scale would enable the benefits of these microbial mechanisms to be properly quantified and valued. At the time of writing there are no peer-reviewed studies quantifying the percentage of UK landowners (non-agricultural) using organic matter inputs, at what rates, in which areas of their gardens and on which soil types. Many studies into OM inputs focus on agricultural systems. Common in the literature are evaluations of farmyard manure and associated nutrient deposition, supply and loss. An increasing number combine this with macro-micro soil biology (Maeda et al., 2017; Moore et al., 2014; Qin et al., 2020; Sizmur et al., 2017). Aside from a rise in studies into biochar applications to soil very few studies compare more than 2 organic matter types and their impact of soil microbial life. Life cycle analyses informed by accurate quantitative data on land-use, soil type and regime will facilitate efficient and targeted management of soil carbon and organic matter, and result in reductions of contributions to unwanted environmental effects.

6.2.5.3 The soil microbiome – Central mediator of the soil system

Even in extreme conditions microbes retain function through a vast and complex system of adaptation and substitution (Ho et al., 2017; van Bruggen et al., 2019b). Research which positions the soil microbiome as the essential epicentre of the system through which all processes essential to life are filtered re-focuses anthropocentric inquiry into Earth's systems. Classification of a plant or a human as the core of a holobiont system can no longer be considered sufficient in an era where microbial life outnumbers and may out-last human life on Earth (Banerjee et al., 2018; Gilbert et al., 2018). The soil microbiome continues without a plant, but a terrestrial plant could not survive in the absence of soil biology. An exception being if all output of microbial processes were replaced by an artificial system e.g. "soilless culture" or hydroponic systems in which addition of nutrients, suppression of pathogens and control of surrounding environment is completed by design (Zamora-Izquierdo et al., 2019).

Research on terrestrial ecosystems is increasingly emphasising the essentiality of the soil microbiome to processes and cycles. This is reflected in an increase of studies focused on functional genes (Lemanceau et al., 2017) and exploration of mechanisms by which microbial life mediates terrestrial nutrient cycling (Craig et al., 2021; Fitch et al., 2020).

This study has evaluated how resilient microbial function is to organic matter inputs and the effects on microbial domains and diversity. Understanding the effects of OM amendment on microbial function will ensure effective management adaptations that have a beneficial legacy for future gardeners.

6.2.6 Conclusions

This thesis has provided an in-depth analysis of long-term legacy and within season changes in soil chemical and microbiological characteristics of a garden related field trial. From the field trial further experimentation at a finer scale examined organic matter amendment effects on changes to microbiological community structure and function.

Collectively UK gardens are a significant proportion of total UK land (7-12%). Changes across UK garden systems can have either a significant contribution to (or mitigation potential of) climate change impacts and drivers. Examples of industry-wide changes, expected to reduce garden related contributions to carbon emissions, include the banning of peat-based composts in order to prioritise carbon sequestration and peat-bog ecosystems.

Quantifying changes to microbial community structure (changes considered to be in response to organic matter input as compared to a control without OM input) and analysing correlations of the changes to microbial function contributes to understanding of the relationship between humans and microbes as dynamic feedback-loops between them continue. Garden management decisions which factor in microbial functionality, and actively facilitate the favourable outcomes of these processes, create more diverse systems with a reduction in negative impacts on the environment at local and global scales.

Most abiotic factors have or can have negative and positive effects on what humans consider essential services (ecosystem services). Microbes have been found in the most uninhabitable places on Earth and constitute a vast portion of the Earth’s biodiversity (Averill et al., 2022). Microbes, not just in soil, are recognised as essential in a vast range of ecosystems. Microbial cycling of nutrients is a continuous process happening on a vast scale. Constant adaptation of the soil microbiome to anthropogenic influence results in changes to globally significant cycles. Whether these changes are positive or negative depends on robust and relevant research transferable and applicable to society, policy and practice.

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APPENDICES

Appendix A

A.1 RHS Wisley Gardens organic matter field trial additional information

Figure A-1 Aerial photo of RHS organic matter field trial (2018). (Photo credit: RHS).

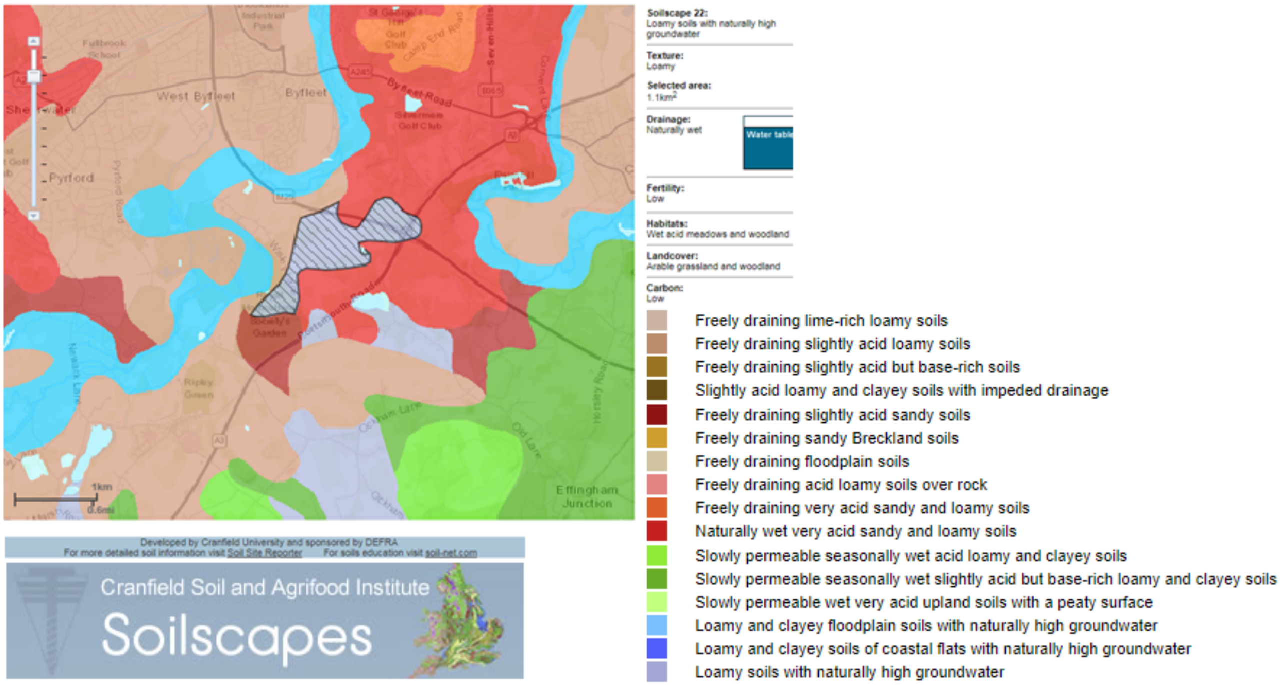
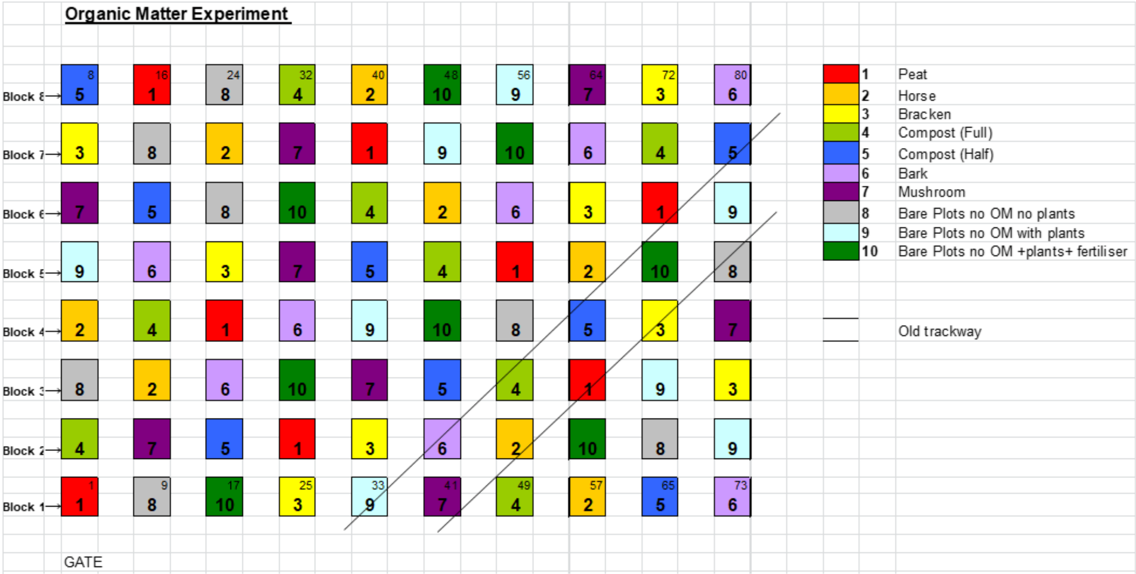


Figure A-2 LandIS Soil-Scape map of RHS Wisley Gardens field trial and surrounding area. (Defra, 2010).



Organic Matter Experiment

Block 8→	8 5	16 1	24 8	32 4	40 2	48 10	56 9	64 7	72 3	80 6
Block 7→	3	8	2	7	1	9	10	6	4	5
Block 6→	7	5	8	10	4	2	6	3	1	9
Block 5→	9	6	3	7	5	4	1	2	10	8
Block 4→	2	4	1	6	9	10	8	5	3	7
Block 3→	8	2	6	10	7	5	4	1	9	3
Block 2→	4	7	5	1	3	6	2	10	8	9
Block 1→	1 1	9 8	17 10	25 3	33 9	41 7	49 4	57 2	65 5	73 6

GATE

1	Peat
2	Horse
3	Bracken
4	Compost (Full)
5	Compost (Half)
6	Bark
7	Mushroom
8	Bare Plots no OM no plants
9	Bare Plots no OM with plants
10	Bare Plots no OM +plants+ fertiliser

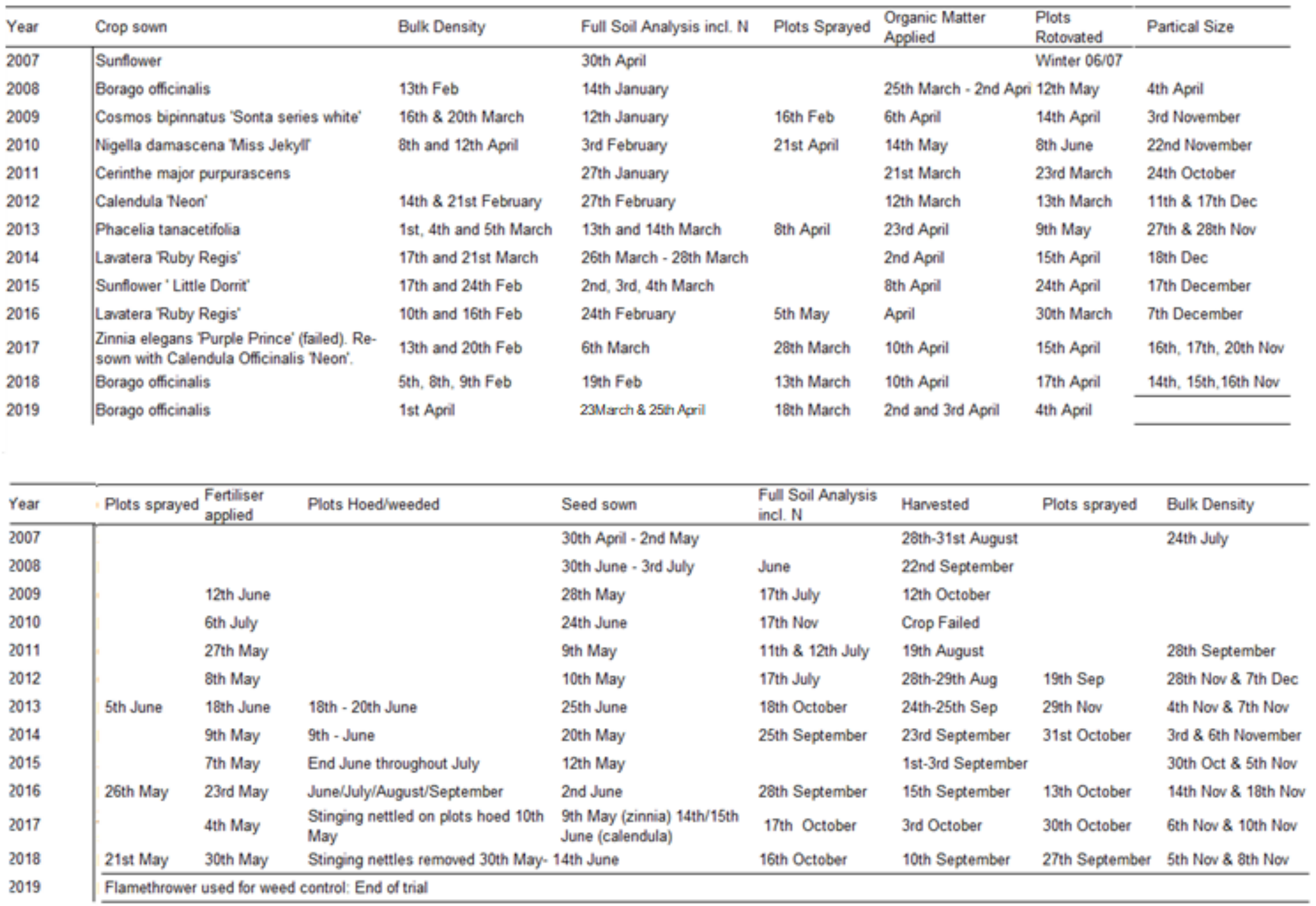
Old trackway

Figure A-3 RHS Wisley OM field trial treatment map

Figure A-4 Photo of RHS Wisley Gardens organic matter field trial 2013. (Photo credit: Rachael Tanner)

Figure A-5 RHS Wisley field trial 2019 prior to the 2019 organic matter application (Photo credit: Rachel Hasler)

Figure A-6 RHS Wisley field trial 2019 following application of organic matter and prior to the incorporation of OM material. (Photo credit: Rachel Hasler)



Year	Crop sown	Bulk Density	Full Soil Analysis incl. N	Plots Sprayed	Organic Matter Applied	Plots Rotovated	Partical Size
2007	Sunflower		30th April			Winter 06/07	
2008	Borago officinalis	13th Feb	14th January		25th March - 2nd Apri	12th May	4th April
2009	Cosmos bipinnatus 'Sonta series white'	16th & 20th March	12th January	16th Feb	6th April	14th April	3rd November
2010	Nigella damascena 'Miss Jekyll'	8th and 12th April	3rd February	21st April	14th May	8th June	22nd November
2011	Cerinthe major purpurascens		27th January		21st March	23rd March	24th October
2012	Calendula 'Neon'	14th & 21st February	27th February		12th March	13th March	11th & 17th Dec
2013	Phacelia tanacetifolia	1st, 4th and 5th March	13th and 14th March	8th April	23rd April	9th May	27th & 28th Nov
2014	Lavatera 'Ruby Regis'	17th and 21st March	26th March - 28th March		2nd April	15th April	18th Dec
2015	Sunflower ' Little Dorrit'	17th and 24th Feb	2nd, 3rd, 4th March		8th April	24th April	17th December
2016	Lavatera 'Ruby Regis'	10th and 16th Feb	24th February	5th May	April	30th March	7th December
2017	Zinnia elegans 'Purple Prince' (failed). Re-sown with Calendula Officinalis 'Neon'.	13th and 20th Feb	6th March	28th March	10th April	15th April	16th, 17th, 20th Nov
2018	Borago officinalis	5th, 8th, 9th Feb	19th Feb	13th March	10th April	17th April	14th, 15th,16th Nov
2019	Borago officinalis	1st April	23March & 25th April	18th March	2nd and 3rd April	4th April	

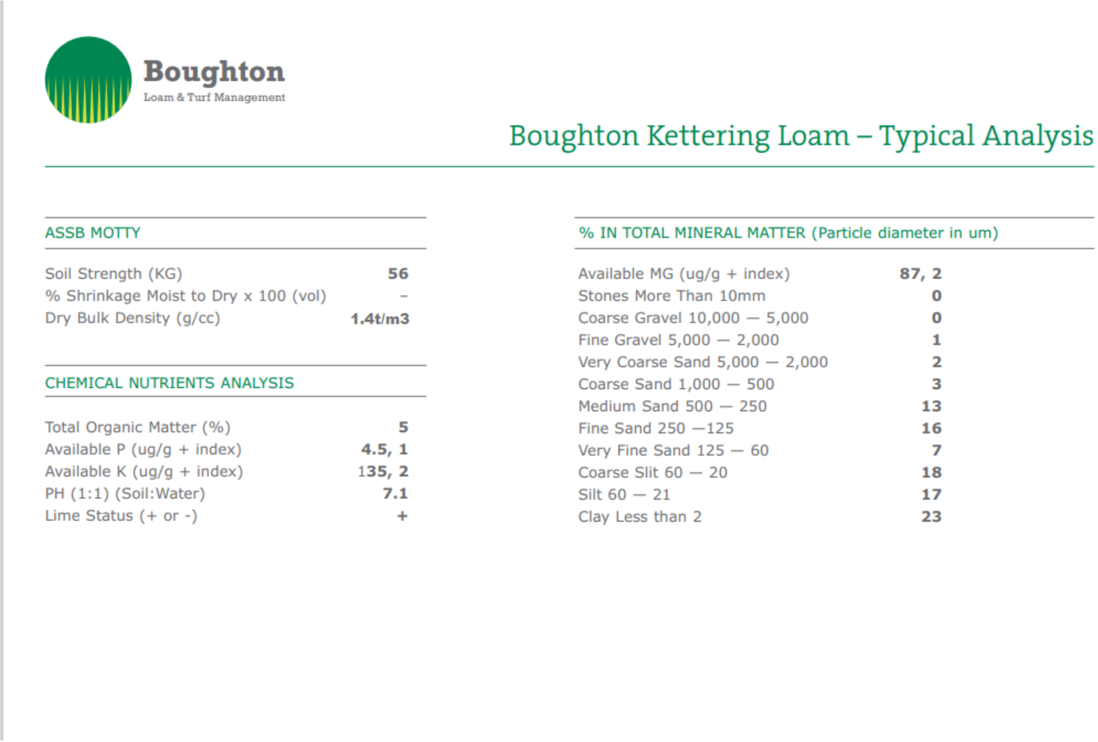
Year	Plots sprayed	Fertiliser applied	Plots Hoed/weeded	Seed sown	Full Soil Analysis incl. N	Harvested	Plots sprayed	Bulk Density
2007				30th April - 2nd May		28th-31st August		24th July
2008				30th June - 3rd July	June	22nd September		
2009		12th June		28th May	17th July	12th October		
2010		6th July		24th June	17th Nov	Crop Failed		
2011		27th May		9th May	11th & 12th July	19th August		28th September
2012		8th May		10th May	17th July	28th-29th Aug	19th Sep	28th Nov & 7th Dec
2013	5th June	18th June	18th - 20th June	25th June	18th October	24th-25th Sep	29th Nov	4th Nov & 7th Nov
2014		9th May	9th - June	20th May	25th September	23rd September	31st October	3rd & 6th November
2015		7th May	End June throughout July	12th May		1st-3rd September		30th Oct & 5th Nov
2016	26th May	23rd May	June/July/August/September	2nd June	28th September	15th September	13th October	14th Nov & 18th Nov
2017		4th May	Stinging nettled on plots hoed 10th May	9th May (zinnia) 14th/15th June (calendula)	17th October	3rd October	30th October	6th Nov & 10th Nov
2018	21st May	30th May	Stinging nettles removed 30th May- 14th June		16th October	10th September	27th September	5th Nov & 8th Nov
2019	Flamethrower used for weed control: End of trial							

Table A-1 Management dates of RHS field trial (some dates missing due to incomplete RHS records)

Appendix B – Additional information: Chapters 4&5

Compound	Name	Retention time
1	14:00	18.01
2	15:0i	20.13
3	15:0ai	20.41
4	15:00	21.4
5	16:0i	23.77
6	16:1w11t	24.21
7	16:1w7c	24.46
8	16:1w5	24.84
9	16:00	25.29
10	Me17:0isomer	26.78
11	Me17:0 isomer2	26.94
12	17:0i	27.18
13	Cyc17:0isomer	27.55
14	ai17:0	27.72
15	17:0br	28.08
16	17:1w8c	28.09
17	17:0c	28.68
18	17:1w8t	28.98
19	17:0w7	29.22
20	17:0(12Me)	29.37
21	UK 29.68	29.62
22	18:2w6,9	32.22
23	18:1w9c	32.46
24	18:1w7	32.7
25	18:1w13	33
26	18:00	33.43
27	19:1w6	33.73
28	18:0(10Me)	35.19
29	19:0c	37.13
30	20:4 (5,8,4,11,14)	38.99
31	20:5w3	39.26
32	UK 42.041	40.99
33	20:00	41.79
34	UK42.41	42.35

Table B-1 List of phospholipid fatty acid (PLFA) biomarkers and retention times



Boughton

Loam & Turf Management

Boughton Kettering Loam – Typical Analysis

ASSB MOTTY	
Soil Strength (KG)	**56**
% Shrinkage Moist to Dry x 100 (vol)	-
Dry Bulk Density (g/cc)	**1.4t/m3**

CHEMICAL NUTRIENTS ANALYSIS	
Total Organic Matter (%)	**5**
Available P (ug/g + index)	**4.5, 1**
Available K (ug/g + index)	**135, 2**
PH (1:1) (Soil:Water)	**7.1**
Lime Status (+ or -)	**+**

% IN TOTAL MINERAL MATTER (Particle diameter in um)	
Available MG (ug/g + index)	**87, 2**
Stones More Than 10mm	**0**
Coarse Gravel 10,000 — 5,000	**0**
Fine Gravel 5,000 — 2,000	**1**
Very Coarse Sand 5,000 — 2,000	**2**
Coarse Sand 1,000 — 500	**3**
Medium Sand 500 — 250	**13**
Fine Sand 250 —125	**16**
Very Fine Sand 125 — 60	**7**
Coarse Slit 60 — 20	**18**
Silt 60 — 21	**17**
Clay Less than 2	**23**

Figure B-1 Kettering Loam specification sheet supplier: Boughton