University of Reading

How can we make better use of crop residues?

PhD Environmental Science

Department of Geography and Environmental Science

Marijke Struijk

December 2019

Declaration: I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Marijke Struijk

Table of contents

Abstract

Crop residues are a widely available on-farm resource that contain calories, carbon and other nutrients. However, decomposition of crop residue soil amendments does not always translate into greater SOM levels, increased nutrient availability, or improved soil structure. Therefore, a better understanding of the decomposition processes involved is needed to improve the management of crop residues in arable and horticultural cropping systems.

In this thesis, an extensive literature review and an investigation of the link between aboveground crop diversity and belowground soil biota suggested two strategies to increase SOM accumulation with crop residue amendments. The potential of these strategies was assessed in two experiments underpinned by ecological theories that have previously been observed in natural systems: (1) a test of the applicability of the home-field advantage (HFA) hypothesis (i.e. litter decomposes faster in soil in which it was grown, *home*, compared to a different soil, *away*) to arable cropping systems; and (2) a trial to exploit litter-mixing effects observed in forest ecosystems, in which crop residues of different chemical qualities were applied as mixtures and as individual residues to a horticultural soil.

Different abundances of soil fauna were observed at the different stages of an arable crop rotation. However, no HFA effect could be detected within this same crop rotation. Soil amendment with mixtures of chemically contrasting crop residues, on the other hand, led to non-additively greater SOM and available N levels within a short time frame (44 days). Crop-residue mixing may therefore be a more suitable strategy to make better use of crop residues in arable and horticultural systems. This strategy may have practical implications, because it would involve the removal, mixing and re-application of crop residues, rather than simply returning them to the soils they were grown in. There is a need for a more mechanistic understanding of HFA effects, which may help explain why no HFA effect was found in this research project. Therefore, I envision a future HFA microcosm experiment in which possible factors that drive HFAs are controlled.

Acknowledgements

Contributions by others and chapter-specific gratitude

Chapter 3: I would like to acknowledge the contributions by two placement students, Emma Bowen and Sion Thomas, who collected most of the soil faunal data with help from Louise Truslove with nematode extractions and from Frank Ashwood with mesofauna extraction and identification; as well as contributions by Erika Degani who supplied relevant data from the Liberation field plots from 2016 (soil pH and total C and N). I would also like to acknowledge Suzanne J. Clark for her help with the design of the statistical analysis. Also see gratitude for Chapter 4 below.

Chapter 4: I would like to thank Hannah Jones, Simon G. Potts, Erika Degani, Samuel G. Leigh, Richard Casebow and Caroline Hadley for their contributions in designing, establishing, maintaining, and studying the Liberation field experiment, and Erika Degani especially as main point of contact while I performed my experiment on the home-field advantage at the Liberation site.

Chapter 5: I would like to acknowledge the contribution by a placement student, Omar El-Huni, who carried out the majority of lab work involved in the assessment of soil aggregate stability.

Gratitude

First of all, I would like to thank my supervisor Tom Sizmur for continued and thorough guidance throughout all phases of my PhD. His calm and structured approach has helped simplify things when they seemed complicated, enabling me to keep moving forward. I could not have asked for a better supervisor. I also thank my co-supervisors Andy Whitmore – for short, prompt and pertinent replies and comments, particularly during the writing-up phase, and thanks to whom I could follow statistics courses at Rothamsted – and Simon Mortimer – for coining the idea of and enabling me to carry out experiments at Sonning farm, and for his complementary feedback on issues otherwise overlooked. I thank my panel chair Joanna Clark for her positive inputs and assessment of my progress.

I thank the internal examiner Chris Collins and the external examiner Davey L. Jones for reading and assessing this thesis by viva voce.

I thank the many people who provided technical assistance: Anne Dudley for general lab assistance, TOC analyses, help with fumigation-extraction, ICP analysis, and a run on the Skalar; Karen Gutteridge for general lab assistance, managing products in stores and locating difficult-to-find items; Geoff Warren for many runs on the Skalar; Pierre-Antoine Ariotti for help with the set-up of the HFA field experiment and general support during the first year of my PhD; Alice Ughi for help with GC-FID during the first batch of PLFA samples; Ilse Kamerling for guidance using the microbalance and for Flash analysis; Fengjuan Xiao for assistance with Flash analysis and GC-FID during the second batch of PLFA samples; Chris Speed for help with laser granulometry; Yiran Zou and Elisa Alonso Lopez for help with milling; Liam Doherty for extra Tüllgren funnels; Mike Charij for repair/adjustments of Tüllgren funnels and wet sieving apparatus.

I thank Sonning farm staff: Richard Casebow for answering many questions, Caroline Hadley for advice on growing wheat indoors, and both of them for crop residues and maintenance of the Liberation field experiment. I thank the employees at G's: Emma Garfield for setting up contact, Simon Gardner and Patrick Phoenix as points of contact regarding field site set-up and issues, Sergio Moreno Rojas for assistance with the GPS device, Elzabieta Witkowska, and special thanks to Giorgi Kamburov and field workers for their cheerful help with residue weighing and application.

I am appreciative of the funding from the University of Reading Faculty of Science and the Waitrose Agronomy group.

I was fortunate with the cohort of other PhD students around me and thank my colleagues for their friendship. Specific thanks to Harriet Robson, who got me started in R; Erika Degani, with whom I shared the lab work involved in PLFA analysis; Xin Shu, who watered my potentially mineralisable N samples while I was away, helped out during a day of field sampling, and shared her method of PLFA data analysis; Adetunji Adekanmbi, who helped out during a day of field sampling; and Alfonso Rodriguez Vila, with whom I shared the work load for a residue digestion.

I am grateful for meeting A and thankful for his general support. Specific thanks for help with R plots.

Last but not least, I am grateful for my upbringing and thank my family for their continued support and guidance throughout my education.

Chapter 1 – General introduction

The majority of biomass harvested in arable cropping systems consists of crop residues: plant material derived from the main crop that remains in the field after harvest or is left over after post-harvest processing, and which includes straw, stalks, leaves, tuber shoots, etc. (Smil, 1999). There are other plant residues, such as weeds and cover crops, but these are beyond the scope of this thesis.

Croplands have added an estimated 98.4 Pg C to the atmosphere between 1850 and 2015 (Houghton and Nassikas, 2017). In the light of future food security and climate change, increasing soil organic matter (SOM) content has been identified as a major factor in improving both the ability of soils to sustain crops and provide ecosystem services (Kibblewhite *et al.*, 2008). Decomposition of SOM feeds the soil food web, the members of which, in turn, are involved in soil aggregation, nutrient cycling, and improving the conditions for primary production.

Worldwide, an estimated 3400 Tg (Lal, 1997) to 3750 Tg (Smil, 1999) of crop residues are produced annually. Wheat residues are probably the most abundant crop residue produced globally, constituting roughly a quarter of the world's crop residues, with an estimated annual production of 847 Tg yr^{-1} (Lal, 1997) to 955 Tg yr^{-1} (Smil, 1999). Wheat residues, consisting mostly of straw, have a high energy and carbon content compared to other soil amendments (Sizmur *et al*., 2017) and therefore have the potential to increase SOM levels and feed organisms in the soil food web, provided the environmental conditions and decomposition processes involved are conductive to those ends. If just 1% of the world's crop residues (3750 Tg yr^{-1} in the mid 1990s; Smil, 1999) could be turned into SOC on arable land (1417 Mha in 2014; FAO, 2014), that would correspond to a 2.6 g C m^{-2} yr^{-1} short-term increase in SOC in arable soils, leading to an estimated 0.3 g C m^{-2} yr^{-1} long-term increase, assuming about 12.5% of the SOC remains stable after 10 years, as was determined for ryegrass by Jenkinson *et al*. (1977). In comparison, no-till agriculture has an estimated potential of 0.03 g C m^{-2} yr^{-1} increase in SOC (Powlson *et al*., 2014).

However, decomposition of soil amendments such as crop residues is not always beneficial in terms of increasing SOM and improving soil conditions for crop growth. Long-term straw applications can lead to a lock-up of N (Recous *et al*., 1995; Silgram and Chambers, 2002) without significant reductions in nitrate leaching or increases in crop yield over the long term (Silgram and Chambers, 2002). Long-term field incorporation of wheat straw at a rate equivalent to standard farm practice (~5 t ha^{-1} yr^{-1}; equivalent to the yield of straw from previous year) at Rothamsted Experimental Farm had no significant impact on earthworm populations compared

to control plots without any straw amendments (Sizmur *et al*., 2017), and minimal impact on SOC, soil N levels (Powlson *et al*., 2011) and yield (Sizmur, T., pers. comm.).

Attempts have been made previously to manipulate crop residue quality to increase contributions to SOM and increase palatability to members of the soil food web. These include (1) physical manipulations, by grinding residues, which significantly increases decomposition of low-quality litters but not high quality litters (Bremer *et al*., 1991) and can make calories contained in straw accessible to earthworms (Sizmur *et al*., 2017); (2) chemical manipulations to reduce 'recalcitrance' of the residues, such as by drying at high temperatures (Mafongoya *et al*., 1997) or by adding inorganic N to soil in order to grow residues with a lower C:N ratio (Handayanto *et al*., 1995); (3) genetic modifications (Bavage *et al*., 1997), and (4) timing of pruning in the case of trees (Vanlauwe *et al*., 1997). However, as will be further discussed in the literature review in the next chapter, these methods are based on an outdated paradigm of decomposition and SOM dynamics in which the contribution of the soil biological community in the creation and stabilisation of SOM is not sufficiently recognised. Therefore, a better understanding of the decomposition processes in arable cropping systems and the soil food web dynamics involved is necessary to be able to devise informed methods to better use crop residues as soil amendments.

The following chapters are an attempt to contribute to addressing this knowledge gap, guided by the following aims:

1. To obtain a better understanding of the relationship between aboveground crop diversity and belowground soil faunal diversity and how these factors interact to affect crop residue decomposition.
2. To determine what changes in the way that crop residues are applied to arable and horticultural soils can be made to increase the abundance and diversity of members of the soil food web, increase SOM, and eventually increase crop yields.
3. To devise a strategy to gain more benefits from crop-residue amendments in arable and horticultural soils.

Following an extensive literature review, including a discussion of some recent paradigm shifts in our understanding of soil food webs and SOM formation (Chapter 2), which demonstrate the pertinence of this research, the three experimental chapters in this thesis (Chapters 3-5) address my aims according to the following objectives:

1. To determine the link between aboveground and belowground biodiversity in arable cropping systems.
2. To determine if the home-field advantage hypothesis applies to the decomposition of crop residues in arable cropping systems.
3. To test if mixing wheat straw with other crop residues increases the benefits that can be obtained from the wheat straw.

Some of the research presented in this thesis was performed at a research farm, and some at a commercial farm. The first two objectives were addressed in experiments that were carried out at the Crop Research Unit of the University of Reading, in Sonning, UK, within a relatively recently (2013) established field trial comparing three crop rotations of varying degrees of crop diversity (Simple, Moderate and Diverse). The experiment that addresses the first objective entailed a survey of the soil biota (microbes, micro-, meso-, and mesofauna) in the soils of each of the crop rotations, and lays the foundations of the experiment addressing the second objective, which was performed in only one of the crop rotations in the field trial and compared the decomposition of two crop residues in different stages of the crop rotation. The third objective was addressed in an experiment performed at G's, a large producer of salads and vegetables based in Cambridgeshire, UK. The farm has encountered a number of challenges, including loss of organic matter and high crop N requirements (Gardner, S., pers. comm.). The experiment addresses some of these challenges by developing a more informed soil amendment strategy by combining a compost produced on-site from post-harvest processing with other materials, including wheat straw. In general, the overarching hypotheses of these three experiments were:

1. The abundance of belowground biota increases along with the diversity of crops grown aboveground.
2. Crop residues decompose at a faster rate when they are buried in a soil underneath the same crop that they originate from than under a different crop, in accordance with the home-field advantage hypothesis.
3. Soil amendments with crop-residue mixtures deliver non-additive effects on soil properties that are beneficial for crop production.

These hypotheses will be evaluated at the end of this thesis in the general discussion (Chapter 6). Each experimental chapter contains more detailed hypotheses according to the specific soil and crop residue analyses carried out. Apart from the three appendices linked to each of the experimental chapters, the reader is also directed to Appendix D that details the planning of an

experiment designed to determine the mechanisms of the home-field advantage hypothesis, which could not be completed during my PhD due to time and funding constraints.

Chapter 2 – Literature review

2.1. Introduction

In this literature review decomposition processes of organic substrates in soil, and existing knowledge on how these relate to the soil food web, are discussed. First the members of the food web and some of the main decomposition factors will be introduced, followed by an introduction to some of the current debates in soil food-web research. Subsequently, mechanisms of interaction between litter and the soil food web will be explored from the perspective of considering crop residues as a food source to sustain a 'healthy' soil food web. This literature review concludes with suggestions on how we could make better use of crop residues, which feeds into the experiments performed for this thesis.

2.2. Members of the soil food web

The soil biota classification employed here distinguishes between microbes – bacteria and fungi – and soil fauna by size (Figure 2.1). A size-type classification based on body width is adhered to, since this determines whether organisms can fit through certain pore spaces in the soil and indicates habitat restrictions and/or sites of protection from predation by larger organisms.

Microbes
Bacteria – prokaryotic
Genera in soils:
Bacillus
Pseudomonas
Arthrobacter
Clostridium
Nitrosomonas
Micrococcus
Rhizobium
Azotobacter

Fungi – eukaryotic
Orders in soils:
Mastigmycotina
Zygomycotina
Ascomycotina
Basidiomycotina
Deuteromycotina(imperfect fungi)

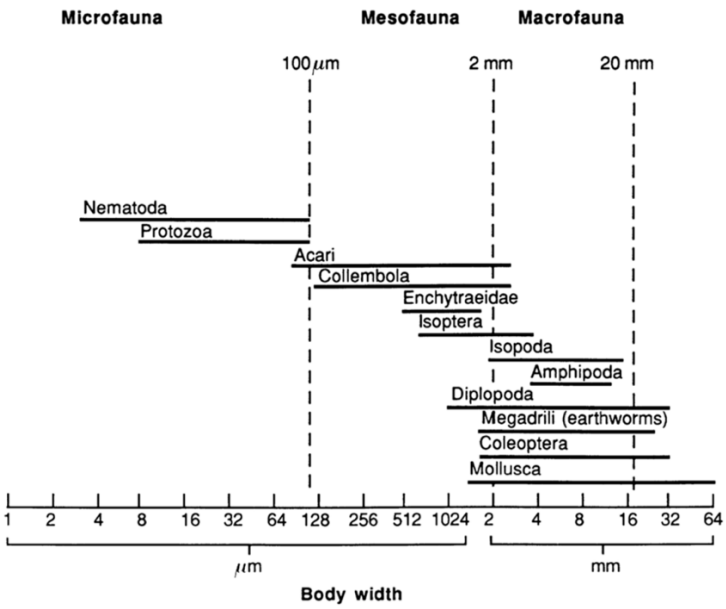


Figure 2.1. A size-type classification of soil fauna (modified from Verhoef and Brussaard, 1990, after Swift *et al*., 1979)

Viruses (about 0.1 µm long), mammals, reptiles and amphibians tend to be disregarded in discussions about soil biology, although they certainly have a significant impact on the soil food web (van Dam and Heil, 2011). The soil microbes include both prokaryotes from the kingdom Monera (bacteria) and eukaryotes from the kingdom Fungi. Soil fauna are eukaryotic and include members of the taxonomic kingdoms Protista (included in microfauna), and Animalia (micro-, meso-, and macrofauna).

2.2.1. Microbes

The group *microbes* includes soil bacteria, actinomycetes and fungi. This group is sometimes referred to as *microbiota* or *microflora*. The use of the term *microflora* is a remnant from the time when bacteria and fungi were classified in the Plant kingdom, but it is technically incorrect because bacteria are not flora.

Most bacteria have a diameter of 0.2 - 2 µm and a length of usually 1-10 µm (Adl, 2003; Hoorman, 2011). They reproduce quickly by binary fission, although a few species employ budding (Killham, 1994). Flagella enable some species to move around, while those without these whips rely on Brownian movement or external factors to be transported and are more likely to live in aggregated colonies (Adl, 2003). Because bacteria are limited in their survival by, sometimes meagre, carbon and nutrient availability in soils, they have developed a number of adaptations: they are able to slow down their metabolic activity to survive in oligotrophic conditions (starvation) and they can surround themselves with a mucous layer that provides protection from desiccation or pH fluctuations, and which plays a role in attachment to soil particles and keeping the colony together (Adl, 2003; Killham, 1994). This mucous layer is often what distinguishes a bacterial species residing in the soil from the same species cultured in a laboratory (Killham, 1994).

Bacteria are very abundant in soils (up to 10^{10} g^{-1} dry soil) and have been found to be active up to 200 - 400 m deep at an abundance of 10^4 - 10^6 g^{-1} soil (Lengeler *et al*., 1999, cited in Adl, 2003), probably brought there by water flow (Adl, 2003). The survival of non-spore-forming bacteria depends on the availability of water, so they tend to reside in smaller pores than spore-forming bacteria which produce dormant structures resistant to adverse environmental conditions (spores) (Killham, 1994). Bacterial communities are generally sensitive to pH changes (Brookes *et al*., 2010).

Many soil bacteria are sorbed to mineral surfaces. Both bacteria and soil clay particles tend to exhibit a net negative charge and are connected to each other via cations (Killham, 1994). Decomposition of organic matter by bacteria and other microbes results in the redistribution of component elements and this process is therefore involved in elemental cycling. This includes nutrient cycling, chemical transformation of metals and minerals, as well as mineral formation and mineral deterioration, which are both involved in soil formation (Gadd, 2010). Examples include iron-oxidizing and -reducing bacteria, manganese-oxidizing and -reducing bacteria, sulphate-reducing bacteria, sulphur-oxidizing and -reducing bacteria, and many other bacterial species as well as prokaryotes and eukaryotes involved in the transformation of minerals (silicates, carbonates, phosphates) (Gadd, 2010).

Via traditional plate count techniques the *Arthrobacter, Pseudomonas* and *Bacillus* genera were found to dominate in most soils (Killham, 1994). *Arthrobacter* can be found in SOM, *Pseudomonas* can metabolise a large range of food sources, including lignin and other aromatics, and *Bacillus* can metabolise hemicellulose (Adl, 2003; Jones *et al*., 2012). The presence or predominance of a certain genus can be used as a biological indicator, e.g. the presence of *Clostridium* indicates anaerobic conditions (Killham, 1994).

The group *Actinobacteria* is exclusive to soils (Adl, 2003). These species produce volatiles (geosmins) that produce the typical "earthy" smell of soils (Killham, 1994). Taxonomically speaking they are bacteria, although they resemble fungi in both their morphology and growth (Adl, 2003; Killham, 1994). They are saprophytes that are able to metabolise many types of substrate – including chitin, cellulose, and other recalcitrant compounds – even when conditions are too stressful for other bacteria and fungi (high pH, water stress, high temperature) (Killham, 1994).

Fungi exhibit a mycelial morphology and grow as branching hyphae that are typically 2 - 10 µm in diameter, and sometimes larger, so they have to reside in larger soil pore spaces than bacteria (Killham, 1994). Some fungal groups can also grow as a thallus or yeast (Adl, 2003). Via the excretion of a wide range of enzymes (including proteases, amylases, cellulose, xylanases, pectin-degrading enzymes, and ligninases), mycelial fungi are able to decompose almost any component of litter (Adl, 2003). Lignin-decomposing fungi are colloquially referred to as 'white rot fungi', and cellulose-decomposers as 'brown rot fungi' (Killham, 1994). Fungi can perform nitrification and sulphur oxidation processes, but unlike bacteria, all fungi are aerobic and they are tolerant to a wider pH range than bacteria, so in acidic forest soils they may be more dominant than bacteria (Killham, 1994; Rousk *et al*., 2009).

Bacteria and fungi distinct from those in the rest of the soil reside in the rhizosphere, including rhizobia and mycorrhizae. Here beneficial microbe-microbe and microbe-plant interactions play a significant role. A discussion of microbes in the rhizosphere is beyond the scope of this text, and the reader is directed to Barea *et al*. (2013).

2.2.2. Microfauna: protists and nematodes

Soil protists are abundant and diverse: thirty to forty thousand protozoa individuals can be found in one gram of arable soil (Killham, 1994) and 365 protozoa species were found in a grassland soil in Scotland (Esteban *et al*., 2006). Originally seen as aquatic species only, protists encompass several groups of mostly unicellular eukaryotes, and are often described as "other eukaryotes" that are not plants, animals or fungi. Historically, many names referring to members of this group have been used in the literature (e.g. animalcules, infusoria) due to changes in taxonomic classification. Until recently, protists were described as a group comprising protozoa, diatoms and other algae, water moulds (oomycetes) and slime moulds (Wilkinson *et al*., 2012). A revised classification of eukaryotes was published in 2012, including the following eukaryotic supergroups: Amoebazoa, Opisthokonta, Excavata, SAR, and Archaeplastida, where the SAR supergroup encompasses Stramenopiles, Alveolates and Rhizaria (Adl *et al*., 2012) (Table 2.1).

Table 2.1. Classification of eukaryotes at the highest ranks, adapted and simplified from Adl *et al*. (2012), with examples mostly from Geisen *et al*. (2018).

Supergroup		**Notable examples**
Amoebozoa		Slime moulds
Opisthokonta		Animals
		Fungi
Excavata		Flagellates
SAR	Stramenopiles	Diatoms
		Oomycetes
	Alveolates	Ciliates
		Gregarines
	Rhizaria	Cercozoa (flagellates and amoeboids)
Archaeplastida		Plants

For convenience and because much of the literature contains the terms as described by Wilkinson *et al*. (2012) mentioned above (even after publication of a revised classification in 2005 by Adl et al., containing the categories Amoebozoa, Opisthokonta, Rhizaria, Archaeplastida, Chromalveolata and Excavata), the rest of this section will introduce some of the main soil protists by their traditional classification.

Protozoa are the most well-known protists, and are represented by flagellates, amoeba and ciliates. Flagellates (4 - 15 µm long; Clarholm *et al*., 2007) exhibit one to four flagella, and ciliates, the largest soil protozoa (20 - 600 µm long; Clarholm *et al*., 2007) are covered in cilia (small hair-like projections), both of which enable them to 'swim' around in water films on soil particles and in pore spaces they can access (Killham, 1994; Wilkinson *et al*., 2012). Similar to bacteria and their mucous membranes, protozoa have also adapted to life in the soil. They are smaller, more flattened and have fewer external projections enabling smoother movement in the soil (Killham, 1994).

Slime moulds have sometimes been classified as fungi, but are now recognized as soil protists preying on bacteria and soil organic matter (SOM) by engulfing the food source (Killham, 1994; Wilkinson *et al*., 2012). This method of catching food, by extending finger-like projections of the protoplasm, is what characterizes amoeboid behaviour. Slime moulds comprise myxomycetes (true slime moulds), and acrasiomycetes (cellular slime moulds), both of which are amoeboid (Killham, 1994).

Diatoms are unicellular algae with a siliceous outer layer. They are not often considered in soil ecology texts, perhaps because finding them between soil particles under a microscope is very difficult (Wilkinson *et al*., 2012). Nevertheless, in a study by Heger *et al*. (2012), they were coined as a possible bioindicator for agricultural soils, because soils subject to more intensive agriculture exhibited fewer diatoms. Diatoms live at the soil surface and are sensitive to dry conditions, so they may be disturbed by ploughing activities in intensively managed soils (Heger *et al*., 2012).

The second group of soil microfauna consists of nematodes, which have a simple morphology with three cell layers and a cuticle that is moulted four times during its life cycle (Adl, 2003). They are often classified functionally by trophic group according to their mouth parts, which indicates what they are able to ingest (Yeates *et al*., 1993) (Figure 2.2). The trophic positions of predator- (Anatonchus and Mononchus), bacterial- (Plectus and Rhabditis), omnivorous- (Aporcelaimidae and Qudsianematidae) and plant feeder (Rotylenchus) nematodes have been assessed by ^{13}C and ^{15}N stable isotope analysis, and are mostly in agreement with prior morphology-based classification (Melody *et al*., 2016).

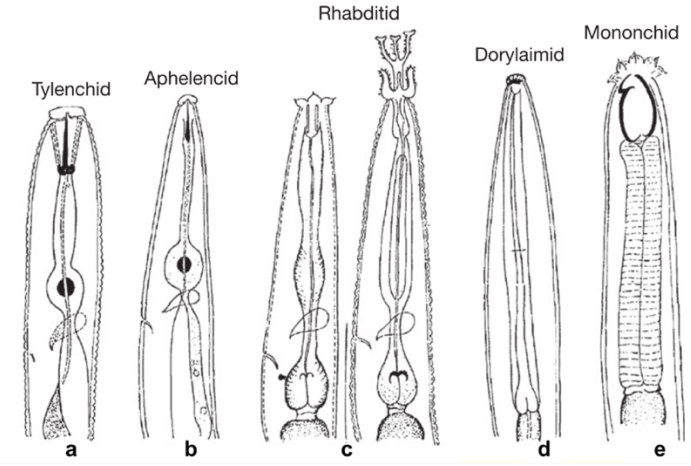


Figure 2.2. Nematode classification based on mouthparts: (a) bacterial feeder (b) predator (c) plant feeder (d) omnivore (e) predator (image adapted from Sharma and Sharma, 1995, cited in Sanderman and Amundson, 2014).

2.2.3. Mesofauna: Collembola, mites, (enchytraeids and termites)

Mesofauna have a body width of 100 µm - 2 mm (though some texts refer to 200 µm – 2 mm; e.g. Menta, 2012) and are represented mainly by the microarthropods Collembola (springtails) and mites (order Acari). Other members of this group include rotifers, tardigrades, small araneidae, pseudoscorpions, opiliones, enchytraeids, insect larvae, small isopods and myriapods (Menta, 2012). The feeding preferences of microarthropods are mainly saprotrophic and microbivorous (mostly fungivorous for Collembola) (Hunt *et al*., 1987; Moore *et al*., 1988; Potapov *et al*., 2016; Walter and Ikonen, 1989), though some also predate on other soil fauna (Petersen and Luxton, 1982). Many species of mites, and to a lesser extent Collembola, are slow to recover after drought stress, so their populations may be disproportionately affected by the impacts of environmental change (Lindberg and Bengtsson, 2005).

Recent publications highlight the importance of mesofauna in nutrient cycling, litter decomposition and SOM formation (Adejuyigbe *et al*., 2006; Carrillo *et al*., 2011; Soong and Nielsen, 2016). Microarthropods are described as litter transformers: fragmentation and comminution, as well as excretion of faecal pellets, increases access and enhances microbial activity (Briones, 2014; Coleman, 2011; Hanlon and Anderson, 1979; Sanderman and Amundson, 2014). The presence of microarthropods and earthworms with decomposing litter has also been shown to increase nutrient mineralization rates, as their grazing activity mineralizes nutrients that are locked up in microbial biomass (Adejuyigbe *et al*., 2006; Ineson *et al*., 1982; Sanderman and Amundson, 2014).

2.2.4. Macrofauna

Macrofauna are 2 - 20 mm wide, including most notably, earthworms, as well as gastropods, isopods, myripods, some araneidae, and most insects (Menta, 2012). Earthworm burrowing activity varies by functional group. Anecic earthworms create permanent vertical burrows, transporting organic matter from soil surface to deeper mineral layers, endogeic species create temporary burrows in the top mineral soil layers, and epigeic species live in the litter layer. Earthworms directly impact the soil physical structure, the chemical processes of decomposition, and the soil biology, and are thus referred to as ecosystem engineers (Ojha and Devkota, 2014).

The burrowing activities of earthworms directly affect the soil structure – by decreasing bulk density, increasing aggregation, and improving aeration, depending on the species (Lavelle *et al*., 1988) – and the distribution and mineralization of carbon and nutrients (Bhadauria and Saxena, 2010; Lemtiri *et al*., 2014; Marinissen and de Ruiter, 1993; Ojha and Devkota, 2014). Mineralization of SOM may be enhanced by fragmentation and formation of organo-mineral aggregates by creating new interaction surfaces between organic matter and microbes (the idea that underpins vermicomposting; Domínguez *et al*., 2010). This may lead to enhanced priming initially (i.e. stimulated microbial activity) (Lavelle, 1988; Six *et al*., 1998), though, depending on land use (Pulleman *et al*, 2005) and the earthworm species (Blouin *et al*., 2013) SOM may eventually become protected in casts (Bossuyt *et al*., 2005; Guggenberger *et al*, 1996; Martin, 1991). These newly formed aggregates can be very stable (Bhadauria and Saxena, 2010; Shipitalo and Protz, 1989) for a long time (McInerey and Bolger, 2000), suggesting earthworms may help improve soil C sequestration. However, in a meta-analysis on the impact of earthworms on soil respiration rates, an overall 33% increase in CO_2 emissions was found (Lubbers *et al*., 2013). Furthermore, a long-term study by Lubbers *et al*. (2017), with maize residues added every six months, demonstrated that while both stabilisation and mineralisation of organic matter by earthworms occur simultaneously, mineralisation rates were higher.

Earthworms increase nutrient availability because higher concentrations of plant available N, P, K, and Ca are found in earthworm excretions than in bulk soil (Bhadauria and Ramakrishnan, 1989; Bhadauria and Saxena, 2010). Earthworms may indirectly impact on soil carbon and nutrient cycling due to (1) their physical impact on soil architecture (affecting soil water and oxygen); (2) fragmentation and redistribution of organic matter; (3) alteration of soil pH (Edwards and Lofty, 1977, cited in Butenshoen *et al*., 2009), and (4) grazing on other soil organisms (Marinissen and de Ruiter, 1993). Moreover, habitat formation by earthworms may increase

collembolan abundance (Hamilton and Sillman, 1989), which in turn may increase plant available nutrients (see previous section).

As earthworms feed on manures and other organic matter present in the soil, they effectively graze on bacteria. A type of mutualism between earthworms and microbes has been suggested as the conditions (moisture, pH) in the earthworm gut are thought to enhance the ability of microbes to break down more complex organic matter that has been ingested (Barois and Lavelle, 1986). Aira *et al*. (2015) report that the ingested microbial community from different types of manure (horse, cow, pig) does not match their cast microbiome, thus suggesting that earthworm guts act as a 'biological filter'.

Cultivation of the land by tillage operations and pesticide/fungicide applications have led to the disappearance of larger earthworm species (e.g. *Lumbricus terrestris, Allolobophora longa*), particularly of the anecic group (Paoletti, 1999). As such, earthworms have been adopted as indicator/monitoring species to indicate ecological impacts of pollutants and land use change (Paoletti, 1999; Lemtiri *et al*., 2014). Indeed, experimental evidence demonstrates high sensitivity of earthworms to different agricultural practices (Emmerling, 2001; Wardle, 1995 cited in Ojha and Devkota, 2014). For example, Paoletti *et al*. (1995) found significantly lower earthworm abundance in conventional compared to organic apple orchards, and a later study showed that both biomass and abundance were considerably lower in tilled versus untilled orchards (Paoletti *et al*., 1998).

2.3. Factors that influence the rate of crop residue decomposition

Decomposition of plant litter drives the soil food web and leads to the release of plant-available nutrients and the respiration of CO_2 (Adl, 2003). Factors determining the rate of crop residue decomposition can be classified into chemical (the chemical quality of the litter, soil nutrient availability), biological (the community of decomposer organisms, trophic interactions), and environmental factors.

2.3.1. Primary decomposers

Primary decomposers use organic matter as a food source and break it down into simpler molecules in the process. Together with saprotrophic bacteria and fungi, the organisms deemed to be most involved in soil decomposition include protozoa, protozoa-invertebrate symbionts,

oomycetes (water moulds), nematodes, rotifers, mites, Collembola, earthworm and enchytraeids (Adl, 2003). Saprotrophic organisms excrete extracellular digestive enzymes to enable the assimilation of nutrients: bacteria obtain nutrients from their environment by diffusion, facilitated diffusion or active transport and fungi take up nutrients by osmotrophy (Adl, 2003). At this level, decomposition is essentially a process of depolymerisation and mineralization that drives a complex web of interactions involved in nutrient and carbon cycles both globally and locally in the soil, and hence primary production based on photosynthesis (Burns *et al*., 2013).

Extracellular enzymes enable microbes to break down organic residues into chemical energy (ATP) and nutrients by means of depolymerization of macromolecules so that they can be assimilated into biosynthetic material (also catalysed by enzymes) while part of the organic compounds are mineralised. Extracellular enzymes form the link between microbes and geochemical cycles and soil nutrient dynamics, so enzyme diversity can represent the functional diversity of the soil (Caldwell, 2005). Soil microbes employ different types of extracellular enzymes, including (1) cell-bound extracellular enzymes, which maintain a connection with the parent cell either inside the cell (oxymoronically), within the periplasmic space, or attached to the cell's outer surface; (2) diffusible extracellular enzymes, which move away from the parent cell – these represent the majority and are more resistant to a range of environmental conditions; and (3) immobilised extracellular enzymes, which form an association with clay minerals, humic acids, or particulate organic matter – these can remain active over long periods of time even when microbial populations are low due to stress (Stursova and Sinsabaugh, 2008), despite their activity being somewhat inhibited by being occluded (Burns *et al*., 2013). Immobilised enzymes tend to be the first to react to new inputs of organic substrates and can produce molecules that signal the microbial community (Burns *et al*., 2013).

As microbes mineralise organic substrates, they use the energy, carbon, and nutrients obtained to grow and form new microbial biomass. Hence, a metabolic pathway involving catabolism of organic residues, followed by anabolism of microbial biomass. The efficiency by which organic residues are converted into microbial biomass determines how much C is retained within the soil system as microbial biomass and how much C is respired as CO_2, referred to as carbon use efficiency (CUE), expressed as a percentage or a fraction, and calculated as the proportion of C used for microbial growth (microbial biomass C) relative to the total amount of C taken up. Higher efficiency equates to greater contribution to SOM in living biomass and smaller losses in terms of CO_2. The terms growth yield, growth efficiency, metabolic efficiency, and substrate use efficiency (Cotrufo *et al*., 2013) are also used in the literature, and different methods are employed by different authors to determine CUE (for a discussion see Sinsabaugh *et al*., 2013), which can lead to both over- (> 0.6) and underestimations (< 0.4) (Geyer *et al*., 2019). Individual microbial

species are physiologically limited in the CUEs at which they can operate, defined as their CUE window (Kallenbach *et al*., 2019). Environmental changes can lead to shifts in CUE within a microbe's inherent CUE window, and can eventually lead to changes in the composition of microbial communities, because when the limits of a microbe's CUE window are reached, these microbes can be outcompeted by other species operating at a window that is more adapted to the changed environment (Kallenbach *et al*., 2019).

Classification of microbes as *r*-strategists and *K*-strategists depends on their growth rate (*r*), where *r*-strategists grow quickly (high *r*) and *K*-strategists grow slowly (low *r*) (Verhulst, 1838). Assuming a trade-off between rate and yield (Pfeiffer *et al*., 2001), Geyer *et al*. (2016) argue that the high growth rate of *r*-strategists is linked to high but inefficient use of organic substrates (low overall CUE), and vice versa for *K*-strategists, such that *r*-strategists are likely to outcompete *K*-strategists in resource rich conditions, and vice versa. Although soil microbial dynamics as a result of competition have not been well studied (Kallenbach *et al*., 2019), there is one paper that suggests that the type of species competition in a microbial community drives the impact of microbial diversity on ecological functioning (e.g. CUE), with intransitive competition (i.e. indirect- or rock-paper-scissors competition in which no one strategy wins but all species continually win and lose) leading to greater efficiency (Maynard *et al*., 2017). Because heterogeneous systems tend to advance diversity and lead to indirect competition (Allesina and Levine, 2011), Kallenbach *et al*. (2019) therefore argue that greater soil heterogeneity could increase the CUE of a system.

2.3.2. Trophic interactions

Soil organisms affect and are affected by other members of the soil food web through trophic interactions. For instance, secondary consumers, such as nematodes and protozoa, regulate microbial populations and reduce competition between different microbial species through their grazing activity (Coleman *et al*., 2004; Scheu *et al*., 2005). Nematodes and other soil fauna also mineralise nutrients by predation of microbes, and aid the distribution of bacterial and fungal spores via ingestion and excretion or by transporting them on their body surface (Sharma and Sharma, 1995, cited in Sanderman and Amundson, 2014). Other litter conditioning activities by soil fauna, including litter comminution also stimulate microbial activity and promote decomposition (Addison *et al*., 2003). Many exclusion litterbag studies (using different mesh sizes to control which organisms can access the litter) have shown an increase in litter mass loss in the presence of microarthropods (García-Palacios *et al*., 2013; Gonzalez and Seastedt, 2001; Seastedt, 1984). However, regardless of the presence of microarthropods, decomposition rates

obtained for litter in coarser mesh bags are higher than litter in fine mesh bags (Bradford *et al*., 2002; Siendtop, 1995, cited in Kampichler and Bruckner, 2009). In a meta-analysis by Kampichler and Bruckner (2009) covering 101 litterbag experiments, the authors applied a mesh size effect to all the results, which turned the positive microarthropod influence typically reported in exclusion experiments into a significant negative effect on litter mass loss, casting doubt over the conclusions of many years of litterbag studies.

2.3.3. Litter quality and soil nutrient status

The quality of residues as an accessible food source for organisms can be described as a combination of physical (e.g. particle size, location on or in soil) and chemical (e.g. C:N ratio, lignin and polyphenol content) conditions of a substrate. Some measure of litter quality forms the basis of the structure of well-known models of SOM dynamics like RothC and CENTURY (Paustian *et al*., 1997). High-quality litter is equivalent to a high content of labile substrates deemed to make it more decomposable and low-quality litter is equivalent to a high content of 'recalcitrant' (for lack of a better term; see discussion of SOM in 2.6.1) substrates deemed to make it less decomposable. Other chemical litter quality parameters that are sometimes used, on their own or in combination as a ratio, are the content of total N, lignin, cellulose, hemicellulose and/or polyphenol (Nicolardot *et al*., 2001). Because none of these factors can successfully predict decomposition and nutrient release in a range of residues, a plant residue quality index (PRQI) and later a PRQIM (M for modified) have been proposed (Kumar and Goh, 2003; Ostrowska and Porębska, 2015; Tian *et al*. 1995):

$$PRQI = \frac{1}{\mathrm{a(C:N) + b(lignin) + c(polyphenols)}}\ 100$$

$$PQRIM = \frac{1}{\mathrm{a(C:N) + b(lignin:N) + c(polyphenol:N)}}\ 100$$

where a, b, and c are the coefficients of the relative contributions of each variable.

The C:N ratio is often used as a simple indicator of litter quality. Decomposer organisms require nutrients to function and produce enzymes, and the availability of N is often a limiting factor. Therefore the C:N ratio of both the soil matrix and the residue can be informative of the decomposability of a residue. Bacteria and fungi exhibit a C:N ratio of around 5 and 8, respectively (Mouginot *et al*., 2014). When they decompose residues, they require available N (in

the form of NH_4^+ or NO_3^-) to start decomposing organic substrates, leading to a temporary immobilisation of N as it is locked up in microbial tissue. As microbial activity and CO_2 respiration increase, the residue is decomposed and becomes part of the soil organic matter while mineralised (plant-available) nutrients like N, S and P are released.

When nematodes and protozoa subsequently graze on bacteria and fungi, they also require N and respire CO_2 while consuming. It has been estimated that only about 10% of the N ingested by bacterial feeding nematodes, and 40% of the N ingested by protozoa is actually required for their own structural maintenance, and the rest is excreted, usually as NH_4^+ (Griffiths and Bardgett, 1997, cited in Scheu *et al*., 2005), thus releasing plant-available N into the soil system. This is the traditional understanding of the role of decomposition in N mineralisation.

According to this paradigm, microbial activity is slowed down when N is limiting, e.g. when litter with a high C:N ratio is added or when N levels in the soil matrix are low, leading to slower decomposition (Fog, 1988). However, the experimental evidence for this supposition is inconsistent. A range of publications over the last two decades has demonstrated a higher complexity of C and N dynamics in relation to microbial decomposition of litter. This shift in our understanding is mostly due to observations in long-term studies that exogenous N additions often result in reduced microbial activity and slower overall decomposition rates (Hobbie, 2015). A range of mechanisms have been proposed to explain this, including (1) inhibition of enzymes required for decomposition of lignin and other recalcitrant C compounds (enzyme inhibition hypothesis) (Fenn *et al*., 1981; Fog, 1988; Gallo *et al.*, 2004; Keyser *et al*., 1978), (2) suppression of OH-radical formation, which can break apart lignin molecules (Forney *et al*., 1982; Kelley and Reddy, 1982); (3) necessity for microbial N "mining" from labile substrates is suppressed, reducing the overall decomposition (N-mining hypothesis) (Craine *et al*., 2007), or similarly, (4) a change in microbial community composition because species adapted to higher N demands and decomposing labile substrates outcompete those species adapted to decomposition of recalcitrant C under N-limiting conditions (copiotrophic hypothesis) (Fontaine *et al*., 2003; Ramirez *et al.*, 2010; Ramirez *et al*., 2012), and most recently suggested is (5) the "Carbon, Acidity, and Mineral Protection (CAMP) hypothesis," which postulates an interaction between these three factors: (a) an increase in microbial biomass and decomposition in response to N addition; (b) this causes the pool of particulate organic matter to decrease while sorption of mineral associated organic matter (e.g. as microbial necromass from a microbial population that now has high turnover rates) onto mineral particles increases; and (c) meanwhile a decrease in soil pH due to N additions inhibit microbes (Averill and Waring, 2017).

Quantifying quality is a tricky activity. Following the discussion of the C:N ratio and the unpredictable effect of N addition on decomposition, litter quality seems to be not only a matter of elemental composition, but also of the nature of chemical compounds within litter and the ease with which their constituent bonds can be broken down by decomposer organisms, which depends on the enzymes they are able to produce. Decomposition of crop residues does not always translate into increased nutrient availability or higher SOM levels, and litter quality parameters do not adequately predict decomposability. Instead, "litter quality is in the eye of the beholder," as stated by Strickland *et al*. (2009), who found that decomposer communities in different habitats underneath different plants "perceived quality differently." More specifically, litter quality is determined by previous litter inputs, such that grass litter was of high quality to decomposer communities in grassland as well as forest soils, while leaf litter was of high quality to decomposer communities of forest soils only (Strickland *et al*., 2009). N.B. This is a phenomenon that can be described as a home-field advantage effect, which will reappear in this literature review and in the experiments presented in this thesis.

2.3.4. Abiotic factors

Abiotic decomposition reactions (i.e. without enzymes) include hydrolysis, oxidation, reduction, and isomerization (McBride *et al*., 1994). An example of abiotic reactions includes, photochemical degradation occurring at the soil surface, which breaks down bonds in organic compounds that absorb wavelengths of 285 nm and higher (UV-B and UV-A) (McBride *et al*., 1994). Abiotic environmental conditions play vital roles in microbial and enzyme activity, and therefore faster decomposition and mineralization rates, including soil pH, availability of sufficient soil moisture, adequate levels of aeration (amount of soil pore space filled with air), temperature, and the availability of key nutrients (e.g. N, P, K, S, Mg, Ca) (Paul, 2015).

Although abiotic factors are deemed to be the main drivers of SOM dynamics (Blagodatskaya and Kazyakov, 2008), they are difficult to assess separately from biological processes. While sterilisation of soil removes microbial activity and reduces enzyme activity, it also chemically changes soils and oftentimes much of the enzyme activity can persist, particularly in soils exhibiting clay minerals with a high specific surface area and/or high cation exchange capacity, e.g. smectites (Carter *et al*., 2007; McBride *et al*., 1994). The complexity of discerning between chemical, biological and abiotic factors is also demonstrated by the uncertainty of the effect of climate change on soil microbes and soil carbon emissions (e.g. Allison *et al*., 2010; Singh *et al*., 2010)

2.3.5. Calculating decomposition rate

The decomposition rate of crop residues can be expressed as a function of temperature as per the Arrhenius equation:

$$k = A\, e^{-Ea/(RT)}$$

where k is the reaction rate constant (decomposition rate in this case), A is the frequency factor, E_a is the activation energy (J mol^{-1}), and R is the gas constant (8.314 J K^{-1} mol^{-1}), and T is the temperature (von Lützow and Kögel-Knaber, 2009). According to this relationship, a crop residue with low litter quality (i.e. recalcitrant) will have a low decomposition rate (k) and a high activation energy (E_a), so it will exhibit a higher temperature sensitivity (and vice versa for a residue with high litter quality) (von Lützow and Kögel-Knaber, 2009). Arrhenius kinetics is less applicable when decomposable matter is scarce, which can be accounted for by Michaelis-Menton kinetics expressed by the following equation:

$$k = \frac{\mathrm{V_{max}}\, X\ [\mathrm{S}]}{Km + [S]}$$

where k is the reaction rate, V_{max} is the maximal rate of enzymatic activity at a given temperature, K_m is the Michaelis-Menton constant (enzyme affinity for substrate), and [S] is the concentration of the substrate (von Lützow and Kögel-Knaber, 2009). The latter parameter can account for soil C stabilization by spatial inaccessibility (aggregation, intercalation in clay minerals, hydrophobicity and encapsulation in organic macromolecules; von Lützow *et al*., 2006) and is indirectly affected by aforementioned environmental conditions (temperature, pH and water, oxygen and nutrient availability) (von Lützow and Kögel-Knaber, 2009).

2.4. Soil food web research

2.4.1. Traditional understanding

Probably the first publication of a soil food web was a paper on the interactions between marine, terrestrial and freshwater organisms in Spitsbergen, Norway (Summerhayes and Elton, 1923) (Table 2.2). A series of food web studies in a range of ecosystems (see references in Table 2.2) highlighted the importance of microbe-fauna interactions in soils for the N, P, and S nutrient

cycles (Gupta and Germida, 1989). The idea that protozoa graze on bacteria was already proposed by Cutler *et al*. (1923), but their significance in nutrient cycling became apparent only later (Coleman *et al*., 2004) (Table 2.2).

Table 2.2. Early progress in soil food web research, roughly chronological (based on Coleman *et al*., 2004). As increasingly more researchers study soil food webs, references mentioned here in the 2000s are non-exhaustive.

Achievement/work	Reference
Description of an arctic food web	Summerhayes and Elton, 1923
Predator-prey relationship between protozoa and bacteria	Cutler *et al*., 1923
Energetics in a forest detrital food web	Bornebusch, 1930
Development of trophic levels	Lindeman, 1942
International Biological Program: Found that most carbon and nutrient flows remain in below-ground food web.	Coleman *et al*., 1976
Food web studies in (semi-)arid grasslands and deserts	Coleman *et al*., 1977; Coleman *et al*., 1983; Parker *et al*., 1984; Whitford *et al*., 1983; Hunt *et al*., 1987; Moore *et al*., 1988
Proposal of fungal and bacterial pathways	Hunt *et al.*, 1987
Food web studies in Sweden	Persson, 1980; Bååth *et al*., 1981; Andrén *et al*., 1990
Food web studies in the UK	Anderson *et al*., 1985
Food web studies in the Netherlands	Brussaard *et al*., 1990; de Ruiter *et al*., 1993; Moore and de Ruiter, 2000
Recognition of protozoa as microbivores	Clarholm, 1985; Kuikman *et al*., 1990
Fungal:bacterial ratio as indicator of ecosystem functioning	Bardgett and McAlister, 1999
Life strategies and feeding preferences of soil microorganisms and fauna more diverse than previously thought.	Fierer *et al*., 2007; Bastian *et al*., 2009; Kramer *et al*., 2016
Greater understanding of trophic interactions from studies applying stable isotope techniques	Chahartaghi *et al*., 2005; Ferlian *et al*., 2015; Maraun *et al*., 2011; Pollierer *et al*., 2009; Potapov *et al*., 2016.

The first complete soil food web, based on calculations of nitrogen cycling in a short-grass prairie, is said to have been presented by Hunt *et al*. (1987), and exhibited a fungal and bacterial pathway. Subsequent studies have built on that understanding: litter inputs are thought to feed into a bacterial- or fungal energy channel, each with their own characteristics (Figure 2.3).

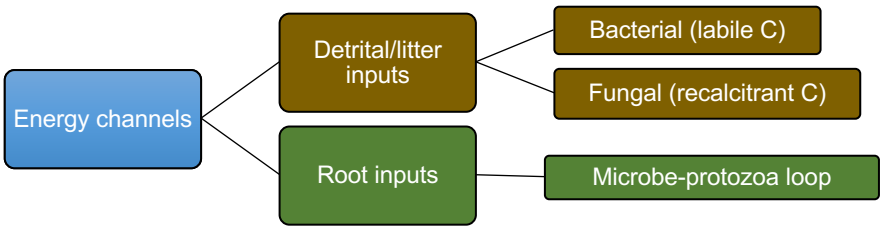


Figure 2.3. Conceptual model of the energy-channel basis to traditional food web models.

The bacterial channel dominates in moist soils and is known for rapid decomposition and nutrient cycling, high N uptake, low C sequestration, and being resource limited (bottom-up control) (Ruess and Ferris, 2004; Scheu *et al*., 2005). The fungal energy channel on the other hand, is known for its slow decomposition and nutrient cycling, ability to handle low quality litter (high C:N, cellulose- and/or lignin-rich), high soil C sequestration, and is limited by predation (top-down control) (Malik *et al.*, 2016; Ruess and Ferris, 2004; Scheu *et al*., 2005). Microarthropod predation on dominant fungal species is associated with higher fungal species diversity due to decreased competition (Kubicek and Druzhinina, 2007). The main bacterial predators are protozoa and nematodes (Scheu *et al*., 2005). The fungal and bacterial channels are considered brown because their energy is derived from detritus (dead OM), and the root channel is considered green because energy is derived from living plants' root litter and root exudates (de Vries and Caruso, 2016).

The root energy channel, feeds into saprotrophic fungi and their consumers, bacteria, mycorrhizal fungi and root-feeding nematodes and their respective consumers (de Vries and Caruso, 2016). A microbial loop is formed (Clarholm, 1994 cited in Scheu *et al*., 2005), in which protists and nematodes control microbial populations by grazing (Scheu *et al*., 2005). The grazers experience little predation (Scheu and Setälä 2002; Wardle 2002, both cited in Scheu *et al*., 2005) resulting in carbon and nutrients becoming temporarily locked up in their tissues (Bonkowski *et al*., 2000; Ruess and Ferris, 2004).

Following such generalisations has led to an understanding regarding the importance of the fungal:bacterial (F:B) ratio as an indicator of ecosystem functioning (Bardgett and McAlister, 1999). Higher fungal biomass (high F:B ratio), e.g. in natural forests, is related to a slow turnover rate in which less N is leached. Mesofauna are traditionally thought of as primarily grazers of fungi, such that the presence of fungi in soils enhances microarthropod communities and are often considered to harbour more diverse soil biota (Beare *et al*., 1997; Scheu and Setälä, 2002, cited in Ruess and Ferris, 2004). When mesofauna are absent, fungal feeding nematodes may take over

their role (Parker *et al*., 1984). As consumer organisms, nematodes have been used as indicators of changes in bacterial and fungal abundance, and to assess the F:B ratio (Ruess and Ferris, 2004). However, in the presence of other consumer organisms, conclusions based on the abundance of different nematode feeding groups may become unreliable.

In systems with a low fungal biomass (low F:B ratio), e.g. in agricultural soils, rapid decomposition and nutrient turnover of the bacterial channel dominate, which may lead to a nutrient release at a rate exceeding plant uptake, such that leaching becomes more likely. High fungal biomass or fungal-dominated soils have been shown to retain more N than bacterial-dominated soils, which can be attributed to fungal hyphae extending out into the soil (de Vries *et al*., 2011; de Vries and Bardgett, 2012). Moreover, Malik *et al*. (2016) demonstrated that a higher F:B ratio (determined by PLFA analysis, RNA sequencing and protein profiling) is associated with lower litter derived CO_2 emissions (^{13}C labelled litter), so the authors conclude that fungal-dominated soils exhibit a greater potential to store C.

It follows from the traditional understanding of soil food webs that energy channels are driven by litter inputs: the addition of high-C:N/recalcitrant litter will stimulate the fungal energy channel and the addition of low C:N/labile litter will stimulate the bacterial energy channel. However, addition of nitrogen to high C:N litter does not increase decomposability of the litter to bacteria by making N less limiting. In fact, N addition to high C:N litter has been observed to stimulate fungal growth and inhibit bacterial growth (Bardgett *et al*., 1999; Rousk and Bååth, 2007). This may be related to lower root biomass in N-amended soils and related changes in the fungal:bacterial ratio (Bardgett *et al*., 1999). While microbes have long been recognized for their importance in decomposition and nutrient cycling, it is still unclear how their populations and activity are affected by both abiotic and biotic factors, and their interactions (Kramer *et al*., 2016).

2.4.2. Recent advances and emergence of new conceptual frameworks

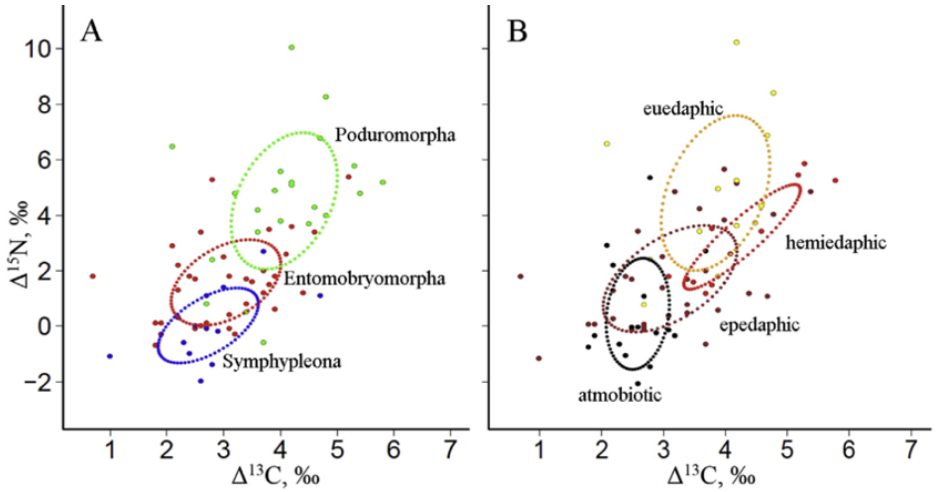
Over the last two decades it has been discovered that the life history traits of primary decomposers are more variable than previously thought. Decomposing litter is colonised initially by copiotrophs and *r*-strategists, succeeded by oligotrophs and *K*-strategists during the later stages of decomposition (Bastian *et al*., 2009). The traditional view of food webs (as previously described) would suggest that bacteria are primarily *r*-strategists and fungi primarily *K*-strategists (Fontaine *et al*., 2003). However, bacterial phyla in soils have been classified as both copiotrophic and oligotrophic, corresponding to r-strategists and *K*-strategists, respectively (Fierer *et al*., 2007). As such, it is unsurprising that the decomposition of recalcitrant substrates is not only

performed by fungi, but also by bacteria (Bastian *et al*., 2009; Kramer *et al*., 2016). Furthermore, studies on the classification of bacterial phyla suggest different phyla predominate under resource limiting conditions. Barnard *et al*. (2013) observed a relative increase in *Actinobacteria* and a relative decrease in *Acidobacteria* under dry conditions. Fierer *et al*. (2007) found a decrease in *Acidobacteria* and an increase in *Bacteroidetes* and *β-Proteobacteria* with increasing rates of C amendments. Soil fauna feeding preferences are also more diverse than originally thought, adding another level of complexity to already intricate soil food web models. For instance, Kramer *et al*. (2016) found that protists are not only bacterivores, but also fungivores and primary saprotrophs, suggesting that the bacterial channel is in fact subject to top-down control as well as being resource limited.

The application of stable isotope techniques is an important technique in unravelling trophic interactions and testing the accuracy of hypotheses on nutrient and energy flows in the original models based on biomass and respiration measurements. While Collembola and mites have often been regarded as mostly fungivorous food generalists (Ferlian *et al*., 2015; Moore *et al*., 1988; Ponge, 2000; Scheu and Simmerling, 2004), trophic specialisations and distinct species diets are increasingly being identified for Collembola and mites (Chahartaghi *et al*., 2005; Ferlian *et al*., 2015; Maraun *et al*., 2011; Pollierer *et al*., 2009).

To confirm the hypothesis that collembolan functional groups and the trophic niches they occupy are linked, Potapov *et al*. (2016) analysed the isotopic ratios of C and N in the primary food sources of Collembola based on the assumptions that (1) the $\delta^{15}N$ ratio is indicative of trophic level; (2) the $\delta^{13}C$ is indicative of basal food sources (Korobushkin *et al*., 2014; Ponsard and Arditi, 2000; Post, 2002); and (3) the concentration of ^{13}C and ^{15}N increases with soil depth (Ponsard and Arditi, 2000). The range of $\delta^{13}C$ and $\delta^{15}N$ values in the data analysed by Potapov *et al*. (2016) differed in a range of 5.1. and 12.1‰ respectively, spanning three trophic levels: primary consumers, secondary consumers (microbial feeders), and tertiary consumers. The main food sources found for Collembola – primary producers, ectomycorrhizal fungi and saprotrophic microorganisms – are, respectively, ^{15}N-depleted, ^{15}N-enriched and ^{13}C-enriched (Layman *et al*., 2007; Tiunov *et al*., 2007). Potapov *et al*. (2016) then looked at what type of classification (by life form or by taxonomic order; Table 2.3) could best predict its trophic niche and isotopic composition (Figure 2.4a) and compiled a classification system based on morphologies of Collembola with three taxonomic orders per life form (Figure 2.4b).

(a)



(b)

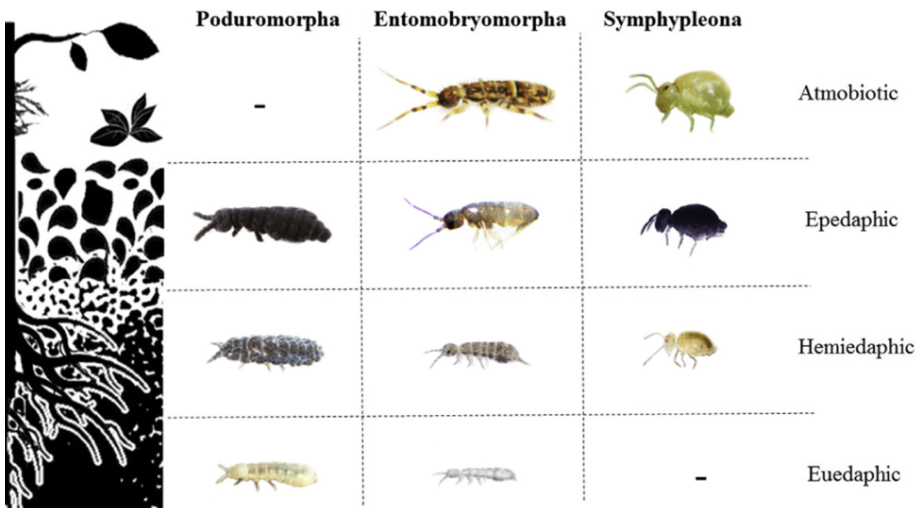


Figure 2.4. (a) Isotopic composition by collembolan order (A) and life form (B). **(b)** Generalized morphologies for collembolan life forms (vertical) across three different taxonomic orders (horizontal), both (a) and (b) taken from Potapov *et al*. (2016).

Table 2.3. Different soil fauna classification approaches

Criterium	Classification		Reference
Habitat	Atmobiotic Epedaphic Hemiedaphic Euedaphic		Potapov *et al*., 2016
Trophic groups	Primary consumers Secondary consumers Third-level consumers		Potapov *et al*., 2016
Taxonomy	For Collembola to order: Poduromorpha Entomobryomorpha Symphypleona Neelipleona	For mites to superorder: Sphaerolichida Prostigmata Oribatida Astigmata	Potapov *et al*., 2016
Functional groups	Soil Fauna: Comminution Microbivory Bioturbation	Nematodes: Bacterivores Fungivores Herbivores Omnivores Predator	Crotty et al., 2015; Grandy *et al*., 2016
Life story tactics			
Reproduction	Sexual/parthenogenesis Ovoposition timing		Siepel, 1994; Briones, 2014
Development	Slow, moderate, fast		
Synchronization	Diapause Aestivation Quiescence		
Dispersal	Phoresy Anemocory		
Feeding regime	For oribatid mites: Macrophytophages Microphytophages Panphytophages Zoophages Necrophages Coprophages		Briones, 2014; Luxton, 1972
Carbohydrase activity	For oribatid mites: Herbivorous grazers Herbivorous browsers Fungivorous grazers Fungivorous browsers Herbo-fungivorous grazers Opportunistic herbo-fungivorous		Briones, 2014; Siepel and de Ruiter-Dijkman, 1993

The biogeochemical importance of mesofauna are now thought to be underrepresented in food web models. Two recent publications describe the mechanisms by which microarthropods are involved in SOM formation (Soong and Nielsen, 2016) and propose conceptual frameworks in which the mechanisms could be incorporated into models (Grandy *et al*., 2016) to address this issue. The main microarthropod contributions to SOM dynamics have been identified as (1) grazing on microbes, meanwhile contributing to nutrient cycling; (2) litter transformation by fragmentation, increasing formation of free particulate OM and increasing surface area available for saprotrophs and leaching of dissolved OM; (3) aggregate formation and turnover involved in nutrient cycling between aggregate occluded- and mineral associated organic matter (Soong and Nielsen, 2016) (Figure 2.5).

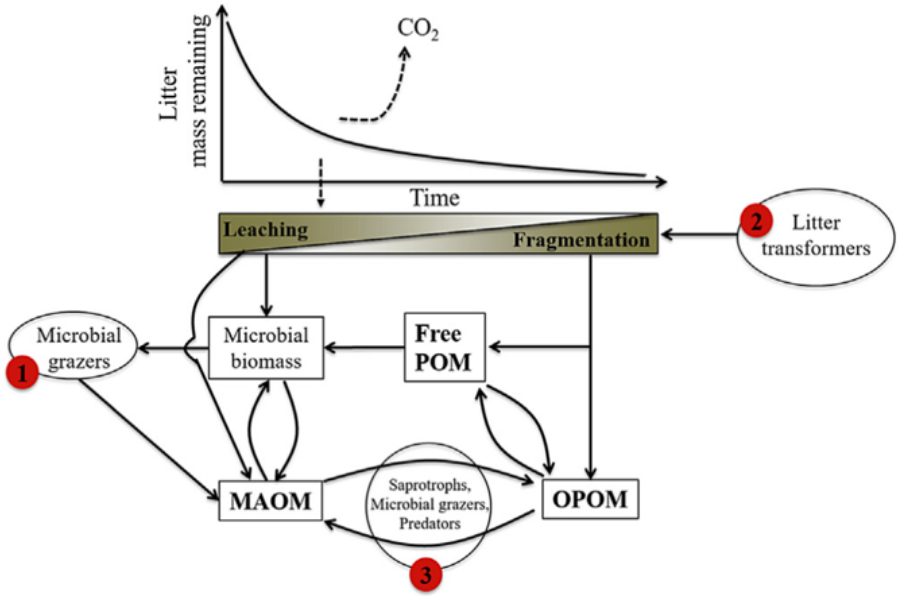


Figure 2.5. SOM formation and dynamics involving microarthropods. Main stabilising mechanisms: MAOM (mineral associated OM) and OPOM (aggregate occluded particulate OM). fPOM (free particulate OM) is not stabilised. Role of microarthropods includes: (1) grazing; (2) fragmentation by Collembola and mites; (3) C and nutrient transfer. Taken from Soong and Nielsen (2016).

Another issue associated with our current understanding of soil food webs that was raised recently is the reliability of the calculations of nutrient and energy flows employed in traditional food web models. Rousk (2016) criticized some of the fundamental assumptions underpinning traditional food-web research. Typically, only population or biomass are measured, while all other variables are drawn or inferred from the literature (e.g. Hunt *et al*., 1987; de Ruiter *et al*., 1994) and used to determine feeding rates of each functional- or trophic group. These assumed variables include feeding preferences, assimilation efficiencies, production efficiencies and nutrient contents of detritus and organisms (Rousk, 2016; de Ruiter *et al*., 1994;). Subsequent determination of the

excretion rate (OM returned to SOM), biomass production rate and mineralisation rate of nutrients and carbon are assumed as fractions of the feeding rate (Rousk, 2016; de Ruiter *et al*., 1994). The modelled mineralisation rate can then be compared to measured values to validate the soil food web model (Hunt *et al*., 1987; de Ruiter *et al*., 1994).

Deriving trophic positions from stable isotope analyses of the organisms in a soil food web also relies on assumptions. Trophic positions are determined using the $\delta^{15}N$ levels of a baseline organism ($\delta^{15}N_{base}$) and a test organism (e.g. $\delta^{15}N_{secondary\ consumer}$ for a secondary consumer) using the following equation:

$$\text{Trophic position} = \lambda + (\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{base})/\Delta_{n,}$$

where λ is the trophic position (e.g. 1 for a primary producer) of the baseline organism, and Δ_n is the $\delta^{15}N$ enrichment per trophic level (usually assumed from the literature to be 3 to 4 ‰) (Post, 2002). Moreover, stable isotope analyses, like a microbial growth rates based on biomass measurements, are but a snapshot in time. Abiotic and biotic conditions (e.g. nutrient availability, temperature, soil moisture, and interactions with other species with different life histories) may lead to different measurements depending on sampling time (Post, 2002).

Lastly, the very existence of discrete energy channels is being questioned (de Vries and Caruso, 2016). Not only is the substrate-driven nature of the energy channels under scrutiny, the types of C inputs consumed by bacteria, fungi and fauna appears to be a lot less discrete than traditionally thought. With the use of stable isotopes, soil fauna have been shown to mostly consume root litter and exudates, rather than leaf litter (Pollierer *et al*., 2007). Therefore, the earlier belief that most of the carbon entering the food web was litter-derived turned out to be false (Pollierer *et al*., 2007). Additionally, there is evidence that saprotrophic fungi are involved in the consumption of root exudates (De Deyn *et al*., 2011).

2.5. Litter – food-web interactions

An area of decomposition research that still remains less explored is the interaction between litter quality and soil microbes and fauna (Carrillo *et al*., 2011). A number of interactive effects that have been observed are introduced here. Research on these effects tends to be more extensive in forest systems, and sometimes in grasslands. A disconnect between forest-oriented and agriculture-oriented research communities has also been noted by Sollins *et al*. (2007), who

suggest that decomposition tends to be more researched by the forest community and SOM by the agriculture community. Naturally SOM and decomposition go hand-in-hand, and if the effects described below are applicable to arable cropping systems, they could help inform strategies to make better use of crop residues.

2.5.1. Litter-mixing effect

While there is ample evidence to show that the diversity of litter species can affect decomposition rates, no consistent results have emerged from studies on litter-mixing compared to decomposition as a single litter (Cong *et al*., 2015; Hättenschwiler *et al*., 2005). This lack of consistency is related to the high complexity of a system involving the decomposition of different litters. Each litter type may respond differently when decomposed in a mixture compared to decomposing as a single litter species (Hättenschwiler *et al*., 2005). Some litter species in the mixture could release allelochemicals, such as such as polyphenols, or glucosinolates in the case of brassicas, which are known to inhibit microbial activity (Brown and Morra, 1997). Microenvironmental conditions can be altered by the presence of one litter type in a mixture, and affect the decomposition of another litter type. For example, in a litterbag study by Wardle *et al*. (2003), the presence of feather mosses (a slowly-decomposing litter) enhanced the decomposition of other litter types in the mixtures because of their high water-holding capacity. Others have suggested that nitrogen released via the decomposition of N-rich litter enhances the decomposition of N-poor litter (Harguindeguy *et al*., 2008; Vos *et al*., 2013; Wardle *et al*., 1997). Such non-additive effects (faster or slower decomposition of the mixture compared to decomposition rates of the individual litters; Cong, 2015) may be further complicated by dynamics in nutrient availability and differences in decomposer communities per microenvironment. Nonetheless, decomposition studies performed by Handa *et al*. (2014), on five sites from the subarctic to the tropics, showed that decreasing diversity of both plant litter and soil organisms involved in decomposition led to a slower C and N cycling.

2.5.2. Legacy effect

The soil legacy effect can be considered as a set of abiotic and/or biotic soil properties that have been inherited from previous land use and has an impact on the capability of the soil food web to perform certain ecological processes (e.g. decomposition, nutrient availability, microbial activity). For instance, Detheridge *et al*. (2016) observed a legacy effect on fungal communities when spring wheat and winter barley were grown on plots with different histories (three years of ryegrass, chicory, red clover or white clover). They found lower abundance of root endophytic

fungi in soils with a white clover history, which may have been due to a legacy of high nitrate-N levels in this soil (Detheridge *et al*., 2016).

Cong *et al*. (2015) attempted to unravel the contributions of crop diversity on decomposition through litter mixing (see section 2.5.1 above) and aboveground plant species richness by performing a decomposition study in which they added different mixtures of grass root litters (litter mixing) to soils supporting a range of grassland species (aboveground diversity – different legacies). Respiration rates were measured as a proxy for decomposition, and were only affected by the legacy effect (not litter mixing) through a higher N availability in soils with higher aboveground diversity (Cong *et al*., 2015).

Marschner *et al*. (2015) studied the impact of a previously applied plant residue's C:nutrient ratio on the microbial activity and nutrient availability following a second residue addition (10-30 days later). Despite the short duration of their pot study, they demonstrate a legacy effect of previous residue additions on the nutrient release and soil respiration from subsequent additions (Marschner *et al*., 2015), and suggest this was brought about by microbes decomposing both the left-overs of the initial residue as well as the new residue (Marschner *et al*. (2015). Because the observed legacy effect was stronger after 10 days than after 30 days, they demonstrated in a follow-up experiment (Zheng and Marschner, 2017) that the amount of initial residue left in the soil determines the strength of the legacy effect when the second residue is added.

2.5.3. Home-field advantage hypothesis

The home-field advantage (HFA) hypothesis refers to the idea that the soil microbial community can quickly metabolise residues from the plants that grow at "home", compared to plant litter originating from different plant species (Gholz *et al*., 2000), also described as positive litter-soil feedbacks (Ayres *et al*., 2009). The HFA hypothesis is a type of legacy effect – the current microbial population has adapted to be able to decompose litter from previous land use and is inherited, even when a different plant is grown. The HFA has previously been observed in forest (Ayres *et al*., 2009) and grassland (Rashid *et al*., 2013) ecosystems, but not within arable cropping systems.

The litter affinity effect described by the HFA is attributed to adaptation and optimization of the soil microbial population, via different metabolic capacities and competition, to be able to quickly degrade litter in the home environment (Austin *et al*., 2014, Ayres *et al*., 2009; Wickings *et al*., 2012). Litter quality parameters usually cannot sufficiently explain observed HFA effects

(Strickland *et al*., 2009; Vivanco and Austin, 2008). The current understanding is based primarily on the observations that the soil microbial community is not functionally redundant (Strickland *et al*., 2009b) and that soil microbes have different metabolic capacities (Keiser *et al*., 2014; Wickings *et al*., 2012). For instance, litter chemistry is not affected in the same way when decomposed by different soil microbial communities (Wickings *et al*., 2012).

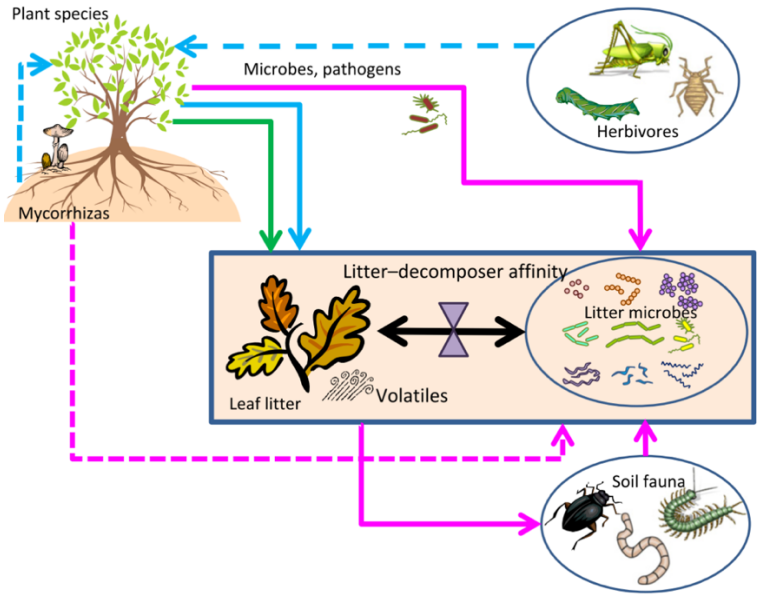


Figure 2.6. Litter-decomposer interactions (taken from Austin *et al*., 2014). Key to arrow colours: pink = microbial community composition; blue = leaf chemistry; green = leaf traits.

To further understand the mechanisms underlying litter affinity effects, it is important to define litter–decomposer interactions (Figure 2.6) (Austin *et al*., 2014): (1) Interactions in the rhizosphere: The microbial community is affected by root exudates, directly or indirectly through inter-organism competition (Cesarz *et al*., 2013; Cesarz *et al*., 2013b). Rhizosphere microbes, in turn, affect plant growth and leaf litter chemistry via nutrient availability (Berendsen *et al*., 2012). (2)"Green leaf hitchhikers:" Saprotrophic microbes may colonize microsites as they persist from green leaves/stalks to the litter stage. Two studies found equivalent fungal communities in decomposed leaf litter and on the original green leaves, supporting this idea (Persoh *et al*., 2013; Voríšková and Baldrian, 2013), although both studies also found significant succession of the microbial communities during decomposition of the leaf litter. (3) Plant litter volatiles: Plant volatile compounds are known to attract or repel other plants and herbivores (e.g. Dicke and Baldwin, 2009) and Austin *et al*., (2014) suggest studying the possibility of plant litter volatiles as repellents or attractants of soil invertebrates. (4) Three-way interactions between plants, microbes and soil arthropods: Plants influence microbial and soil arthropod communities via root exudates, litter, and volatiles. Herbivorous arthropods living in the canopy produce frass

(arthropod excrement), which provides nutrients that feed decomposer microbes (Austin *et al*., 2014; Hillstrom *et al*., 2010; Mattson and Addy, 1975; Meehan *et al*., 2014). Grazing herbivores in grasslands can encourage root exudates through their grazing activity (Hamilton *et al*., 2008), again, affecting decomposer microbes.

2.5.4. Priming effect

The priming effect is a short-term change in SOM decomposition after the addition of fresh organic substrates (Jenkinson *et al*., 1985; Kuzyakov *et al*., 2000) due to a response in microbial activity to changes in C availability. Priming effects can be positive (increase in C and/or N mineralisation rate) or negative (decrease in C and/or N mineralisation rate), and consist of two components: (1) apparent priming, which is a change in the metabolism and turnover of microbial biomass and tends to occur shortly after substrate addition (hours to days); and (2) real priming, which is a change in SOM mineralisation and tends to occur later (weeks to months) (Blagodatskaya and Kuzyakov, 2008). Apparent priming occurs because previously dormant microbes respond to the substrate addition, and their activation leads to decomposition of the substrate (apparent) and can also lead to co-metabolism of SOM (real), such that real and apparent priming overlap (Mondini *et al*., 2006), which is not always measured correctly (Blagodatskaya and Kuzyakov, 2008). Another source of real priming is the mineralisation of soil N to fulfil microbial metabolic requirements, which is thought to depend on the substrate composition (quality) (Blagodatskaya and Kuzyakov, 2008) as per the N mining hypothesis presented in section 2.3.3.

2.6. How can we make better use of crop residues?

We can make better use of crop residues by increasing the ability of the soil to sustain crops, while decreasing detrimental environmental impacts ensued by agricultural practices, which could be achieved by increasing the proportion of crop residues that is turned into SOM and decreasing the proportion that is respired as CO_2 (or CH_4, or NO_2).

2.6.1. Soil organic matter (SOM)

Soil organic matter includes microbial biomass and necromass, decaying plant and animal remains, excrements, and decomposition products of these. Traditionally these products were thought to turn into forms with different degrees of recalcitrance, with humus, a fully decomposed form of SOM, being least decomposable and therefore more stable (Cagnarini *et al*., 2019).

However, over the last decade or so, there has been a paradigm shift (e.g. see Sollins *et al*., 2007) away from this 'humic' theory.

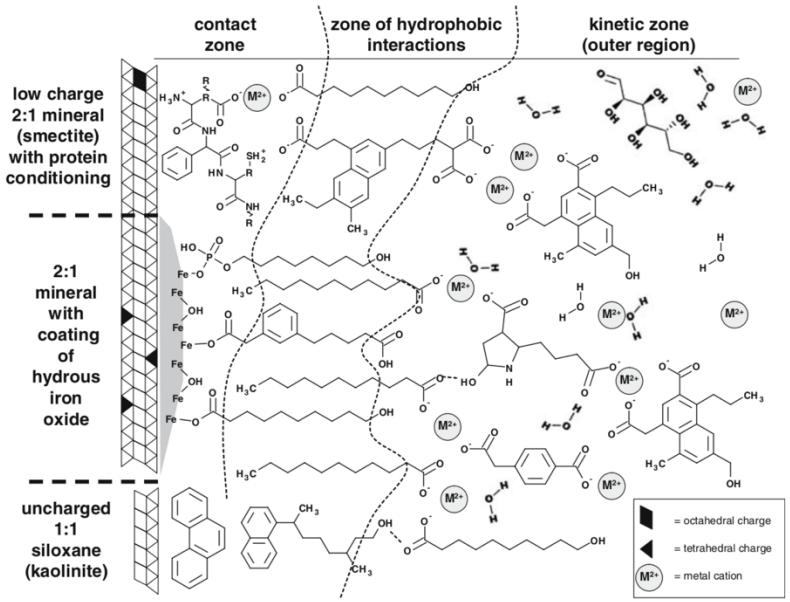


Figure 2.7. Zonal model proposed by Kleber *et al*. (2007). Image taken from Kleber *et al*. (2007).

One of the shifts has been towards a zonal or multilayer theory as proposed by Kleber *et al*. (2007) (Figure 2.7). This new view includes the following suppositions: (1) SOM consists of compounds with varying degrees of amphiphillicity; (2) N in the form of proteins enables organo-mineral associations in a layer structure around the mineral particle (informed by the second law of thermodynamics); and (3) configuration of amphiphiles with the polar part pointing outwards, forming a protection from water by means of a bilayer (Kleber *et al*., 2007). A hydrophobic character of coatings on soil aggregates was also found by FT-IR spectroscopy (estimated from intensity of C-H band) of loamy arable soil samples (Ellerbroc and Gerke, 2004). An important difference articulated in the new paradigm is that the traditional humic theory predicts large polyaromatic compounds, which we now know only represents a fraction of SOM (Rabbi *et al*., 2014), while the zonal theory predicts more simple decomposition products.

A second shift is the rejection of the idea that chemical recalcitrance makes soil C stable (in most cases), and instead inaccessibility to microbes is recognised as the main C stabilisation mechanism, regardless of the chemical composition (Dungait *et al*., 2012), including by occlusion within aggregates, intercalation, hydrophobicity, encapsulation, and interaction with mineral surfaces and metal ions (Lützow *et al*., 2006). Ekschmitt *et al*. (2005) state that a large portion of SOM is accessible and yet not decomposed, which they propose is due to a negative feedback

loop of microbial activity that arises when the energy incurred by producing enzymes exceeds the energy gained from metabolising SOM. This energy preservation mechanism arises because the majority of enzymes employed by microbes are diffusive (see section 2.3.1), so decomposition products may end up far away from the microbial cell.

Thirdly, a shift in the role of microbiology in decomposition has occurred due to recognition of the role of microbial metabolites and necromass in soil C accumulation (Ma *et al*., 2018), as well as the effect of the microbial community structure on C cycling in the rhizosphere and detritus layer, thus affecting C entering mineral soil (Schimel and Schaeffer, 2012). Recent attempts to quantify the contribution of microbial necromass have shown that it can make up more than half of SOM (Liang *et al*., 2019). Plaza *et al*. (2013) separated SOM from 25-year old no-till and chisel-till plots continuously cropped with barley into different SOM pools by physical fractionation (dissolved OM, free OM, intra-macroaggregate OM, intra-microaggregate OM and mineral associated OM) to understand the characteristics of these pools. No-till soils contained 16% more organic C than chisel-till soils, which was mostly due to higher C in the mineral-associated OM pool (65%), followed by intra-microaggregate OM (18%), intra-macroaggregate OM (14%) and free OM (11%) (Plaza *et al*., 2013). The mineral-associated OM pool was mostly of microbial origin, while the free and intra-aggregate OM fractions were mostly derived from crop-residues, highlighting the importance of microbes in SOM preservation and the interaction of SOM with mineral surfaces to form organo-minerals (Plaza *et al*., 2013). However, Barré *et al*. (2018) reported that they found both microbial and plant-derived persistent organic compounds in the soils of various long-term bare fallow experiments (Askov, Rothamsted, Versailles and Ultuna).

Finally, based on evidence from arable systems, root inputs (45%), compared to litter inputs (8.3%), have been approximated to be five times more likely to be stabilised as SOM, which is attributed to both the chemical composition of roots and their exudates (more aliphatic compounds), and the more favourable location within the soil (Jackson *et al*., 2017).

Apart from containing nutrients, SOM has a high surface area and cation exchange capacity which increases nutrient and water retention in the soil. Furthermore, it improves the soil structure by promoting soil aggregation. Therefore, increasing SOM can decrease greenhouse gas emissions associated with soil respiration, and the production of chemical fertilisers (although such externalities are beyond the scope of this thesis), within the limits of a soil's capacity to increase soil C any further (Stewart *et al*. 2009). In this thesis two methods of increasing SOM in arable soils is envisioned, by means of changing business-as-usual to manipulating the system's biology

and enable greater benefits to be obtained from crop residues as soil amendments: (1) manipulation of CUE; (2) manipulation of decomposition rate.

2.6.2. Manipulation of resource chemistry to increase CUE

Recent publications (e.g. Allison, 2014; Bradford *et al*., 2013; Geyer *et al*., 2016 and 2019; Jones *et al.*, 2019; Sinsabaugh *et al*., 2013 and 2016; Spohn *et al*., 2016) show an increased interest in microbial CUE. This is likely due to a change in our understanding of SOM – from a view based on humus and recalcitrance to a view of a more dynamic system that recognizes the microbial biomass and necromass as pivotal factors controlling what C stays within the soil system and what C is emitted – and CUE serves as a useful measure to be able to determine how to maximise microbially derived soil C. Kallenbach *et al*. (2019) argue that environmental changes, such as altering the C:N ratio of organic matter inputs, can lead to either a change in the CUE of the existing microbial community (trait moderation) or, if the inherent CUE windows of certain species within the original microbial community are exceeded, a shift to a different microbial community through competition (trait filtering). Hence, this could lead to an educated approach to create an 'optimal' crop-residue mixture to maximise the soil microbial community's CUE.

2.6.3. Manipulation of decomposition rate by 'confusing' microbes

After harvest, crop residues are typically applied to the same soil they originate from, i.e. where there may be a HFA. Therefore, assuming the HFA applies, microbes are adapted to quickly decompose the organic amendment and mineralise nutrients at a rate that may exceed plant demand and/or the requirements of other soil biota with relatively long life cycles (e.g. meso- and macro-fauna). Moreover, transition into and out of a dormant state incurs an energetic cost (Lennon and Jones, 2011), which may be reduced by promoting slower yet more steady decomposition.

If the HFA applies to arable cropping systems, so does its inverse: an 'away-field disadvantage,' where decomposition of residues 'imported' from *away* occurs more slowly. Therefore residue-derived resources will be released more slowly into the soil system. In resource-poor conditions *K*-strategists, which tend to exhibit a higher overall CUE, are more likely to outcompete r-strategists, which tend to exhibit a lower overall CUE (Kallenbach *et al*., 2019). The release of nutrients from the more slowly decomposing residues may match the life cycle of soil fauna occupying higher trophic niches, such as Collembola and earthworms. If decomposition rate can

be manipulated like this, the release of nutrients could be timed to achieve better synchrony between plant and microbial demand (Myers *et al*., 1997).

2.7. Conclusions

The decomposition subsystem, through the recycling of nutrients and the formation of SOM is an integral part to aboveground primary productivity. With a view on crop residues as soil amendments, the factors that affect the rate of decomposition and the complexity of the food web involved were discussed in this literature review. The many knowledge gaps identified in this review alone, particularly those identified by new analytical techniques that have led to recent advances in food web research, are in line with the supposition by Coleman (2011) that "soils are one of the last great unknown realms on earth, despite decades of extensive research." In an attempt to decipher a piece of the seemingly ∞-pieced puzzle that is the soil system, the experimental chapters of this thesis follow.

Chapter 3 – Is the belowground soil food web affected by the diversity of plants in aboveground crop rotations?

Note on publication strategy: This chapter is intended for publication with the following author list:

Marijke Struijk[1,2], Erika Degani[3], Samuel Leigh[3], Simon Mortimer[3], Andrew P. Whitmore[2], Simon Potts[3], Hanna Jones[3], Emma Bowen[1], Sion Thomas[1], Frank Ashwood[4] and Tom Sizmur[1]

[1] Department of Geography and Environmental Science, University of Reading, Reading, UK.
[2] Department of Sustainable Agriculture Sciences, Rothamsted Research, Harpenden, UK.
[3] School of Agriculture, Policy and Development, University of Reading, Reading, UK.
[4] Forest Research, Alice Holt Lodge, Farnham, Surrey, UK.

Abstract

The effect of aboveground botanical biodiversity on the belowground soil food web remains poorly understood. In this experiment the soil microbial community structure and biomass, by means of phospholipid fatty acid (PLFA) analysis, and the abundance of microfauna (nematodes), mesofauna (Collembola and mites), and macrofauna (earthworms) were assessed in three crop rotations with varying degrees of botanical diversity (Simple, Moderate and Diverse). Soils subjected to more diverse crop rotations had a smaller microbial biomass and a slightly lower fungal:bacterial ratio compared to the other treatments, although these effects were not significant and the overall soil microbial community structure was found to be similar in all crop rotations. Soil faunal abundance was not significantly affected by the different crop rotations, although the abundance of Collembola and mites tended to be greatest in Simple > Moderate > Diverse. The lack of a significant effect of crop rotations on soil biochemical parameters and biota could be related to the recent establishment of the field experiment, three to four years prior to these measurements, so a legacy effect from the different crop rotations may be yet to develop. The lower microbial biomass and abundance of Collembola and mites in the Diverse crop rotations could be associated with higher levels of disturbance in these soils from more frequent drilling to establish a greater diversity of crops. Statistical analysis did reveal a significant effect of crop stages within the Simple rotation for Collembola and mites, suggesting it may be individual crop types rather than crop rotations that favour certain biota.

3.1. Introduction

Diversification of farming systems has been proposed as a method to increase their ecosystem service delivery and thereby reduce environmental damage ensued from modern agriculture (Kremen *et al*., 2012) – primarily the pollution of waterbodies, the emissions of greenhouse gases, and biodiversity loss – and increase the resilience of our food production systems (Tilman *et al*., 2006; Lin, 2011). It is relatively well established that agricultural intensification leads to decreases in associated biodiversity (Hooper *et al*., 2005) and that increasing diversity of arable crop rotations is linked with greater diversity of other aboveground species, with reports of increased bird populations (Peach *et al*., 2001), a greater diversity of butterflies (Weibull *et al*., 2000), beetles (Östman *et al*., 2001) and other insects (Holland and Fahrig, 2000), and these in turn improve natural pest control (Gurr *et al*. 2003; Bianchi *et al*., 2006). However, the relationship between aboveground and belowground biodiversity is not as well established (Bardgett and van der Putten 2014).

On a global scale, there appears to be an inverse relationship between aboveground and belowground biodiversity (Wu *et al*., 2011) and belowground diversity is often much greater than aboveground (Wardle, 2006). In natural ecosystems, observations are emerging that the species richness of earthworms and small invertebrates is greater at higher latitudes, in contrast with the high levels of biodiversity nearer the equator for larger organisms (mammals, birds, plants) (de Deyn and van der Putten, 2005). Even the species richness of plants aboveground versus roots and rhizomes belowground are not linearly related, with belowground richness in grasslands exceeding levels observed aboveground because aboveground shoots are not present for some roots and rhizomes (Hiiesalu *et al*., 2012). On the other hand, on a local scale, and especially within anthropogenically managed arable cropping systems, the relationship between aboveground and belowground biodiversity appears to differ from natural ecosystems.

Farming systems can be diversified by increasing botanical diversity temporally (e.g. crop rotations) and spatially (e.g. intercropping, establishment of field margins, hedgerows and other landscape features) (Kremen *et al*., 2012). Diversification at the field scale can be realised by growing a combination of different crops (polyculture), e.g. by means of intercropping or under-sowing, and/or growing different genetic varieties of the same crop (Kremen *et al*., 2012). Different plant species in forage systems have been shown to affect the soil food web, with a greater abundance of earthworms and some collembolan species under clover and chicory crops (stolon/tap root system) compared to a greater abundance of herbivorous species under ryegrass (fine/extensive root system) (Crotty *et al*, 2015). Several authors report differences in properties

of soils under crop rotations compared to continuous monocultures, such as increased total C content of the soil (Lange *et al*., 2015), increased microbial biomass C (McDaniel *et al*., 2014), increases in certain microbial communities (Tiemann *et al*., 2015), and increased soil faunal diversity and biomass (Tresch *et al*., 2019). Greater diversity of belowground microbial community composition, in turn, has been associated with agro-ecosystem multifunctionality, including increases in plant diversity, decomposition rate, and retention and cycling of nutrients (Wagg *et al*., 2014; 2019). Diversification of arable systems by increasing the number of plant species has also been linked to higher diversity (Simpson's evenness) of nematodes (De Deyn *et al*., 2004), different diversities of mites (Badejo and Tian, 1999), but a lower diversity (Shannon index) of arbuscular mycorrhizal fungi (Johnson *et al*., 2004), and greater C and N pool due to altered decomposition dynamics of crop residue amendments (McDaniel *et al*., 2016).

The main mechanism explaining increased belowground biodiversity from more diverse cropping systems is related to the production of different qualities of plant-derived organic matter, including aboveground inputs in the form of plant residues/litter, and belowground inputs in the form of root exudates and root litter. These inputs create a larger and more biochemically heterogeneous resource base that reduces interspecific competition and feeds into a greater number of trophic niches and therefore a differently structured soil food web (Wardle, 2006; Armbrecht *et al*., 2004). However, as noted by De Deyn *et al*. (2004), it can be the plant identity rather than diversity or biomass that mainly affects species diversity belowground. Also, due to spatial isolation and dormancy (de Deyn and van der Putten, 2005) and the presence of not only resource-driven but also consumption- and competition-based populations (Wardle, 2006), this proposed mechanism may not always apply to soil microbes.

In this study, the link between aboveground and belowground biodiversity was investigated in an arable cropping system by comparing the microbial community structure and biomass in crop rotations with different degrees of diversity: Simple, Moderate and Diverse. Due to differences in the diversity of organic inputs (root exudates and plant litter) in the three rotations, the following is hypothesised: (1) different soil microbial community structures in the three rotations; (2) a greater microbial biomass in the Diverse rotation due to the production of a greater number of resources and niches from a more diverse input of organic substrates; (3) greater microbial biomass and more diverse litter inputs will lead to greater populations of both microbivorous and detrivorous soil fauna, as reflected in the populations of nematodes, microarthropods and earthworms.

3.2. Methods

3.2.1. Field site

Measurements were made in the summers of 2016 and 2017 on a field plot experiment established in 2013 at the Crop Research Unit, University of Reading, Sonning, UK (51.481152, -0.902188), as part of a larger EU-funded project, succeeding many years of grass ley followed by one season of winter barley and one season of winter wheat (Appendix A.1).

The experiment is a split-plot randomised complete block design that incorporates three different four-year crop rotations of varying degrees of diversity (n = 4): Simple, Moderate and Diverse (Table 3.1). Every block consists of three rotations, each rotation consists of four 12 m × 10 m sub-plots, each at different stages of the crop rotation sequence (i.e. for each of the three crop rotations there are four crop stages), and each plot consists of five 1.9-m wide sub-plots where crops are planted (Appendix A.1). The design of the experiment relies on a space-for-time substitution, so that each stage in the crop rotation is represented by one of the four plots in the rotation at any one time (see Appendix A.1 for maps with plot designation for each year).

Table 3.1. Sequence of crops in each rotation in the field experiment.

	Simple	Moderate	Diverse
Stage 1	Winter wheat	Winter wheat	Winter wheat under-sown with legume mixture
Stage 2	Winter wheat	Oilseed rape	Oilseed rape
Stage 3	Winter wheat	Winter wheat	Winter wheat under-sown with legume mixture
Stage 4	Oilseed rape	Winter beans	Brassica winter cover crop followed by spring beans

Nitrogen fertilisation was performed at 50% recommended rate (i.e. 50 kg N + 50 kg SO_3 ha^{-1}, applied as ammonium nitrate (34.5% N) and ammonium sulphate nitrate (26% N, 37% SO_3)), fungicide was applied at 50% recommended rate and herbicide at 100% recommended rate (following RB209, fertiliser manual, Defra, UK), except for the Diverse plots, which were not treated with a second herbicide dose in 2013 or 2015 crops to encourage establishment of the legume understorey.

3.2.2. General soil characterisation

Soils were sampled from the middle three sub-plots of every plot in June 2016, taking five 15 cm deep cores in a zig-zag fashion and homogenising these into one composite sample per plot. The soil samples were sieved to 2 mm and air-dried. Subsamples of 10 g each were shaken in 25 ml Ultrapure (> 18.2 ΩW.cm) water for 15 min and the pH measured (Hanna HI 11). For

measurement of total C and N, subsamples were ball-milled (Fritsch Pulverisette 4) and analysed by flash combustion (Flash 2000, Thermo Fisher Scientific, Cambridge, U.K.).

3.2.3. Soil microbial community assessment

Soil samples for PLFA analysis were collected in July 2016 from the third stage of each rotation (i.e. winter wheat) (see Table 3.1 and box in Table 3.2). In the Simple rotation these plots were previously cropped by two years of wheat, in the Moderate rotation by a year of oilseed rape (OSR) following a year of wheat, and in the Diverse rotation by a year of beans (after a brassica winter cover crop) following wheat under-sown with a legume mixture (Table 3.2). Cores of 15 cm depth were used to collect 5 samples per plot, in a zig-zag fashion across the three middle sub-plots of each plot, using a gauge auger. Soils were stored in a cool box during field sampling and subsequently transferred to a cold room and stored at 4 ºC prior to sieving to 4 mm , freezing, and freeze-drying.

Table 3.2. Crop sequence in the plots of each rotation included in the PLFA analysis in this experiment, which was performed during the growing season of the crops planted in 2015. The year indicates the beginning of the season, with harvest taking place during the summer of the next year.

Year	Simple	Moderate	Diverse
2013-14	Winter wheat (*Triticum aestivum* var. Solstice)	Winter wheat (*Triticum aestivum* var. Solstice)	Winter wheat (*Triticum aestivum* var. Solstice) under-sown with a legume mixture (*Trifolium repens*, var. Aberpearl and *Medicago lupilina*, var. Virgo pajbjerg)
2014-15	Winter wheat (*Triticum aestivum* var. Scout)	Oilseed rape (*Brassica napus*, var. Amalie)	Brassica winter cover crop (*Sinapsis alba, Eriogonum umbellatum, Lamium purpureum, Senecio vulgaris, Secale cereale*) followed by spring beans (*Vicia faba*, var. Fuego)
2015-16	Winter wheat (*Triticum aestivum* var. Solstice)	Winter wheat (*Triticum aestivum* var. Solstice)	Winter wheat (*Triticum aestivum* var. Solstice) under-sown with a legume mixture (*Trifolium repens*, var. Aberpearl and *Medicago lupilina*, var. Virgo pajbjerg)

Microbial community structure and biomass were assessed using phospholipid fatty acid (PLFA) profiles (Tunlid and White, 1992). This method exploits the fact that fungi, gram-negative (G–), gram-positive (G+), mycorrhizal fungi and actinomycetes exhibit PLFAs with different structural compositions. Soils were extracted using Bligh and Dyer solvent (Bligh and Dyer, 1959) according to Frostegård and Bååth (1996). Extracted phospholipids were derivatized according to Dowling *et al*. (1986) and analysed as fatty acid methyl esters by gas chromatography (Agilent 6890N, flame ionization detector and a 30 m × 0.25 mm capillary column with a 0.25 μm film of 5% diphenyl, 95% dimethyl siloxane) according to Frostegård *et al*. (1991).

The internal standards used were methyl tetradecanoate (C14:0; Sigma-Aldrich) and methyl nonadecanoate (C19:0; Sigma-Aldrich; 96.0% purity). All solvents were HPLC grade. Glassware was baked out at 450ºC for at least 4 hours, except for volumetric flasks and graduated cylinders, which were rinsed in hexane and left to dry, and volumetric pipettes, which were rinsed with the relevant solvent prior to use. A 0.15 M citrate buffer (pH 4) was prepared by mixing 0.15 M citric acid monohydrate with 0.15 M tri-sodium citrate to the ratio of 1:0.7 (v:v). The Bligh and Dyer solvent was prepared by mixing citrate buffer, chloroform and methanol to the ratio of 0.8:1:2 (v:v:v) with 50 mg L^{-1} butylated hydroxytoluene (BHT; ≥99% purity). Centrifugation was performed on a Mistral 3000i at 1500 for 10 minutes.

PLFA chromatograms were integrated and peaks identified based on BAME and FAME chromatograms provided by the supplier of the standards (Supelco, Supelco UK, Poole, UK), as well as soil-sample chromatograms previously identified at Cranfield University (Pawlett, M., pers. comm.). A broad selection of peaks between C14 and C20 were included in further data analyses, namely bacterial PLFAs i15:0, a15:0, 15:0, iC16:0, 16:1ω7t, 16:1ω5, 10me-16:0 (actinomycete), i17:0, 17:0cy, 17:0, 10me-18:0 (actinomycete) and 19:0cy; fungal PLFAs 18:2ω6c and 18:1ω9c; and general PLFA indicators 14:0, 14:1ω9t, i16:1, 3-OH-C14:0, 16:1ω11t, 16:1ω7c, 16:0, 17:0brα, 17:0brβ, 17:1ω8c, 17:1ω7, 12me-17:0, 18:1ω9t, 18:1ω13, 18:1ω10or11, 18:0, 19:1ω6, 19:1ω8, 19:0, 20:4ω6 (protists), 20:5ω3, 20:1ω9, 20:3w3, 20:2, and 20:0 (Bååth and Anderson, 2003; Frostegård and Bååth, 1996; Kaur *et al*., 2005; Frostegård *et al*., 1993; Bardgett *et al*., 1999). Concentrations of PLFAs were calculated based on FAME calibration standards, and data were adjusted to blanks and the internal standard 19:0.

3.2.4. Soil fauna survey

Soils were sampled from all 48 plots of the experiment for members of different soil faunal size classes in June 2017. We sampled for nematodes to represent microfauna, Collembola and mites to represent mesofauna, and earthworms to represent macrofauna.

Soils were sampled for nematodes using a gauge auger, collecting five 30-mm diameter topsoil cores from the middle three passes of each plot combined in one composite sample. Duplicate subsamples were then prepared per plot for extraction of nematodes using a modified version of the Baermann funnel method (Baermann, 1917). A 100 ml sample of soil was placed in a plastic supporting sieve lined with one ply of tissue paper. The sieve was placed in a plastic 18-cm diameter pot saucer. Ultrapure water was added to the soil to keep it moist but not saturated. Samples were left for 24 hours, after which the clear solution collected in the saucer was gently

stirred to ensure nematodes were floating in suspension, and subsequently collected (adding more water if necessary). These samples were left to rest for 2 hours to allow nematodes to sink to the bottom, after which excess water could be poured off the top and/or additional water added to ensure the same volume for each sample. Finally, two or three (depending on concordance) 1 ml aliquots were analysed for nematodes in a petri dish with an inverted microscope to determine nematode abundance. The first 25 specimens counted were identified to trophic level (bacterial feeder, plant parasite, and predator) based on mouthparts, to determine the proportion of each group.

A 10 cm deep core of 9.8 cm diameter (754 cm^3) was collected from each plot to collect microarthropods (Collembola and mites). Each core was then placed upside down and extracted for three days under a hot lamp in Tüllgren funnels, allowing microarthropods to drop through a with 2 mm mesh into collection receptacles containing 70% ethanol. Collembola specimens were identified by microscope to the orders *Poduromorpha, Entomobryomorpha* and *Symphypleona*, and mite specimens were identified to the suborders *Prostigmata*, *Mesostigmata* and *Oribatida*.

A 20 cm × 20 cm × 20 cm soil pit was excavated from each plot and transported to the lab, where it was hand sorted for earthworms. Juveniles were distinguished from adults based on the absence of a saddle and then adults (and some juveniles) were identified to species level, following Sherlock (2012). The biomass of each species was recorded. Five litres of mustard solution (6 g L^{-1} Coleman's mustard powder) was poured into each soil pit immediately after excavation to retrieve deep-burrowing anecic earthworms, but none were retrieved from any of the plots sampled.

3.2.5. Data analyses

Statistical analyses were performed in R 3.5.1 (R Foundation for Statistical Computing) using RStudio 1.1.456 (RStudio, Inc.) and GenStat 18.2.0.18409 (VSN International, 2016).

PLFA data were converted into a proportion, and analysed by nonmetric multidimensional scaling (NMDS) ordination and subsequent permutational analysis of variance (PERMANOVA). The fungal:bacterial (F:B) ratio was calculated based on the classification of PLFAs specified above to provide some indication of the presence of these microbial groups, although we recognise the shortcomings of the F:B calculation from PLFA profiles (see e.g. Strickland and Rousk, 2010). ANOVAs (with experimental blocking structure) were performed on the biomass of all fatty acids as well as F:B, G+:G–, actynomycetes, and total PLFA biomass. Assumptions of the ANOVA

test were assessed via the relevant statistical tests: homoscedasticity was evaluated with a Levene test of the data set, and normal distribution of the residuals was evaluated with a Shapiro-Wilk test of the residuals of the ANOVA. Since ANOVA tests are robust to considerable heterogeneity of variances as long as the sample sizes are nearly equal (Zar, 1999), ANOVAs were still carried out if Levene test results showed $0.04 < p < 0.05$, which occurred only once (for 20:5ω3).

Soil faunal abundance and biomass were analysed by a nested ANOVA with treatment structure Rotation/(Simple + Moderate + Diverse), where Rotation indicates whether a plot is in the Simple, Moderate or Diverse rotation, and the other factors indicate which of the four crop stages within the rotations the plot was in. Assumptions of the nested ANOVAs were assessed graphically and the data of most variables tended to exhibit unequal variance of the residuals. If a factor had a significant or near-significant effect ($p < 0.07$) and did not fulfil assumptions, data were cube-root transformed (also see statistical output in Appendix A.2). Pearson correlations were performed to investigate relationships between different variables.

3.3. Results

3.3.1. General soil characterisation

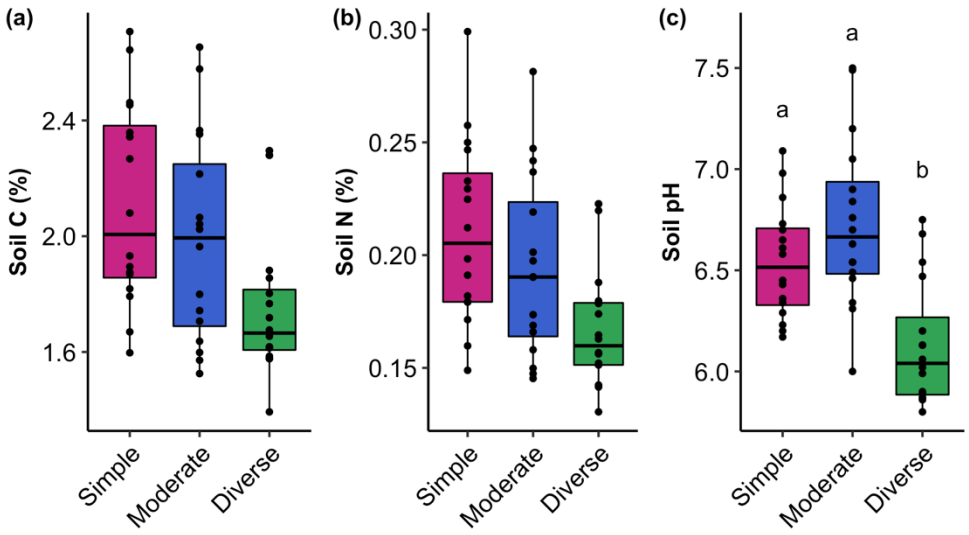


Figure 3.1. Total C **(a)**, total N **(b)**, and pH **(c)** of the soils in the different rotations of the 2015-2016 season. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints, occasionally overlapping (n = 16). Different letters indicate significant difference ($p < 0.05$; post-hoc Tukey HSD).

Total soil C and N levels differed noticeably but not significantly between rotations ($F = 4.59$, $p = 0.062$; $F = 5.02$, $p = 0.052$, respectively) and were highest in the Simple, then the Moderate,

and then the Diverse rotation (Figures 3.1a and 3.1b). Soil pH differed significantly between rotations ($F = 27.95$, $p < 0.001$) and was highest in the Moderate, then Simple and then Diverse rotation (Figure 3.1c).

3.3.2. Soil microbial community structure

Ordination of the PLFA profiles of the wheat plots revealed very similar soil microbial community structures in all rotations (treatment $R^2 = 0.151$, $p = 0.78$; PERMANOVA) (Figure 3.2). It appears that the microbial community in the plots of the Diverse rotation are more similar to each other than those in the Simple and Moderate rotations. The total PLFA biomass was highest in the Simple rotation, and was lower in more diverse crop rotations (Figure 3.3a), although these differences were not statistically significant ($F = 2.426$; $p = 0.150$). Likewise, the F:B ratio was highest in the Simple rotation (Figure 3.3b), but was not significantly different between rotations ($F = 0.573$, $p = 0.586$).

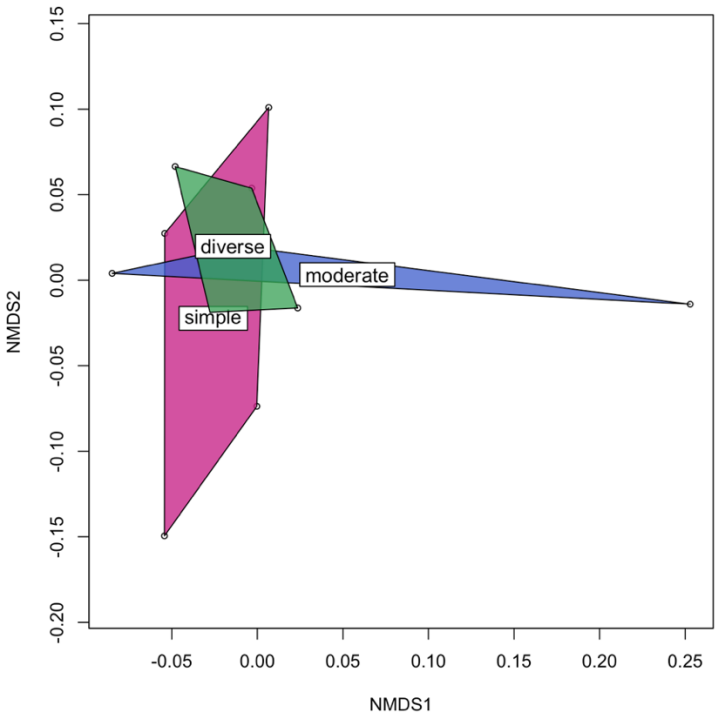


Figure 3.2. Nonmetric multidimensional scaling (NMDS) ordination of relative abundances of identified fatty acids (based on Bray-Curtis dissimilarity matrix; stress = 0.042). Each dot represents a PLFA profile in a replicate plot of the rotations. Dots that are closer to each other represent more similar microbial community structures.

In two plots of the Simple rotation (5 and 23; see Appendix A.1) the F:B was considerably higher than in all other plots, which may have skewed the results of this metric. Total soil C content was

strongly and positively correlated with PLFA biomass, fungal biomass, bacterial biomass, actinomycetes, G+ biomass and G– biomass ($p < 0.05$, $r > 0.60$). Total soil N only had significant ($p < 0.05$) correlations with bacterial biomass ($r = 0.60$) and actinomycetes ($r = 0.67$). Soil pH did not exhibit noteworthy correlations with the variables obtained via PLFA analysis. See Table A.2 in Appendix A.2. for all Pearson correlations between soil biochemical and PLFA parameters.

Treatment differences in the biomass of each fatty acid were determined by ANOVAs, and fatty acids with a notable treatment effect ($p < 0.1$) were included in Table A.1 in Appendix A.2, with indications of their origin. The treatment effects on each of these fatty acids exhibited a similar pattern, with greater biomass in the Simple rotation and smaller biomass in the Diverse rotation. This pattern was also observed when adding the biomass of these fatty acids together (Figure 3.3c).

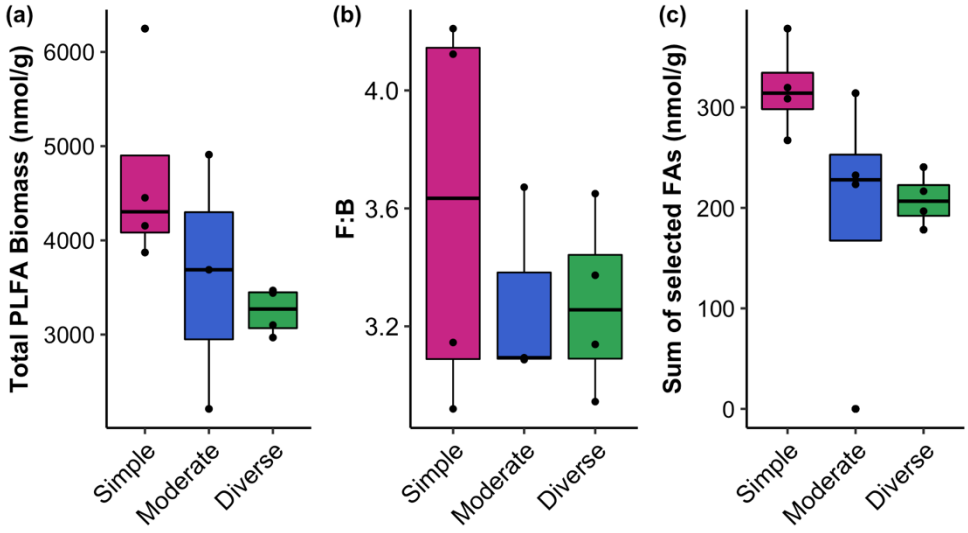


Figure 3.3. Boxplots per rotation of **(a)** total PLFA biomass based on identified fatty acids, **(b)** fungal:bacterial ratio, and **(c)** the sum of fatty acids (FAs) with a notable treatment effect ($p < 0.1$) (C16:1ω7t, C17:0brα, C17:0brβ, C18:1ω10or11, C20:4ω6, and C20:5ω3; see Table A.1 in Appendix A.2). Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints, occasionally overlapping ($n = 4$).

3.3.3. Soil faunal abundance

We found similar abundances of nematodes in the different rotations and similar proportions of trophic groups that made up the nematode populations in each rotation. Nematode abundance was not significantly influenced by the crop rotation ($F = 0.20$, $p = 0.833$) (Figure 3.4a), or the crop stages in either of the rotations (see Table A.3 in Appendix A.2 for statistical outputs). In all crop rotations bacterial feeders were the most dominant trophic group, followed by plant parasites and

a small proportion of predatory species (Figure 3.4b). The proportion of nematodes belonging to each trophic group was not significantly ($p > 0.05$) influenced by the crop rotation or crop stage.

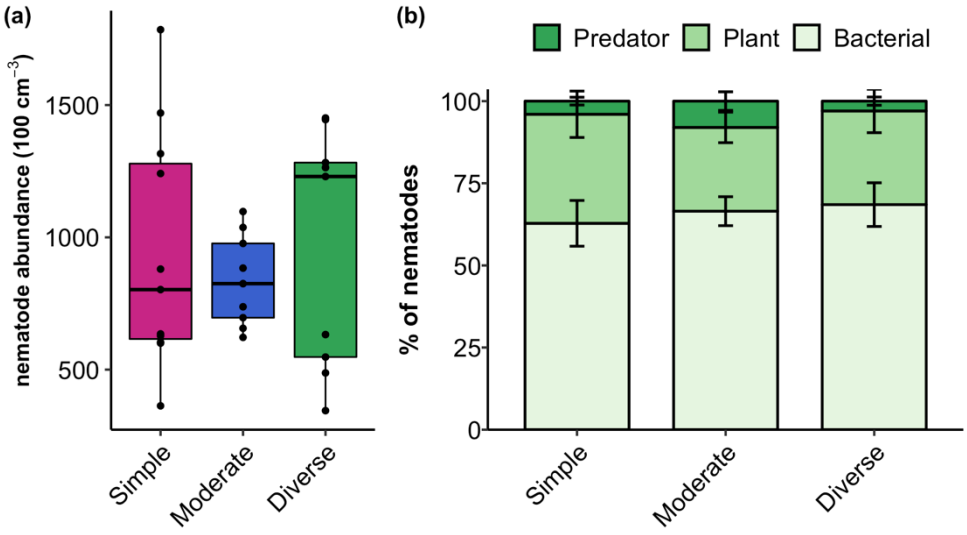


Figure 3.4. (a) Nematode abundance per rotation. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints, occasionally overlapping (n = 16). **(b)** Mean proportions (%) of trophic levels of the nematode populations per rotation. Error bars represent standard error of the mean (n = 10 for Simple; n = 8 for Moderate and Diverse).

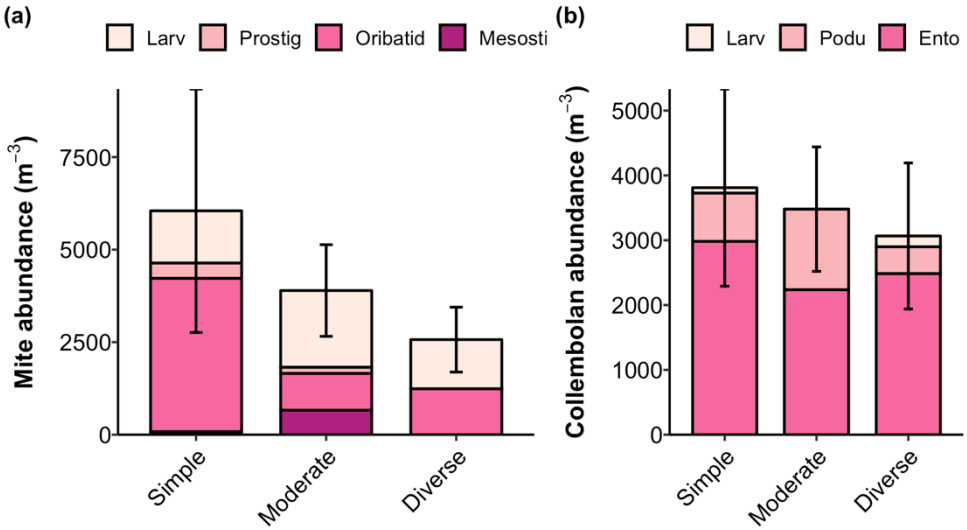


Figure 3.5. Mean mite **(a)** and collembolan **(b)** abundance per rotation. Error bars represent standard error of the mean of total abundance (n = 16). Abbreviations: Larv = larvae, Prostig = *Prostigmata*, Oribatid = *Oribatida*, Mesostig = *Mesostigmata*, Podu = *Poduromorpha*, Ento = *Entomobryomorpha*.

Both mite abundance and collembolan abundance were highest in the Simple rotation followed by the Moderate and Diverse rotations (Figure 3.5), although there were no statistically significant

differences between rotations (F = 0.14, p = 0.855 and F = 0.14, p = 0.872, respectively). The stage of the crop rotation in the Simple rotation significantly affected mite and collembolan abundance (F = 4.61, p = 0.01; F = 3.16, p = 0.041, respectively) and this was particularly the case for *Oribatida* mites and *Entomobryomorpha* Collembola (F = 10.56, p < 0.001; F = 4.21, p = 0.014, respectively). Within the Simple rotation, first wheat plots hosted a significantly greater abundance of Collembola and mites (Table 3.3; Figure 3.7). The collembolan order *Symphypleona* was absent from all soil cores. Total mite and collembolan abundance had a significant positive correlation (r = 0.33, p = 0.021). Out of the different mite suborders, Mesostigmata were most significantly correlated with Collembola (r = 0.15, p = 0.088). Pearson correlations between different faunal groups are summarised in Table A.4 in Appendix A.2.

Table 3.3. Mean mite and collembolan abundances (m^{-3} soil) in the different stages of the Simple rotation. Significant letters indicate significant differences in faunal abundance between crop stages (post-hoc Tukey HSD, p < 0.05), assessed after cube root transformation. Data presented in this table are not transformed.

Variable	First wheat	Second wheat	Third wheat	OSR
Collembolan abundance	8617^{b}	994^{ab}	4972^{b}	663^{a}
Entomobryomorpha	7292^{c}	331^{a}	3646^{b}	663^{a}
Mite abundance	17897^{b}	3314^{a}	1326^{a}	1657^{a}

Earthworm abundance and biomass were marginally greater in the Moderate rotation, compared to the Diverse or Simple rotations, but there were no significant differences between rotations (F = 0.34, p = 0.725; F = 0.76, p = 0.509, respectively) (Figure 3.6). Earthworm biomass was significantly affected by the crop stage in the Diverse rotation (F = 4.91, p = 0.008), increasing in the order spring beans (stage 4)^{a} < first wheat (stage 1)^{ab} < OSR (stage 2)^{ab} < second wheat (stage 3)^{b} (see Table 3.1; different superscript letters indicate that treatments are significantly different from each other). We identified two adult earthworm species in the plots, *Aporrectodea rosea* and *Octolasia cyaneum*, juveniles of *Allolobophora chlorotica*, and numerous other unidentifiable juvenile specimens. All identified earthworms were soil dwelling endogeic species. Adult earthworms were rare and only appeared in the Moderate rotation. Earthworms correlated positively with plant parasitic nematodes (r = 0.46, p = 0.017) and negatively with bacterivorous nematodes (r = -0.48, p = 0.013). Pearson correlations between different faunal groups are summarised in Table A.4 in Appendix A.2.

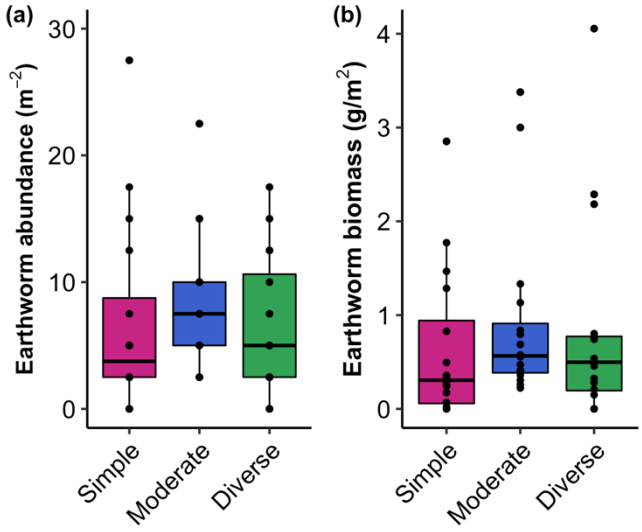


Figure 3.6. Earthworm abundance **(a)** and biomass **(b)** per rotation. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints, occasionally overlapping (n = 16).

3.3.4. Effects of crop stages within rotations

The stage of rotation within the Simple crop rotation significantly affected collembolan and mite abundances ($p < 0.05$), and the stage of rotation within the Diverse crop rotation significantly affected earthworm biomass ($p < 0.01$) (see Table A.3. in Appendix A.2). The effects of different crop stages within a crop rotation were further investigated by plotting relative effects of the four crop stages in each rotation on various soil faunal abundances and chemical soil parameters as a proportion of the total abundance/value across all the plots within the rotation (Figure 3.7).

The greatest contrast between crop stages was observed within the Simple rotation, where a greater abundance of mites and Collembola was observed in the first wheat plots than in other crop stages of the rotation (Figure 3.7a) (also see Table 3.3). Within the Moderate rotation, more earthworms and fewer mites were found in the OSR plots compared to other crop stages of the rotation (Figure 3.7b). Within the Diverse rotation, earthworms were more abundant in the second wheat and then in the OSR plots, nematodes were least abundant in the second wheat plots, and mites and Collembola were more abundant in the beans (which followed a brassica cover) and second wheat plots (Figure 3.7c). Within the rotations, all crop stages had similar levels of total soil C, total soil N and pH.

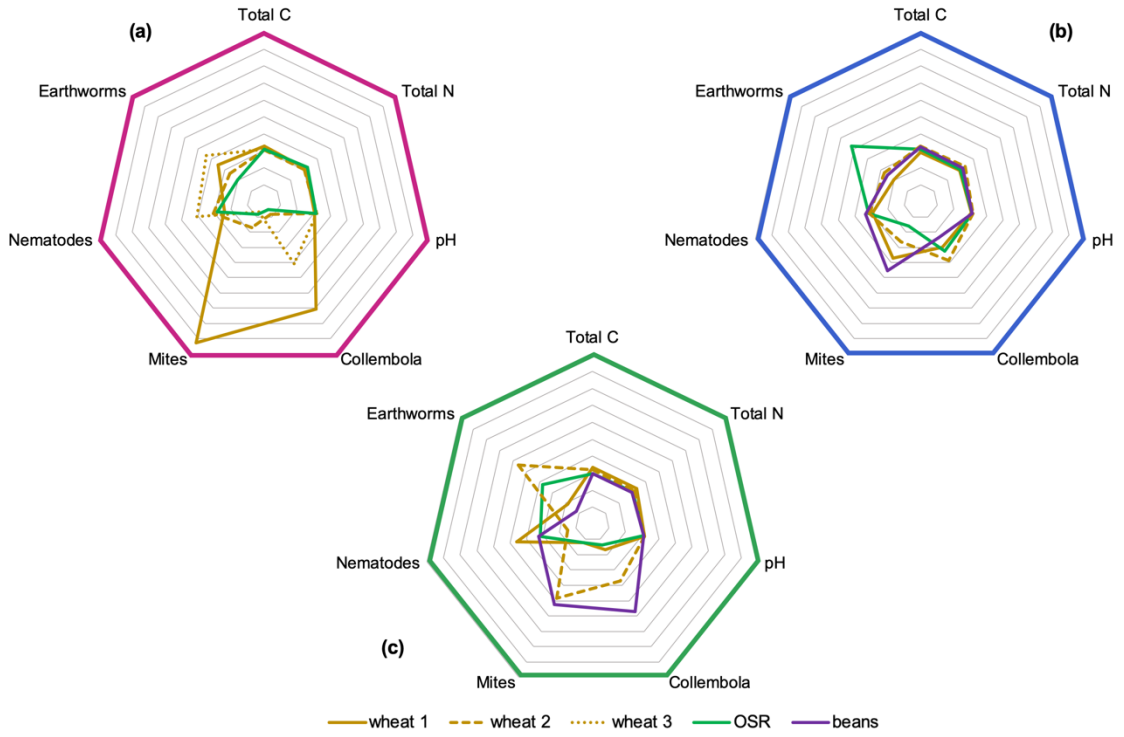


Figure 3.7. Radar plots of the mean relative effect of crop stage (2017) on soil faunal abundances and soil chemical parameters as a proportion of the total abundance/value across all the plots within the rotation in the Simple **(a)**, Moderate **(b)**, and Diverse **(c)** rotation (n = 4). All data were normalised per variable. Greater distance from the centre of the plot corresponds to a greater effect. Concentric polygons denote proportions increasing with steps of 0.02 up to the outer polygon which corresponds to a fraction of 0.2.

3.4. Discussion

3.4.1. Soil microbial community

Contrary to hypothesis 1, ordination of PLFA data detected no significant differences in the soil microbial community structures in the wheat plots (third stage in each crop rotation; see Table 3.1) between crop rotations of varying degrees of diversity. This could be related to the relatively recent establishment of the field experiment (three years prior to sampling for PLFA analysis). The legacy effect of more diverse crop rotations might require a longer-term experiment. The absence of a treatment effect (i.e. rotation effect) on the structures of the soil microbial communities suggests that, at least in the short term, temporal diversification of plant biomass inputs in arable soils does not lead to a different soil microbial community structure. This finding could be due to a number of reasons: (1) The soil microbial community could be primarily composed of generalist species in terms of habitat or diet, which are not affected by the creation of more niches, i.e. the greater diversity of resources is not perceived as such by the consumer species because they are equally as adapted to one resource as they are to the other (Armbrecht *et al*., 2004). (2) A more diverse mixture of plants aboveground does not produce a more diverse

mixture of substrates belowground, and therefore does not create more metabolic niches. As noted by Hooper *et al*. (2000), one plant species can create the same diversity of litter qualities and chemical substrates as a mixture of plant species, so it is the diversity of resource types rather than species that matters. Indeed, a review of the literature by Wardle (2006) indicates that the effect of plant diversity on soil biology is inconsistent. (3) The soil microbial community was sampled in the summer, towards the end of the growing season, so the soils underneath all wheat crops may have adapted to the main crop (wheat) independent of crop rotation, i.e. there was no legacy effect lasting throughout the whole growing season of the more diverse inputs prior to establishment of the wheat crop in the Diverse rotation. (4) PLFA analysis may not have been able to capture the changes in the microbial community taking place in this ecosystem, because the cell walls of those microbial species that may have responded to an increase in resource diversity in the Diverse rotation contain similar fatty acids to those microbial species present in the Moderate and Simple rotations.

In contrast to hypothesis 2, soil microbial biomass was highest in the wheat plots of the Simple rotation and lowest in the Diverse rotation, although this was not statistically significant, and followed the same trend as the total soil C and N levels in each rotation. This is contrary to previous studies on crop diversification and soil C and N levels. In a meta-analysis on the impact of crop diversity on soil properties, for instance, increases in soil microbial biomass C, and total soil C and N were found in systems with a polyculture of crops compared to monocultural systems, regardless of the crop type or management practices in place (McDaniel *et al*., 2014). It may be that the lower microbial biomass and soil C content in the Diverse rotation resulted from greater soil disturbance due to more passes of a seed drill to drill additional understorey or cover crops as well as cash crops, increasing aggregate turnover, and increasing decomposition of soil organic matter (Six et al., 2000). Alternatively, the lower microbial biomass and C and N in the Diverse rotation plots may be related to excessive adaptation to more frequent changes in aboveground plant species in the Diverse and Moderate rotations which did not enable one specialised microbial community to emerge.

Low soil C and soil microbial biomass levels in the Diverse rotation could also be related to differences in priming activities in the different crop rotations. Priming of organic matter involves microbial decomposition of labile C substrates. Microbes produce extracellular enzymes based on the chemical composition rather than energy content of labile organic matter (Di Lonardo *et al*., 2017), and these enzymes can remain stable and active in soil after secretion (Allison, 2006). We assume that the crop residues added to the soil in the Diverse plots had a greater biochemical diversity of C substrates compared to Moderate and Simple rotations because the Diverse rotation includes plants from different plant families which produce residues that are biochemically

different to one another due to the synthesis of plant family-specific primary and secondary metabolites (Wink, 2008). These biochemically more diverse crop residues can lead to increases in the activity of some extracellular enzymes (Hernández and Hobbie, 2010), and chemically complex substrates have been linked to a greater priming effect (Wu *et al*., 1993). Extracellular enzyme activity is widely considered to be a rate limiting step in soil organic matter (SOM) decomposition, and subsequent C and N mineralisation (Schimel and Weintraub, 2003; Allison, 2005). Previous studies at this study site showed that the N mineralisation rate in the Diverse plots was greater than in the Moderate and Simple rotations (Degani, 2018). Therefore, the lower C content in the Diverse soils might be related to a greater priming rate associated with a greater diversity of labile C substrates, and subsequently greater C and N mineralisation rates in these soils. McDaniel *et al*. (2014) also found greater processing rates of C and N in more diverse crop rotations, including greater rates of decomposition of crop residues and greater levels of cellulose (labile biopolymer) degrading enzymes compared to lignin (recalcitrant biopolymer) degrading enzymes.

We did find that a selection of fatty acids tended to be affected by the rotations with different degrees of diversity (Table A.1 in Appendix A.2). Unlike the soil fauna data, PLFA analysis was only performed on soils sampled from the wheat plots in each rotation, so the effect of crop stages on PLFA data could not be tested. Therefore, the effect of rotation on some of the fatty acids could be due to both the general diversity of crops in a rotation and/or be confounded by the effect of the previous year's crop, which was beans in the Diverse rotation, OSR in the Moderate rotation, and wheat in the Simple rotation.

3.4.2. Soil fauna

Contrary to our hypothesis, the abundance of none of the soil faunal groups sampled in this experiment was significantly influenced by aboveground botanical diversity in the different crop rotations (hypothesis 3). This could be related to the relatively recent establishment of the field experiment.

Microarthropods (Collembola and mites) did exhibit a clear pattern, similar to microbial biomass and soil C and N, with higher population abundance in the Simple, then Moderate and then Diverse rotation. Lower abundance in the Diverse rotation may be due to greater soil disturbance from drilling additional intercrops and cover crops, as proposed for soil C and microbial biomass. This disturbance may have reduced microarthropod abundance either (1) directly, by disturbing their habitat, which is also suggested by other authors reporting lower microarthropod abundance

in organically managed soils than in conventionally managed soils attributed to disturbance from tillage activities replacing herbicide applications for weed control (Mazzoncini *et al*., 2010); or (2) indirectly, by reducing food resources in the form of soil organic matter and/or microbial biomass. Microarthropods may have been more abundant in the Simple rotation because they graze on microbes and these soils contained more C and a greater microbial biomass, and therefore provided greater food resources (Beare *et al*., 1997).

Microarthropods are known to transform plant residues into a more decomposable state by increasing surface area by fragmentation and comminution, as well as excretion of fecal pellets, which increases microbial activity (Addison *et al*., 2003; Briones, 2014; Coleman, 2011; Sanderman and Amundson, 2014). Therefore, the presence of a greater abundance of microarthropods may have also increased microbial activity and the formation of soil organic matter from crop residues. For mites, it was mostly the *Oribatida* that were of higher abundance in the Simple rotation. The abundance of the other mite suborders were similar in all three rotations. *Oribatida* mites are known to be food generalists, occupying three to four trophic levels, as determined by stable isotope studies (Schneider *et al*., 2004), so they may be able to better adapt to a lower diversity of resources available in the Simple rotation compared to other faunal groups that might occupy more specialist niches.

Plots in the Diverse rotation may have harboured more insects among the more diverse crop assemblage, which may have increased predation on Collembola and mites. Ants have been found to predate on mites (Masuko, 1994; Wilson, 2005), and beetles and spiders on Collembola (Bilde *et al*., 2000; Lawrence and Wise, 2000).

Populations of earthworms and nematodes did not exhibit a clearly distinguishable pattern between crop rotations, although a slightly higher average nematode abundance could be observed in plots of the Diverse rotation (Figure 3.4a). Since nematodes tend to reside near roots (Coleman and Wall, 2015; Ingham *et al*., 1985), this observation could be related to more numerous and more diverse rooting systems in the Diverse rotation. Earthworms feed on organic matter present in the soil, effectively grazing on bacteria in the process (Pokarzhevskii et al., 1977). Earthworm biomass and abundance tend to increase with greater inputs of organic matter (Deibert and Utter, 1994; Fraser and Haynes, 1996). Considering the lower soil C and microbial biomass present in the Diverse soils, it is surprising that there is no corresponding drop in earthworm abundance. Perhaps the greater quantity and diversity of crop residues produced in the Diverse plots counteracted the lack of microbial biomass.

We found significant Pearson correlations between Collembola and mites, and also between earthworms and some trophic groups of nematodes, but not between microarthropods and nematodes or between microarthropods and earthworms. This could suggest that they – microarthropods on the one hand, and earthworms and nematodes on the other hand – each respond to different environmental factors that were not measured in this study. Their seemingly different responses may be related to them occupying different parts of the soil. The earthworms identified in this experiment were all endogeic, therefore occupying somewhat deeper soil levels, and nematodes are known to reside closely to the root zone of plants (Coleman and Wall, 2015; Ingham *et al*., 1985). Microarthropods tend to inhabit more shallow soil layers (top ~5 cm) than earthworms or nematodes (Sharma and Parwez, 2017). They can be atmobiotic, living in plants; epedaphic, living in the upper soil layer among plant residues; hemiedaphic, living among more decomposed plant residues; or euedaphic, living in the upper mineral layer of the soil (Potapov *et al*., 2016). It has been suggested that microarthropod abundance is related to soil pore volume (Nielsen *et al*., 2008), although nematodes have also been found to have a soil pore size preference (30–90 μm diameter; Hassink *et al*., 1993), and they are generally known to be sensitive to desiccation, even to short-term drought (Frampton *et al*., 2000). Therefore, even minor levels of soil disturbance or drying of the top soil layer in the summer may have affected microarthropods, while endogeic earthworms at slightly deeper levels and nematodes closer to the root zone are less easily affected. Soil pore volume stability may have been increased by the higher organic matter content in the soils of the Simple rotation (Barral *et al*., 1998), which may have also kept collembolan and mite abundances at higher levels than in the Moderate- and Diverse-rotation soils.

3.4.3. Crop stage effects

Although we found fewer effects than expected from the crop rotations of differing botanical diversities, different stages of the same crop rotation revealed some significant effects on soil fauna (Table A.3 in Appendix A.2). The importance of plant identity rather than plant diversity has been noted in numerous studies, including on nematodes (Kostenko *et al*., 2015; Viketoft *et al*., 2009; Wardle *et al*., 2003), mesofauna (Beugnon *et al*., 2019; Salamon *et al*., 2011; Wissuwa *et al*., 2012) and earthworms (Gastine *et al*., 2003). Often leguminous plants are considered to provide higher-quality resources that positively affect the soil faunal groups studied (Spehn *et al*., 2000), but differences in the quantity of resources provided by different plant species has also been coined as a mechanism for greater soil biota abundance (Salamon *et al*., 2011; Wissuwa *et al*., 2012).

In this study, a significantly higher abundance of mites and Collembola was observed in the first wheat plots of the Simple rotation, compared to the other stages in the rotation. This crop-stage effect was most significant for the collembolan order *Entomobryomorpha* and the mite suborder *Oribatida*. Continuous wheat can decrease the number of meso- and macropores (Zimmermann, 1984; Schönhammer and Fischbeck, 1987, both cited in Sieling and Christen, 2015), so perhaps the preceding OSR break crop had beneficial effects on the soil structure. Root assessments at 17 winter wheat and 40 OSR sites in the UK found longer, more dense and deeper roots for OSR compared to winter wheat, and OSR exhibited greater topsoil root length density (i.e. root length divided by root volume, equivalent to the number of roots) (White et al., 2015). Root length density has been shown to positively affect the abundance of herbivorous and detrivorous mesofauna (Beugnon *et al*., 2019). Microarthropods require sufficient microhabitats and heterogeneity in the top layer of the soil, which may have been more abundant after OSR (Nielsen *et al*., 2010). Moreover, plant leaf area has been found to positively affect the abundance of herbivorous and detrivorous mesofauna (Beugnon *et al*., 2019). We did not measure leaf area, but other authors who assessed the leaf areas of wheat and OSR crops found it to be higher in wheat (Dreccer et al., 2000). These factors combined, may have made the first wheat crop stage more favourable for microarthropods. Earthworm biomass (but not abundance) was significantly lower in bean plots than second wheat plots in the Diverse rotation. This could be due to a biofumigant effect on soil microbes from the brassica cover crop directly preceding the spring beans. However, the reasons for crop preferences of some soil faunal groups remains unclear at this point.

Generally, differences in scales of the processes that influence aboveground and belowground systems make it difficult to distinguish different mechanisms from each other (Hooper *et al*., 2000). Scales of soil food web processes differ (1) spatially, as species reside at different depths and in different pore spaces; (2) temporally, as species have different life cycles and respond differently to changes in temperature, moisture and other abiotic conditions; and (3) functionally, as species each fulfil different roles in a community or ecosystem. Although we suggested that the lack of an effect from crop rotations in this experiment could be related to the relatively short legacy of this field experiment, the significant effects of crop stages within rotations indicates that some effects occur on a much shorter temporal scale, which warrants further investigation.

3.5. Conclusions

Although some differences in the soil microbial community and faunal abundance were observed in the different crop rotations, such as greater total PLFA biomass, greater F:B, a greater biomass of certain fatty acids, and greater mite and collembolan abundance in the Simple rotation soils,

these effects were not statistically significant. Moreover, the overall structure of the soil microbial population was similar in all soils sampled. Significant effects were observed within the Simple rotation, where crop stage significantly affected mite and collembolan abundance, and in the Diverse rotation, where crop stage significantly affected earthworm biomass (but not abundance). Based on this initial investigation on the link between aboveground plant diversity on belowground microbial and faunal communities, no direct impact of crop rotational diversity could be detected, but within crop rotations, crop stage seemed to affect the abundance of certain members of the food web.

Chapter 4 – Absence of a home-field advantage within a short-rotation arable cropping system

Note on publication strategy: This chapter is intended for publication with the following author list:

Marijke Struijk[1,2], Andrew P. Whitmore[2], Simon Mortimer[3], Xin Shu[1] and Tom Sizmur[1]
[1] Department of Geography and Environmental Science, University of Reading, Reading, UK.
[2] Department of Sustainable Agriculture Sciences, Rothamsted Research, Harpenden, UK.
[3] School of Agriculture, Policy and Development, University of Reading, Reading, UK.

Abstract

The home-field advantage (HFA) hypothesis, which predicts faster decomposition of plant residues in the soil in which they were grown (i.e. at *home*) compared to a soil where different plants were grown (i.e. *away*), has been demonstrated in forest and grassland ecosystems. It is not yet known whether this legacy effect applies to the decomposition of crop residues within different stages of an arable rotation, which could improve our understanding of decomposition dynamics in these soils and may be useful in optimising the use of crop residue amendments in arable systems.

Here, we test the HFA in a reciprocal transplant experiment with mesh bags containing wheat and oilseed rape (OSR) residues in soils at three stages of a short-rotation (wheat, wheat, wheat, OSR) arable cropping system. Subsets of mesh bags were dug up every month for six months throughout the growing season to determine residue decomposition rates. Soils were sampled concomitantly for measurement of soil available N (KCl extraction), microbial biomass C (fumigation-extraction) and microbial community structure (phospholipid fatty acid analysis), to assess how plants influence litter decomposition rates via alterations to soil biochemical properties. Soil microbial activity was assessed via the Tea Bag Index (TBI) protocol (Keuskamp *et al*., 2013).

The crop residues decomposed at similar rates at all stages of the crop rotation. After thorough investigation of the data using several statistical approaches, we did not find an HFA effect *within* the crop rotation considered here, and the soil microbial community structures were similar at all stages of the rotation. We attribute the absence of an HFA to the shortness of the rotation and soil disturbance involved in intensive agricultural practices. We did observe a higher decomposition rate of wheat residues compared to OSR residues, which could be explained by residue chemistry.

4.1. Introduction

Soil organic matter (SOM) has been identified as a major factor for improving the ability of soils to sustain crops and provide ecosystem services like climate regulation (Kibblewhite *et al*., 2008). However, global loss of SOM has added 78±12 Pg C to the atmosphere since the industrial revolution (Lal, 2004). Crop residues – straw, stalks, leaves, etc. – comprise the majority of plant materials harvested worldwide, with an estimated annual production ranging from 3.4 Pg to 3.8 Pg (Lal, 1997; Smil, 1999) and thus represent a considerable opportunity to increase SOM. Wheat residues in particular, have a high carbon content (around 46%) compared to other soil amendments (Sizmur *et al*., 2017). With an annual production estimated as 0.85 Pg yr^{-1} to 0.96 Pg yr^{-1} they constitute roughly a quarter of the world's crop residues (Lal, 1997; Smil, 1999). Current thinking recognises the important role of microbial metabolites and necromass in soil C accumulation, as opposed to unmineralized residues (Liang *et al*., 2019; Ma *et al*., 2018). Just a small increase in the proportion of wheat straw, and other crop residues, that is converted into SOM, mainly via microbial decomposition pathways, could have a large impact on the global atmospheric carbon loading.

Apart from being used as biofuels, and animal feed and fodder, crop residues represent a major on-farm resource that could be applied as a soil amendment. Transformation of unmineralized residues into SOM feeds the soil food web, the members of which are involved in soil aggregation, nutrient cycling, and improving the conditions for primary production. Unfortunately, straw incorporation is often inefficient in terms of SOM formation, as demonstrated by studies that compare straw to other soil amendments for their C accumulation potential (Powlson *et al*., 2012) or that compare CO_2 savings by SOC formation from straw amendments to biofuel use to displace electricity generated from fossil fuels (Powlson *et al*., 2008). However, these results are based on experiments in which crops are grown and their residues applied to soils directly after that crop's harvest. Perhaps a different application strategy could be devised, based on a better understanding of decomposition processes in arable cropping soils, to increase the amount of straw-derived C persisting in the soil and to decrease the amount of C that is respired.

According to the legacy effect described by the home-field advantage (HFA) hypothesis, plant residues are predicted to decompose faster in the soil in which they were grown (*home*) compared to a soil where different plants were grown (*away*) (Gholz *et al*., 2000). This has previously been observed within forest (Ayres *et al*., 2009) and grassland (Rashid *et al*., 2013) ecosystems, as well as between cropland, grassland and forest biomes (Di Lonardo *et al*., 2018), but to our knowledge the HFA hypothesis has not been tested within a rotational arable cropping system. The HFA

hypothesis is attributed to adaptation and optimisation of the soil microbial community to *home* plants' residues (Austin *et al*., 2014; Ayres *et al*., 2009), which is based primarily on the observations that the soil microbial community is not entirely functionally redundant (Strickland *et al*., 2009a; 2009b) and that different soil microorganisms have different metabolic capacities (Keiser *et al*., 2014; Wickings *et al*., 2012). For instance, the chemical composition of litter changes as it is decomposed, and it has been observed that different soil microbial communities affect this change in litter chemistry differently (Wickings *et al*., 2012). However, the mechanism involved in the formation of a *home* microbial community remain poorly understood (Austin *et al*., 2014) and therefore validity of the HFA between different years of typical arable cropping systems, which are different in nature to forests and grasslands in terms of the time that the soil is exposed to a particular crop, is difficult to predict.

In forest and grassland conditions, the litter decomposition rate could theoretically be manipulated by selecting *home* or *away* litter. If *away* litter is applied, effectively realising an "away-field disadvantage," the soil microbial community is not adapted to being able to easily decompose the *foreign* litter. At this lower decomposition rate, the unfamiliar organic substrates are not easily decomposable, temporarily realising resource-poor conditions in which *K*-strategists, which tend to exhibit a higher overall CUE than *r*-strategists, are favoured (Fierer *et al*., 2007; Kallenbach *et al*., 2019). Therefore, realising an "away-field disadvantage" might increase the net accumulation of SOM from crop residue amendments compared to application of litters in *home* soil. However, this would be easier to accomplish in an arable cropping system, if a HFA is found in this environment. Therefore, determination of the applicability of the HFA hypothesis to arable cropping systems could inform strategies for optimal crop residue applications in arable cropping systems.

In this experiment we tested the HFA hypothesis within an intensively managed arable cropping system of continuous wheat with an oilseed-rape (OSR) break crop every four years. Each stage in this crop rotation was represented in the experimental plots by using a space-for-time substitution. Wheat and OSR residues were buried in 1st wheat, 2nd wheat and OSR plots, and their mass loss measured over time over a period of six months (during the growing season). Soil available N, microbial biomass C and the microbial community structure (by phospholipid fatty acid analysis) were assessed as explanatory variables. In line with the HFA hypothesis, we hypothesised that (1) wheat straw incorporated in a soil after a wheat crop would decompose faster than wheat straw incorporated after an OSR crop. Likewise, OSR straw incorporated after an OSR crop would decompose faster than OSR straw incorporated after a wheat crop; and that (2) the belowground soil microbial community would be altered by the crops grown aboveground.

4.2. Methods

4.2.1. Study site

This experiment was carried out in 2016 on a field site at the Crop Research Unit, University of Reading, Sonning, UK (51.481152, -0.902188), which was established in 2013, succeeding many years of grass ley followed by one season of winter barley and one season of winter wheat. The HFA was tested in a simple crop rotation (Figure 4.1a) representative of an intensive agricultural system in the UK which was part of a larger experiment described fully elsewhere (see Chapter 3 and Degani *et al*., 2019). Three stages of the crop rotation (microsites) were selected: 1st wheat, 2nd wheat (i.e. the first wheat crop after OSR and the second wheat crop after OSR; *Triticum* sp., var. Solstice) and OSR break crop (*Brassica napus* sp., var. Tamarin) (indicated in bold in Table 4.1). We employed microsite labels as [previous crop]-[current crop], as follows: OSR-WW for 1st wheat, WW-WW for 2nd wheat, and WW-OSR for OSR (Table 4.1). For clarity, this microsite designation was used because we hypothesised that an HFA effect would be based on a legacy effect of the previous year's crop. The 3rd wheat crop rotation stage was used in a different experiment and was therefore excluded from this study.

Figure 4.1. (a) Aerial photograph of the field site, indicating the plots (12×10 m^2) of the intensive arable rotation included in this study. Image taken by Richard Casebow. **(b)** Mesh bags containing wheat (left) and OSR (right) straw. **(c)** Mesh bag buried at 15-cm depth (bottom left). **(d)** A pass in an OSR plot with 15-cm deep holes for mesh bags, locations marked with peg.

Table 4.1. Cropping history per rotational stage included in this study. Rotation stages and microsite labels used in this study are indicated in bold.

Up to 2011	Grass ley	Grass ley	Grass ley
2011-2012	Winter barley	Winter barley	Winter barley
2012-2013	Winter wheat	Winter wheat	Winter wheat
2013-2014	3rd wheat	OSR	2nd wheat
2014-2015	OSR	1st wheat	3rd wheat
2015-2016	**1st wheat**	**2nd wheat**	**OSR**
Treatment label	**OSR-WW**	**WW-WW**	**WW-OSR**

Abbreviations: OSR is oilseed rape, WW is winter wheat.

Nitrogen fertilisation was performed at 50% recommended rate (according to RB209 Fertiliser Manual; Defra, UK), i.e. 50 kg N + 50 kg SO_3 per ha, applied as ammonium nitrate (34.5% N) and ammonium sulphate nitrate (26% N, 37% SO_3). Wheat plots were fertilised on 8 April and OSR plots were fertilised on 20 April (Table 4.2).

Table 4.2. Schedule of the experiment with the months and days referred to in text and figures.

Event	Sampling month	Experimental day	Date
Mesh bags buried (blocks 1, 2, 4)		0	10 February 2016
Mesh bags buried (block 3)		2	12 February 2016
Initial soil samples (blocks 3 and 4)	Initial	2	12 February 2016
Initial soil samples (blocks 1 and 2)	Initial	5	15 February 2016
PLFA soil samples (start)		23	4 March 2016
Mesh bag retrieval 1	1	26	7 March 2016
Mesh bag retrieval 2	2	54	4 April 2016
Fertilisation of wheat plots		58	8 April 2016
Fertilisation of OSR plots		70	20 April 2016
Mesh bag retrieval 3	3	82	2 May 2016
Teabags buried		102	22 May 2016
Mesh bag retrieval 4	4	110	30 May 2016
Mesh bag retrieval 5	5	138	27 June 2016
PLFA soil samples (end)		146	5 July 2016
Mesh bag retrieval 6	6	166	25 July 2016
Teabags retrieved		180	8 August 2016

4.2.2. Mesh bag preparation, burial and retrieval

A series of wheat- and OSR straw samples (residues) were buried, as specified in the following paragraphs, in plots cropped with 1st wheat, 2nd wheat and OSR (microsites; OSR-WW, WW-WW and WW-OSR, respectively). Sub-samples of the crop residues were dried at 60 ºC, milled to a fine powder, and analysed for total C and N (Flash 2000, Thermo Fisher Scientific, Cambridge, U.K., 109% recovery of both C and N for in-house reference material traceable to certified reference material GBW 07412) and the total concentrations of micronutrients were determined by ICP-OES (inductively coupled plasma optical emission spectroscopy; Perkin

Elmer Optima 7300 Dual View, recovery rates of 99% (P), 94% (K), 102% (Mg), 114% (Fe), 104% (Zn), 96% (Ca), and 92% (Mn) of in-house hay reference material traceable to certified reference NCSDC 73349) analysis of 0.5 g residues samples digested in 8 ml of nitric acid using MARS 6 microwave digestion system (Table 4.3).

Table 4.3. Residue characterisation (SEM included in parentheses; n = 3).

Nutrient	Wheat straw	OSR straw	Rooibos tea[1]	Green tea[1]
C (g/kg)	454.1 (1.60)	463.0 (1.16)	505.1 (1.7)	490.6 (0.6)
N (g/kg)	6.6 (0.05)	5.0 (0.03)	11.9 (0.3)	40.2 (0.3)
C:N	68.5 (0.41)	93.3 (0.6)	42.9 (1.06)	12.2 (0.07)
P (mg/kg)	1433 (7.0)	694 (10.1)		
K (mg/kg)	14341 (59.3)	2965 (34.5)		
N:P	0.0046	0.0072		
Mg (mg/kg)	649 (1.9)	196 (1.1)		
Fe (mg/kg)	653 (11.5)	132 (3.0)		
Zn (mg/kg)	13 (0.3)	5 (0.3)		
Ca (mg/kg)	6578 (22.6)	17077 (216.3)		
Mn (mg/kg)	65 (0.5)	10 (0.1)		

[1] Data taken from (Keuskamp *et al*., 2013)

Mesh bags with the residues were prepared on 6 and 7 February 2016, and buried at 15 cm depth on 10 (i.e. day 1 of experiment) and 12 (block 3 only) February 2016 (Table 4.2). Twelve mesh bags containing wheat residues and twelve containing OSR residues were buried in each plot. Every four weeks two replicate wheat mesh bags and two replicate OSR mesh bags were retrieved, resulting in six retrievals over 24 weeks in total. Along with a numbered colour-coded identification tag, 5 g (±5%) of residue – OSR or wheat straw (internodes only) – were inserted into each mesh bag (Schwegmann Filtrations-Technik, polyamide monofilament, 500 µm mesh size, 12 cm × 12 cm; Figure 4.1). A 500 µm mesh was chosen to allow access to microorganisms but exclude most mesofauna (Appendix B.4 for complete rationale of choosing 500 µm mesh-size). Mesh bags were closed up with 100% polyester sewing thread. Each mesh bag was stored in a plastic zip-lock bag until burial. Mesh bag "blanks" were also performed by burying and directly thereafter retrieving 10 bags of each residue, to account for mass loss in the process of transporting, burying and retrieving mesh bags without decomposition of the residues.

Mesh bags were retrieved with a spade on days 26, 54, 82, 110, 138, and 166 of the experiment (Table 4.2), dried in a drying cabinet at about 50 ºC, and subsequently cut open to sort the residues. This involved removing soil and roots that had entered the mesh bag as much as possible and placing the residues in a pre-dried and pre-weighed paper bag for final drying of residues at 60 ºC overnight. Dried bags were cooled in a desiccator and weighed. Drying, desiccating and weighing were repeated once more and the average weight taken. Finally, because some soil had entered

the mesh bags and was stuck to the residues, each residue sample was ashed overnight in a crucible at 550 ºC to account for the mineral content and determine the ash-free dry mass.

To assess the baseline decomposition rate in each plot, and by extension the soil microbial activity, Lipton Green and Rooibos teabags were buried on 22 May 2016 (day 102) and retrieved on 8 August 2016 (day 180), following the Tea Bag Index (TBI) protocol (Keuskamp *et al*., 2013). During this period, all labile substrates from Green tea are considered to be decomposed, but the actual decomposable fraction may deviate because environmental factors may lead to stabilisation (recalcitrance) of some of the labile compounds. This allows for calculation of the stabilisation factor S_{TBI}. Rooibos tea is less decomposable and labile fractions are still decomposing when the teabags are retrieved, allowing for estimation of the decomposition rate constant k_{TBI} (for further details, see Keuskamp *et al*., 2013). Tea has never been grown at the site, so it was deemed to be a foreign substrate to the soil microbial community in all the plots, such that an HFA effect would not apply. Thus, the TBI serves as a general assessment of the inherent activity of the soil microbial community.

4.2.3. Soil sampling

Topsoil sampling was performed in a zig-zag fashion using a 30-mm diameter gouge auger. Soils were sieved to 4 mm immediately after sampling and stored at 4 ºC. Initial soil samples were taken on 12 (blocks 3 and 4) and 15 February 2016 to assess baseline conditions. Subsequent soil samples were taken concomitant with each mesh bag retrieval and analysed for soil available N and microbial biomass C to determine soil conditions in each plot over time.

Available N (i.e. sum of NO_3^- and NH_4^+) was extracted from 40 g of dry-soil equivalent shaken for 30 minutes in 200 ml 1 M KCl (99.5% purity). Extracts were filtered through Whatman no. 2 filters and analysed colourimetrically for nitrate and ammonia on a Skalar San^{++} continuous flow analyser. Available N was calculated per gram of dry soil extracted and taken as the sum of the NO_3^- and NH_4^+ measured in the extract.

Soil microbial biomass C was determined by fumigation-extraction (Vance *et al*., 1987). Samples of 50 g dry-soil equivalent were weighed into borosilicate glass beakers that were placed in a vacuum desiccator lined with moist filter paper along with ~50 ml ethanol-free chloroform and some anti-boiling chips. The desiccator was evacuated until the chloroform boiled for ~2 minutes, kept under vacuum in the dark for 24 hours, the chloroform removed and the desiccator evacuated 4 times the following day, leaving the samples to vent off any remaining chloroform. Fumigated

and unfumigated soils were extracted in 200 ml 0.5 M K_2SO_4 (> 99.5% purity), shaking for 30 min and filtered using Whatman no. 42 filters. Diluted (10 ×) samples were analysed for organic C on a TOC analyser (Shimadzu TOC-L CPH).

4.2.4. PLFA analysis

Additional soil samples were taken at the beginning (4 March 2016) and end (5 July 2016) of the growing season, immediately frozen and subsequently freeze-dried to assess microbial community structure using phospholipid fatty acid (PLFA) profiles, following Sizmur *et al*. (2011). This method exploits the fact that fungi, gram-negative, gram-positive, mycorrhizal fungi and actinomycetes each exhibit PLFAs with different structural compositions. Soils were extracted using Bligh and Dyer solvent (Bligh and Dyer, 1959) according to Frostegård and Bååth (1996), extracted phospholipids were derivatised according to Dowling *et al*. (1986) and analysed as fatty acid methyl esters by gas chromatography (Agilent 6890N, flame ionisation detector and a 30 m × 0.25 mm capillary column with a 0.25 μm film of 5% diphenyl, 95% dimethyl siloxane) according to Frostegård *et al*. (1991). The internal standards used were methyl tetradecanoate (C14:0; Sigma-Aldrich) and methyl nonadecanoate (C19:0; Sigma-Aldrich; 96.0% purity).

PLFA chromatograms were integrated and peaks identified according to the retention time and peak area based on BAME and FAME chromatograms provided by the supplier of the standards (Supelco, Supelco UK, Poole, UK), as well as soil-sample chromatograms with peaks previously identified using GC-MS at Cranfield University. A broad selection of peaks between C14 and C20 were included in further data analyses, namely bacterial PLFAs i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7t, 16:1ω5, i17:0, 17:0cy, 17:0 and 19:0cy; fungal PLFAs 18:2ω6c and 18:1ω9c; and general PLFA indicators 14:1ω9c, i16:1, 16:1ω11t, 16:1ω7c, 16:0, 17:0brα, 17:0brβ, 17:1ω8c, 17:1ω7, 12me-17:0, 10me-17:0 (actinomycetes), 18:3(5,10,12), 18:1ω9t, 18:1ω13, 18:1ω10or11, 18:0, 19:1ω6, 10me-18:0 (actinomycetes), 19:1ω8, 19:0, 20:4ω6 (protists), 20:5ω3, 20:1ω9, and 20:0 (Bååth and Anderson, 2003; Bardgett *et al*., 1999; Frostegård *et al*., 1993; Frostegard and Bååth, 1996; Kaur *et al*., 2005; Kominoski *et al*., 2009). Concentrations of PLFAs were calculated based on FAME calibration standards, and data were adjusted to blanks (noise/contamination) and the internal standard C19:0. Although we recognise the shortcomings of this approach (see e.g. Strickland and Rousk, 2010), the fungal:bacterial (F:B) ratio was calculated based on the classification of PLFAs specified above and using the biomass of PLFAs calculated from the FAME calibration. Previous studies have attempted to determine a conversion factor for fungal biomass from PLFA analysis (e.g. Bååth and Anderson, 2003; Frostegård *et al*., 1991; Klamer and Bååth, 2004), but due to the lack of agreement, we have not applied any conversion factor

and calculate F:B simply as the quotient of the biomass of fungal and bacterial fatty acids as determined by the method specified above. This will provide some indication of the differences between microsites and over time in these microbial groups.

4.2.5. Data analyses

Statistical analyses were performed in R 3.5.1 (R Foundation for Statistical Computing) using RStudio 1.1.456 (RStudio, Inc.), GenStat 18.2.0.18409 (VSN International, 2016; used for repeated measures ANOVAs only), and SAS 9.4 (Intel Corporation, 2016; used for HFA model proposed by Keiser *et al*. (2014) only).

Initial and final weights of residues (adjusted for ash content) were fit to a first-order rate of decay function: $M_t = M_0 e^{-kt}$ (M_t – residue mass at time t, M_0 – initial residue mass, k – rate of decay per day). The decomposition rate constant (k) was derived per residue per plot and the mean k was calculated per treatment (residue × microsite) to assess the presence of an HFA effect for each residue type.

The main test to determine if the HFA hypothesis applied, was a two-way analysis of variance (ANOVA) of k, using the factors microsite and residue, where a significant interaction between microsite and residue would indicate presence of a HFA effect. Blocking was accounted for as an error factor. Subsequently, multiple one- and two-way ANOVAs with and without a range of covariates (e.g. TBI, the average of the available N levels) and with blocking as an error factor were performed to find out if any sign of an HFA effect could be detected for both or either of the residues. The (observed) M_t/M_0 over time was analysed by a repeated measures ANOVA, with and without a range of covariates. We also analysed the data according to the model developed by Keiser *et al*. (2014), which accounts for the ability of the soil microbial community to decompose substrates, the decomposability of the residue, and the HFA effect by defining mass loss = soil ability + litter ability + home interaction (HFA).

The TBI was calculated according to (Keuskamp *et al*., 2013), producing the decomposition rate constant (k_{TBI}) and stabilisation factor (S_{TBI}) of the soil in each plot. In addition, the mass loss of Green and Rooibos tea was calculated individually based on the initial and final tea mass.

Using a k_{EC} factor of 0.36 (Martens, 1995), the microbial biomass C (MBC) was determined per kilogram dry soil as: MBC = k_{EC} × (organic C extracted from fumigated soil – organic C extracted from unfumigated soil).

Quantified PLFA data were normalised, and analysed by nonmetric multidimensional scaling (NMDS) ordination using the vegan package in R.

4.3. Results

4.3.1. Residue decomposition

Both wheat and OSR residues decomposed at the same rate as each other over time (Figure 4.2) and between microsites (Figure 4.3). Decomposition of wheat and OSR residues buried in the different microsites followed a first-order rate of decay (Figure 4.2). About 62% of OSR and 66% of wheat residues decomposed during the experiment. The relative mass loss (M_t/M_0) over time did not differ significantly between microsites for each residue (OSR: $F = 0.17$, $p = 0.84$; wheat: $F = 1.02$, $p = 0.40$; repeated measures ANOVA) and data analysis with covariates (available N and microbial biomass C) also did not result in the emergence of significant differences between microsites.

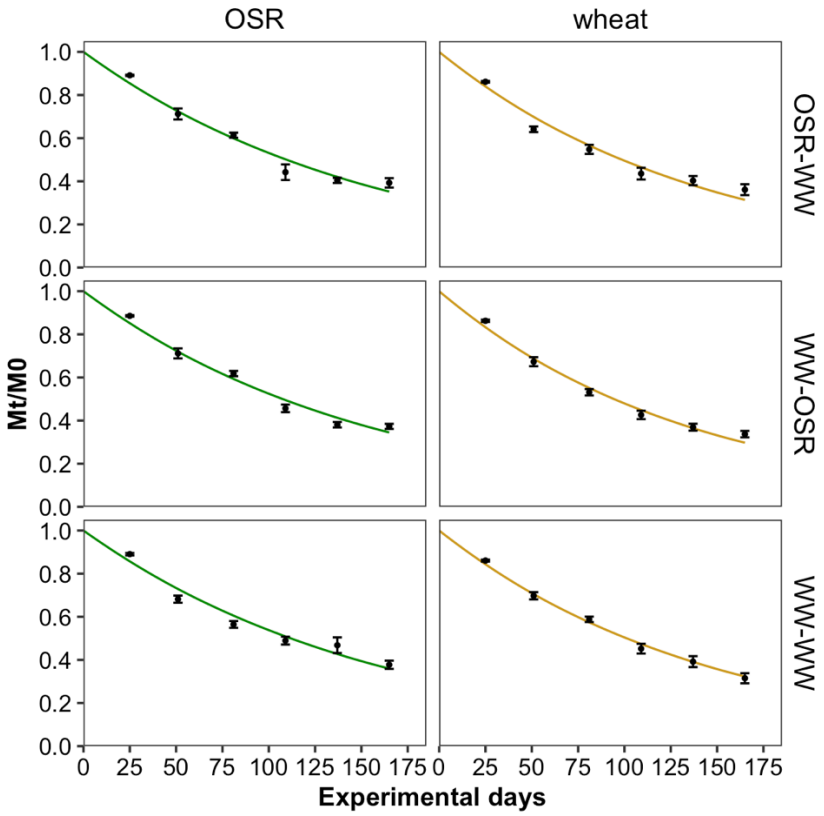


Figure 4.2. Average remaining residue fraction (M_t/M_0) over time with the observed residue fraction remaining (●) fitted to a first order rate of decay model (—). Residue types and microsites are indicated along the top and right-hand side. Error bars represent standard error of the mean (n = 8).

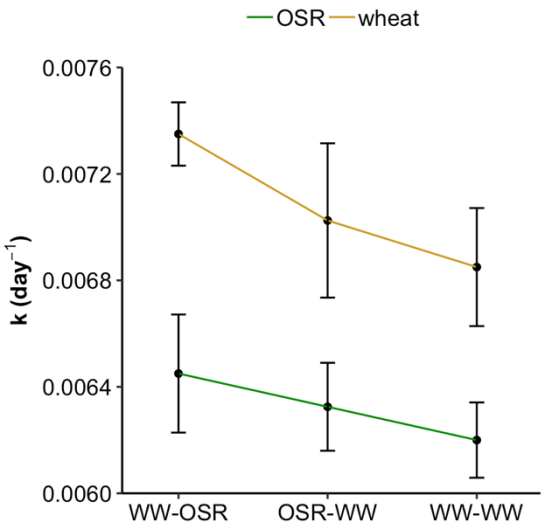


Figure 4.3. Mean optimised decomposition rate constant (*k*) to assess presence of an HFA effect between microsites per residue. Error bars represent standard error of the mean (n = 4).

The decomposition rate constant (*k*) of both residues followed a similar pattern (Figure 4.3), decomposing fastest in WW-OSR > OSR-WW > WW-WW (see Table 4.1 for microsite labels), although there were no significant differences between microsites ($F = 1.582$, $p = 0.238$). Decomposition rates did differ significantly between the residues ($F = 18.738$, $p < 0.001$; two-way ANOVA). After thorough investigation of the data by means of ANOVAs with a range of covariates (Appendix B.1), no HFA effect could be detected, since there was no significant interaction between microsite and residue type. In addition, the relative mass (M_t/M_0) of residue remaining at the end of the experiment was analysed according to the model proposed by Keiser *et al*. (2014), which takes the ability of the soil decomposer community and the decomposability of the residues into account. This revealed no HFA effect either.

4.3.2. Soil biochemical properties

Soil available N was monitored during the experimental period concomitant with each mesh bag retrieval instance. Available N levels differed significantly over time ($p < 0.001$; repeated measures ANOVA) increasing after fertilisation and then decreasing to the original level (Figure 4.4). The wheat crops were fertilised earlier than the OSR crops. While this may have caused a disproportionately greater available N level in the WW-OSR microsite in month 3, the greater available N levels in the WW-OSR plots persists during the subsequent months, suggesting the

OSR crops did not grow well and/or greater N retention in the WW-OSR plots. This caused significantly different available N levels between microsites ($F = 25.02$, $p < 0.001$), over time ($F = 31.07$, $p < 0.001$) and a significant interaction of time × microsite ($F = 14.48$, $p < 0.001$; repeated measures ANOVA). Fluctuations in the microbial biomass C were similar in all microsites ($F = 0.12$, $p = 0.89$; repeated measures ANOVA), but changed significantly over time ($F = 21.32$, $p < 0.001$; repeated measures ANOVA) (Figure 4.5).

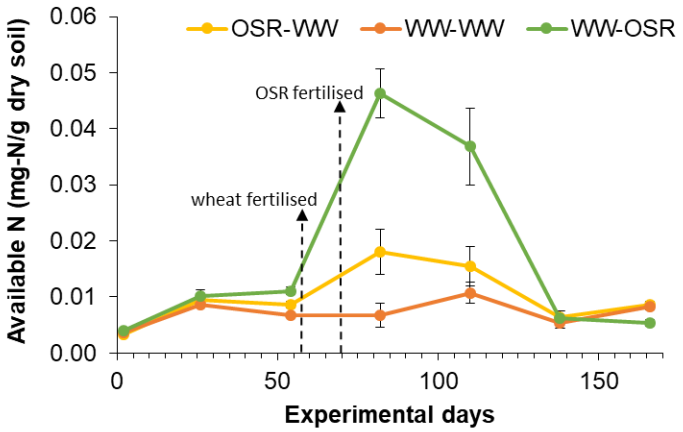


Figure 4.4. Soil available N per microsite over the course of the experimental period. Error bars represent SEM (n = 4). Timings of fertilisation indicated.

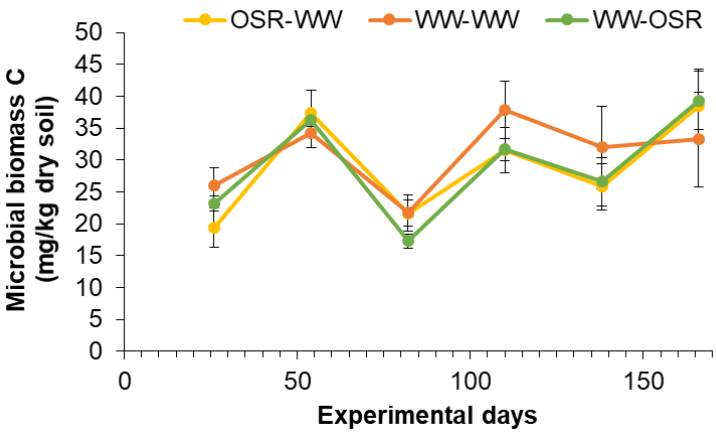


Figure 4.5. Microbial biomass C per microsite over the course of the experimental period. Error bars represent SEM (n = 4).

At the start of the growing season (day 23), the total PLFA biomass was highest in WW-OSR and lowest in WW-WW (Figure 4.6a). At the end of the growing season (day 146), the total PLFA biomass was slightly higher in OSR-WW compared to WW-OSR, and still lowest in WW-WW. This is somewhat different from the microbial biomass C at those time points in terms of the comparisons between treatments, but differences in PLFA biomass between microsites were not

significant (start: F = 0.92, p = 0.45; end: F = 2.14, p = 0.20; one-way ANOVA). In all microsites, the total PLFA biomass increased significantly by the end of the experimental period (F = 18.22, p < 0.001; two-way ANOVA), with the greatest increase observed in OSR-WW.

The fungal:bacterial (F:B) ratio (Figure 4.6b) was greater at the start than at the end of the experimental period, although this was not statistically significant (F = 1.84; p = 0.19; two-way ANOVA). In the WW-OSR microsite the decrease in F:B over time was lowest. Between the microsites the F:B ratios were quite similar (start: F = 0.202, p = 0.823; end: F = 0.171, p = 0.847; one-way ANOVA), although WW-WW microsites exhibited the highest F:B ratio at both the start and end of the experimental period. There was a relatively greater increase in bacterial fatty acids during the experimental period compared to fungal fatty acids (Appendix B.3). The ratio of Gram positive to Gram negative (G+:G–) fatty acids (Figure 4.6c) also differed significantly over time (F = 8.68, p = 0.008; two-way ANOVA), but not between microsites (start: F = 0.996, p = 0.423; end: F = 0.549, p = 0.67).

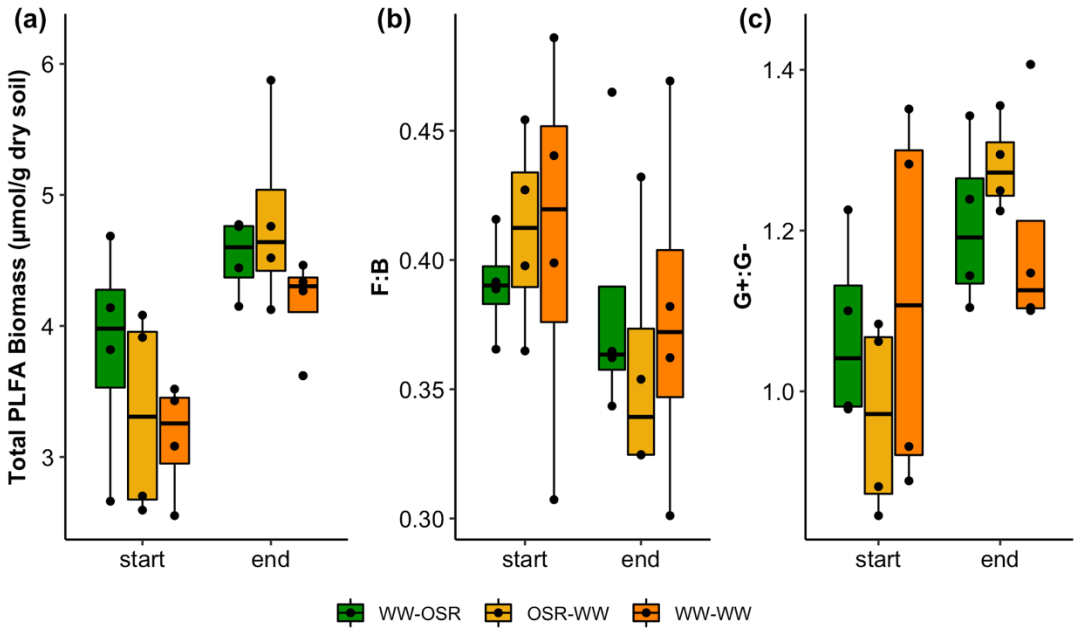


Figure 4.6. Total PLFA biomass per microsite at the start (March 2016, day 23) and end (July 2016, day 146) of the growing season **(a)**; fungal:bacterial ratio of fatty acids per microsite at the start (March 2016) and end (July 2016) of the growing season **(b)**; Gram+:Gram- ratio of fatty acids per microsite at the start (March 2016) and end (July 2016) of the growing season **(c)**. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints (n = 4).

Ordination of the PLFA profiles enables comparison of the microbial community structure between microsites (Figure 4.7). This reveals a clear separation in the microbial communities that were present at the start versus the end of the growing season (F = 11.24, p < 0.01). At each time point (start and end) the polygons in the ordination plot show considerable overlap, indicating

that the microbial communities did not differ notably between the microsites per time point ($F = 0.91$, $p = 0.50$; PERMANOVA). This suggests a change over time in the microbial community structure that was similar in all microsites.

The biomass of some individual fatty acids did differ significantly between microsites: C15:0ai, C16:0i, C16:0, C17:0brβ, C17:1ω7, C18:1ω13, C19:0cy, and C20:0 (a combination of G+ and G– bacterial biomarkers). The biomass of these fatty acids followed a similar trend between microsites; they were highest in WW-OSR > OSR-WW > WW-WW at the start of the growing season, and highest in OSR-WW > WW-OSR > WW-WW at the end of the growing season. A two-way ANOVA of the effects of time and microsite on these fatty acids combined shows that WW-OSR and WW-WW microsites are significantly different ($p = 0.037$, post-hoc Tukey HSD) (see Appendix B.3 for more details).

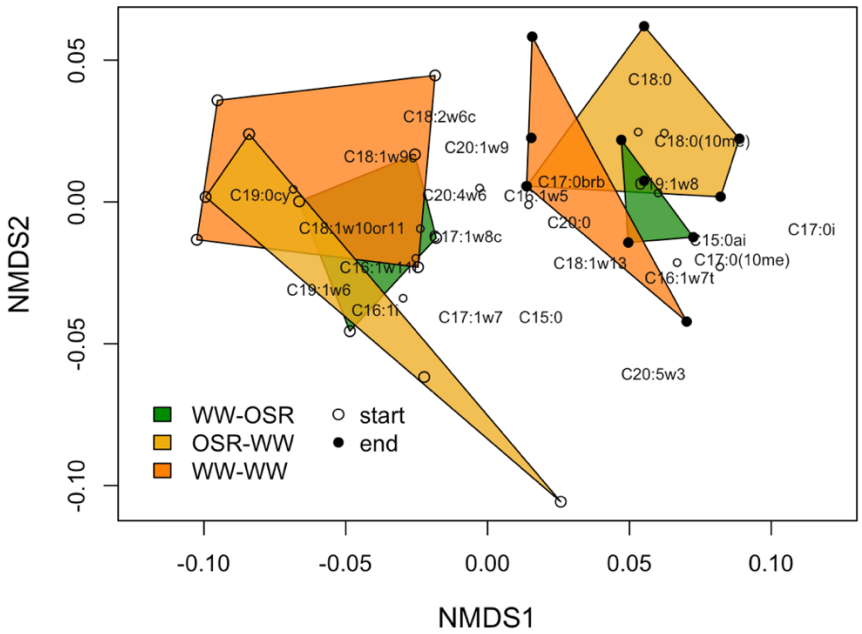


Figure 4.7. Nonmetric multidimensional scaling (NMDS) ordination of relative abundances of identified fatty acids at the start (day 23) and end (day 146) of the experimental period (based on Bray-Curtis dissimilarity matrix; stress = 0.12). Each circle represents a PLFA profile of a replicate plot of the microsites. Circles that are closer together exhibit more similar microbial communities. Vectors of individual fatty acids are also plotted with labels.

4.3.3. Tea Bag Index

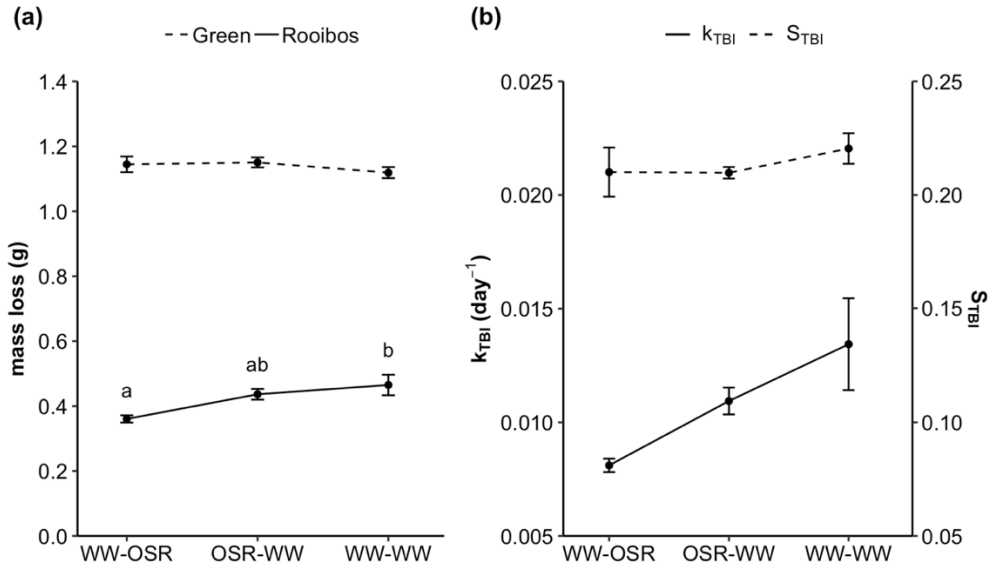


Figure 4.8. Mass loss of each tea type after 78 days in the different microsites **(a)**; and the baseline decomposition rate constant (*k*) and stabilisation factor (S), as determined by the TBI protocol, for the different microsites **(b)**. Error bars represent SEM (n = 4). Different letters indicate significant differences (post-hoc Tukey HSD, $p < 0.05$).

As expected from the principles that underlie the TBI protocol (Keuskamp *et al*., 2013), Green tea underwent more decomposition during the 78-day period than Rooibos tea (Figure 4.8a), which can be attributed to the lower C:N ratio and higher hydrolysable fraction of Green tea. Green tea mass loss, which was used to calculate the stabilisation factor (S_{TBI}), did not differ significantly between microsites ($F = 0.55$, $p = 0.60$). Rooibos mass loss, which was used to calculate the baseline decomposition rate (k_{TBI}) of the different microsites, differed significantly between microsite ($F = 5.25$, $p = 0.048$). The values of k_{TBI} differed somewhat between microsites ($F = 4.20$, $p = 0.072$), increasing in the order WW-OSR < OSR-WW < WW-WW, with the greatest contrast between WW-OSR and WW-WW plots ($p = 0.062$, Tukey HSD) (Figure 4.8b). This decomposition pattern observed for tea is contrary to the decomposition of OSR and wheat residues, which decomposed fastest in WW-OSR. The values of S_{TBI} did not differ significantly between microsites ($F = 0.66$, $p = 0.55$) (Figure 4.8b).

4.4. Discussion

4.4.1. Absence of HFA within a short-rotation arable cropping system

We tested the HFA hypothesis within an arable cropping system using OSR and wheat residues in a reciprocal transplant experiment in an intensive crop rotation. According to the HFA

hypothesis, which postulates a faster decomposition rate of *home* residues compared to residues that come from *away*, we would have expected OSR straw to decompose fastest in plots previously cropped with OSR (i.e. OSR-WW; see Table 4.1), and straw to decompose fastest in plots previously cropped with wheat (i.e. WW-OSR and WW-WW; see Table 4.1). However, we observed no such differences and found that the decomposition of both residues followed the same trend over time (Figure 4.2) and between microsites (Figure 4.3), decomposing fastest in WW-OSR, then OSR-WW and then WW-WW. Differences in the decomposition rates (k) between the microsites were not statistically significant, and more importantly, interactive effects of residue type × microsite that would indicate existence of an HFA were not statistically significant ($p > 0.05$), even after thorough investigation of the data (see Appendix B.1). Therefore, we do not accept the HFA hypothesis in this experiment.

Greater chemical inputs in arable farming have decreased dependency on legume crops for maintenance of sufficient soil N levels and on non-cereal crops for pest control, which has allowed for a move towards shorter and simpler crop rotations (Robinson and Sutherland, 2002; Tisdale *et al*., 1985). These intensively managed systems differ from forests and grasslands in a number of ways: (1) they are frequently tilled, disturbing the soil structure and therefore habitats for soil faunal species; (2) plant species are established for only one growing season and tend to grow in a monoculture; (3) removal of plant materials at harvest time reduces the amount of plant litter returned to the soil compared to natural systems; (4) soils are often left fallow for a period of time, as opposed to more continuous establishment of vegetation in forests and grasslands; and (5) application of exogenous fertilisers, pesticides and fungicides impacts on the soil microbial community. It has been suggested that an HFA may not be detected in soils that have been recently disturbed or where succession is relatively young (Austin *et al*., 2014; Gießelmann *et al*., 2011). Austin *et al*. (2014) point out that HFA effects are mostly observed in ecosystems without human disturbance, which is not the case in intensively managed arable cropping systems that are subject to ploughing, chemical inputs of fertilisers and pesticides, periods of fallow following crop harvest, and perhaps most importantly, short vegetation establishment compared to perennial systems. All these activities impact on the soil microbial community. Gießelmann *et al*. (2011) tested the HFA in forests at three successional stages with different tree species composition and attribute the absence of an HFA to a soil microbial community that rapidly adapts to changes in litter quality additions. However, Veen *et al*. (2018), who also studied HFA at different successional stages, showed that the influence of successional stage on HFA depends on the conditions in the system considered, with high C:nutrient ratios in the litter and high SOM:nutrient ratios in the soil leading to greater HFA effects. Other studies identified contrasting litters and contrasting microsites as determinants of HFA effects (Li *et al*., 2017; Veen *et al*.,

2015). Therefore the absence of a HFA effect here may be due to similarity between the residue types and between the microsites.

4.4.2. Residue quality and nutrient availability

The faster decomposition of wheat straw, compared to OSR residues, could be related to differences in the chemical characteristics of the residues. The chemical quality of wheat straw was higher because it had a lower C:N ratio (Table 4.3). The relatively high C:N ratios of neither residue could fulfil the stoichiometric N requirements of soil microbes, although based on their C:N ratios, less soil-derived N would have been necessary to decompose the wheat residues compared to the OSR residues. Whether inorganic N additions enhanced or inhibited decomposition probably depends on the C:N ratio of the residues and the initial C:N ratio of the soil (Finn *et al*., 2015; Hobbie, 2005). In this study, greater levels of available N were retained in the WW-OSR microsite compared to OSR-WW and WW-WW microsites (Figure 4.4), which may have facilitated decomposition of both wheat and OSR straw under OSR by satisfying microbial N demand. However, the impact of N fertilisation on decomposition dynamics, and by extension on the existence of any HFA effects, is complex. Addition of N by fertilisation can increase decomposition rates by alleviating microbial N demand and by stimulating the production of cellulose-degrading enzymes, but also decrease decomposition rates by reducing fungal biomass and suppressing lignin-degrading enzymes (Carreiro *et al*., 2000; Frey *et al*., 2014; Hobbie, 2005; Waldrop *et al*., 2004).

Another attribute that may have made straw easier to decompose is its higher manganese (Mn) content. Many studies have shown the significance of Mn in decomposition of forest litters (e.g. Berg *et al*., 2007, 1996; Keiluweit *et al*., 2015) as well as the role of initial Mn concentration of litter, particularly when lignin decomposition is involved, because manganese peroxidase is an enzyme employed by white-rote fungi to degrade lignin (Berg *et al*., 2015; Sun *et al*., 2019). Wheat residues contained more than six-fold the amount of Mn present in OSR residues, which may further help explain the higher decomposition rate of wheat compared to OSR. Additionally, as a brassica species, OSR releases isothiocyanates (biofumigant glucosilonate compounds) upon decomposition, which may have inhibited the decomposition of OSR residues. Residues of the OSR crops grown in plots were incorporated at the end of previous growing seasons, but on the microsite scale, no inhibiting effects were observed in the microbial biomass C or the microbial community structure or biomass. The microbial biomass C and the total PFLA biomass at the start of the season in OSR-WW or the end of the season in WW-OSR microsites were not noticeably lower than other plots (Figures 4.5 and 4.6a), except maybe for the microbial biomass

C in the OSR-WW plots at the start of the experimental period, but not significantly so. Moreover, both residues actually decomposed fastest in the WW-OSR microsite, further suggesting the biofumigant effect did not suppress decomposer organisms. Only k_{TBI} was low in WW-OSR compared to the other microsites.

4.4.3. Soil microbial community structure

In this study, the soil microbial community structure, determined by PLFA analysis, was similar in all microsites and changed similarly over time regardless of the crop rotation phase (Figure 4.7), contrary to our hypothesis. Ordination plots of the PLFA data do not identify the formation of a specific *home* microbial community that can be distinguished from the other microsites, nor do determinations of the PLFA biomass, F:B ratio or G+:G– ratios. The lack of an identifiably different microbial community further supports the finding that an HFA effect could not be found in this system.

We did observe a shift over time in the microbial community structure that was similar for all microsites, suggesting an adaptation to environmental factors that changed equally across all plots, such as temperature and moisture. A shift over time was also observed in the ratio of G+:G– bacteria. Fanin *et al*. (2019) found that the G+:G– ratio increased when sources of labile C were removed and suggest a relationship of G– bacteria with simple organic substrates, and of G+ bacteria with more complex organic substrates, thus the ratio of G+:G– bacteria may indicate availability of carbon. In our experiment, both the biomass of G+ and G– bacterial fatty acids increased over time, but the increase was greater for G+ bacterial fatty acids, suggesting the soil microbial community may have adapted to being able to decompose more complex forms of C. Easily decomposable substrates were probably consumed first, such that the more complex organic compounds were left towards the end of the experimental period.

Optimisation of the soil microbial community, to which the existence of an HFA is attributed, is typically considered to be a long-term process because specialisation may decrease the functional redundancy of a community (Keiser *et al*., 2011) and therefore decrease the ability of the community to adapt to environmental changes, like inputs of organic substrates with different chemical qualities. There tends to be a link between habitat specialisation and life strategies, with *r*-strategists being generalists and *K*-strategists being specialists (McKinney, 1997; Sakai *et al*., 2001). It has also been suggested that even low remaining abundances of species adapted to the decomposition of crops that grew in its soil several seasons earlier can regenerate relatively quickly in response to a new litter input (Gießelmann *et al*., 2011). Therefore, it may be that the

soil microbial community in the arable cropping system in this experiment is composed of mostly *r*-selected species that are not specialised for a particular environment, which can be attributed to soil disturbance and changes in residue inputs from year to year that have prevented the establishment of a soil microbial community including specialised *K*-selected species.

Fungi represent one group of microbes that is typically thought of as *K*-strategists (Fontaine *et al*., 2003), although this is not always the case (Fierer *et al*., 2007). Lin *et al*. (2018) found fungi to play a prominent role in driving HFA effects for not only low-quality but also high-quality litters in broadleaf and bamboo forests. This could be due to the fungi being K-selected and significantly contributing to specialisation of the soil microbial community. There were no significant differences in fungal biomarkers in the microsites in our study (also see Appendix B.3). Inorganic fertilisers have been shown to lead to decreased fungal- and increased bacterial richness (Chen *et al*., 2016), and higher F:B ratios are generally observed in less disturbed systems (Gregory *et al*., 2009). Therefore the role of fungi and/or *K*-strategists in general, might be downplayed in intensively managed arable cropping systems, reducing the chance of observing an HFA.

4.4.4. Tea Bag Index

The Tea Bag Index can be used as a measure of microbial activity, where k_{TBI} is representative of the ability of the soil decomposer community and S_{TBI} is an indication of the degree of inhibition from environmental conditions on the decomposition of labile substrates (Keuskamp *et al*., 2013). The k_{TBI} and S_{TBI} values reported in this experiment are within the range of values expected from the protocol (Keuskamp *et al*., 2013). There were no significant differences in k_{TBI} in the different microsites, suggesting the microbial activity and ability to decompose were similar in all plots and unaffected by the release of glucosilonates in the WW-OSR plots (Figure 4.8b). However, the mass loss of Rooibos tea was lower in the WW-OSR plots compared to the wheat plots, and significantly lower compared to the WW-WW plots (Figure 4.8a). Therefore, decomposition may have been inhibited in the WW-OSR plots. This was taken into account by testing HFA with the mass loss of Rooibos tea as a covariate, but like all other statistical analyses, did not result in detection of an HFA (Appendix B.1).

It is interesting that the decomposition patterns of the residues and tea contradicted each other: k_{TBI} followed the order WW-OSR < OSR-WW < WW-WW, while k of both crop residues followed the order WW-WW < OSR-WW < WW-OSR. On the mesh-bag scale, the decomposing OSR residue may have had a biofumigant effect and negatively influenced decomposer activity

leading to lower decomposition rate of the OSR residues themselves (see above), but because the residues in the mesh bags were retained in a small space, this would not have affected the decomposition in teabags, and therefore cannot explain the low k_{TBI} in WW-OSR.

4.4.5. HFA in different arable crop rotations

Despite not finding any HFA effect in our experiment, studies on the HFA hypothesis are highly variable in their results, with many reports on both the presence (Ayres *et al*., 2009; Lin *et al*., 2018) and absence (Ayres *et al*., 2006; Gießelmann *et al*., 2011; St. John *et al*., 2011) of an HFA, both within and between ecosystems. Therefore, despite not observing an HFA effect in this experiment, we do not reject the possibility of there being an HFA in some arable cropping systems. Perhaps the prolonged cropping of wheat increased specificity of the soil microbial population for being able to decompose wheat residues, while decreasing the ability to decompose chemically different residues like tea. Although we found no HFA between the different microsites, wheat straw exhibited a higher decomposition rate than OSR straw in all plots, which could be related to a historically greater exposure to wheat crop and residues in the field site (legacy effect). If that is the case, the HFA effect might still apply to arable cropping systems, but between different crop rotations rather than within a crop rotations.

The HFA hypothesis could be further tested in a future experiment where (1) both residue types are buried in a crop rotation dominated by wheat as well as in a different crop rotation dominated by another crop; or (2) even within a rotational system that includes more contrasting crops; or (3) in a perennial system where plants are established for longer periods of time. Where and how an HFA applies depends on the mechanisms that underlie the optimisation of the microbial community involved in the HFA, which remain poorly understood (Austin *et al*., 2014). Proposed mechanisms include litter-decomposer interactions ranging from (1) microbial selection via root exudates or plant litter volatiles, (2) “green-leaf hitchhikers” that persist from green leaves/stalks to the litter stage, and (3) three-way interactions where plants influence soil microbial and microarthropod communities, whose frass (arthropod excrement) production further selects for microbes (Austin *et al*., 2014). To more fully assess the applicability of the HFA to arable cropping systems, we need a better understanding of the mechanism (see Appendix D) underlying the optimisation of the soil microbial community to which the HFA is attributed. Further experiments in different arable cropping systems and alternative research methods (e.g. field-scale amendment with residues instead of litterbags) are needed to determine in what circumstances (if any) an HFA can be observed within an arable cropping system.

4.5. Conclusions

The decomposition rates of wheat and OSR residues in a reciprocal transplant experiment within a short-rotation arable cropping system did not reveal an HFA effect. We mainly attributed this to the similarity in the soil microbial community structures in the different microsites (stages of the crop rotation), which could be related to high levels of soil disturbance and short duration of crop establishment in these intensively managed systems. We further suggest that the decomposition rates observed here may be better explained by the chemical quality of wheat residues compared to OSR, and the levels of soil available N in each microsite.

Chapter 5 – Obtaining more benefits from crop residues as soil amendments by application as chemically heterogeneous mixtures

Note on publication strategy: This chapter is intended for publication with the following author list:

Marijke Struijk[1,2], Andrew P. Whitmore[2], Simon Mortimer[3] and Tom Sizmur[1]
[1] Department of Geography and Environmental Science, University of Reading, Reading, UK.
[2] Department of Sustainable Agriculture Sciences, Rothamsted Research, Harpenden, UK.
[3] School of Agriculture, Policy and Development, University of Reading, Reading, UK.

Abstract

Crop residues are valuable soil amendments in terms of the carbon and other nutrients they contain, but incorporation of residues does not always translate into increases in nutrient availability, soil organic matter (SOM), soil structure, and overall soil fertility. Studies have demonstrated accelerated decomposition rates of chemically heterogeneous litter mixtures, compared to the decomposition of individual litters, in forest and grassland systems. Mixing high C:N ratio with low C:N ratio amendments may result in greater carbon use efficiency and non-additive benefits in soil properties (i.e. mixture ≠ sum of the parts).

We hypothesised that non-additive benefits would accrue from mixtures of low-quality (straw or woodchips) and high-quality (vegetable-waste compost) residues applied before lettuce planting in a full-factorial field experiment. Properties indicative of soil structure and nutrient cycling were used to assess benefits from residue mixtures, including soil respiration, aggregate stability, bulk density, SOM, available and potentially mineralisable N, available P, K and Mg, and crop yield.

Soil organic matter and mineral nitrogen levels were significantly and non-additively greater in the straw-compost mixture compared to individual residues, which mitigated the N immobilisation occurring with straw-only applications. Addition of compost significantly increased soil available N, K and Mg levels. Together, these observations suggest that greater nutrient availability improved the ability of decomposer organisms to degrade straw in the straw-compost mixture.

We demonstrate that mixtures of crop residues can influence soil properties non-additively. Thus, greater benefits may be achieved by removing, mixing, and re-applying crop residues, than by simply returning them to the soils *in situ*.

5.1. Introduction

Intensive agricultural systems, with a monoculture of crops and relying on external inputs of fertilisers and pesticides/herbicides, are criticised for their negative environmental impacts. These include the degradation of soil – particularly degradation of soil organic matter (SOM), biodiversity loss, and over-application of N and P (Malézieux *et al*., 2009; Tilman *et al*., 2002). Implementation of multispecies cropping systems (e.g. Malézieux *et al*., 2009) and increasing functional diversity via trait-based approaches (Garnier and Navas, 2012) are some methods that have been proposed to increase biodiversity and functional complementarity of the variety of species present in arable cropping systems. These approaches can lead to more sustainable nutrient cycling, reduced soil erosion, stabilised crop production, and improvements to a system's innate capacity to resist pests, diseases and other environmental disturbances (Gurr *et al*., 2003). However, some farming systems prevent the cultivation of more than one crop in a field at any one time, and so applying mixtures of crop residues may provide an alternative route to obtaining the benefits of multispecies cropping within monocultural arable cropping systems.

Crop residues comprise the majority of plant materials harvested worldwide (Medina *et al*., 2015; Smil, 1999) and are readily available on arable farms. Containing carbon and other nutrients, they present a valuable resource as soil amendments with the potential to increase SOM and nutrient levels, which feed the soil food web (Kumar and Goh, 1999) and may increase soil aggregation and improve soil structure (Cosentino *et al*., 2006; Martin *et al*., 1955). Unfortunately, while these changes in soil properties are likely to lead to increased crop yield, decomposition of residue soil amendments does not always translate into such benefits and is instead followed by loss from the system, with lower soil N retention and C levels than expected (Catt *et al*., 1998; Powlson *et al*., 2011; Thomsen and Christensen, 2006).

Rather than applying a single crop residue, mixtures of crop residues could form a better soil amendment. Complementarity in mixtures of different residues has been previously shown in research on the decomposition rates of mixtures of moss and leaf litters in forest ecosystems and grass clippings in grassland ecosystems (Gartner and Cardon, 2004; Hättenschwiler *et al*., 2005). Synergistic non-additive mixing effects are frequently observed, i.e. decomposition of the mixture is greater than would be predicted from the rate of decomposition of individual litter types (mixture > sum of the parts), especially when the litters are chemically heterogeneous (Pérez Harguindeguy *et al*., 2008; Wardle *et al*., 1997).

Suggested mechanisms for non-additive decomposition rates of mixtures include physical, chemical and biological processes (Gartner and Cardon, 2004). Frequently cited is the mechanism that N-rich residues are thought to accelerate the decomposition of N-poor residues (Seastedt, 1984) by inter-specific transfer of nutrients in the residue mixture (Berglund *et al*., 2013; Briones and Ineson, 1996). Additionally, more heterogeneous and improved micro-environmental conditions increase habitat and resource options for decomposer organisms (Hättenschwiler *et al*., 2005), also known as the improved micro-environmental condition theory (Makkonen *et al*., 2013).

However, whether synergistic decomposition rates in mixtures are related to benefits in terms of soil nutrient and carbon management is unclear because studies on the C and N dynamics in decomposing residue mixtures are limited (Redin *et al*., 2014). It has been shown that increased plant species richness can promote soil C and N stocks via higher plant productivity (Cong *et al*., 2014) and to increased diversity and functionality of soil microbes (Lange *et al*., 2015) as well as the whole soil food web (Eisenhauer *et al*., 2013). Quemada and Cabrera (1995) found non-additivity in the C and N dynamics when mixtures of leaves and stems were decomposed compared to individual residues, with the C:N ratio of the residues playing an important role in N mineralisation. Nilsson *et al*. (2008) report synergistic effects on soil available N as well as on plant productivity when mixing *Populus tremula* litter (C:N = 40, known to decompose quickly) with *Empetrum hermaphroditum* (C:N = 77, known to decompose slowly). These experiments suggest that non-additivity in decomposition rates and changes to other soil properties could go hand-in-hand. After all, the transfer of organic matter and nutrients from crop residues to the soil requires decomposition, which involves the activity of decomposer organisms, primarily microbes.

Increasingly more evidence is emerging that SOM accumulation is primarily derived from the production of microbial residues (Ludwig *et al*., 2015; Simpson *et al*., 2007), and this microbially-derived SOM seems to be produced at the early stages of plant-residue decomposition (Cotrufo *et al*., 2015). Microbial carbon use efficiency (CUE) describes a functional trait of microbes that refers to the fraction of carbon assimilated from organic matter additions to the soil system compared to C losses to the atmosphere via microbial respiration (Allison *et al*., 2010). Different microbial species exhibit an inherent CUE window, so that they can operate at different CUE levels to fulfil their maintenance and growth C requirements depending on environmental factors (Schimel *et al*., 2007). Organic substrates can feed into different microbial metabolic pathways (e.g. anabolism vs. catabolism) or microbial communities that exhibit different overall inherent CUE levels (e.g. fungi vs. bacteria, or copiotrophs vs. oligotrophs) (Jones *et al*., 2018). Therefore, an increase in the amount of SOM from microbial activity is not linearly related to CO_2

production, or to the quantity of C applied to the soil, but depends also on the CUE of the decomposer community.

Fertilisation practices typical of intensively managed arable soils stimulate copiotrophic microorganisms (Fierer *et al*., 2012) with boom-bust population dynamics. These microbial communities tend to exhibit a lower inherent CUE window than slower growing oligotrophic communities (Ho *et al*., 2017; Roller and Schmidt, 2015). In intensively managed arable soils, the decomposition of soil-applied crop residues can lead to a large portion of residue-derived C being respired as CO_2 rather than turned into SOM (Bailey *et al*., 2002; Six *et al*., 2006). Decomposition of high-C:N residues requires microbes with a relatively high CUE, but due to N-limitation they operate towards the lower end of their CUE window (Kallenbach *et al*., 2019). Low-C:N residues, providing relatively more N, may increase the CUE of individual microbes, but can also shift the composition of the soil microbial community to one that exhibits an inherently lower CUE (Kallenbach *et al*., 2019). As suggested by Kallenbach *et al*. (2019), a mixture of crop residues of different C:N ratios could therefore achieve a more diverse microbial community comprising organisms fulfilling niches of both high and low inherent CUE windows, and may enable all species to operate at their maximum CUE. Other authors have also suggested the possibility of manipulating the functionality of the soil microbial community with soil amendments, such as Li *et al*. (2019) who report that eutrophic microbes are stimulated by organic carbon amendments and oligotrophic microbes are stimulated by chemical fertilisers. Studies have also demonstrated that changes in tree litter diversity affect both fungal and bacterial diversity (Otsing *et al*., 2018; Santonja *et al*., 2018).

Low-quality plant materials with high C:N ratios constitute the majority of crop residues produced by arable farming practices worldwide, typically involving cultivation of corn, wheat and rice (Medina *et al*., 2015). The potential of crop residue soil amendments to deliver benefits to crops would be better exploited if the decomposition processes were manipulated for C to persist in the soil biomass, necromass or other forms of (semi-)stabilised SOM, such as in soil aggregates. Generally soil amendments consisting of one large amount of a single crop residue do not always deliver benefits. We suggest that the non-additive decomposition rates observed in forest litter mixtures reinforced by recent insights into the link between CUE and the difference of C:N ratio of soil organic co-amendments, can inform strategies to obtain more benefits from crop residues as soil amendments. Mixing these crop residues to create chemically diverse crop-residue mixtures with a CUE-optimised C:N ratio to generate a greater diversity of functionally complementary microbial niches and to enable each member of the microbial community to function at a maximised CUE, could be a relatively simple method to obtain more benefits from this precious, but ubiquitous, resource. If this approach can attain higher CUE levels for high-

C:N residues, a considerable increase in net SOM could be realised in arable cropping systems, along with other beneficial changes in soil properties, leading to greater soil fertility, meanwhile increasing nutrient retention and biodiversity in otherwise monocultural arable cropping systems.

The aim of this study was to investigate the potential of chemically heterogeneous mixtures of crop residue amendments to improve soil properties for crop production. A field experiment was set up on an intensive organic arable cropping farm. Amendments of mixtures and individual crop residues were applied: vegetable waste compost was used as low-C:N (high-quality) residue, and wheat straw and woodchips were used as high-C:N (low-quality) residues. Properties indicative of soil structure and nutrient cycling were used to assess benefits from residue mixtures compared to individual residues, including lettuce crop yield, soil respiration, soil aggregate stability and bulk density, SOM, available and potentially mineralisable N, and available P, K and Mg. We predicted higher decomposition rates when mixtures of crop residues were applied compared to individual residue amendments, leading to non-additive effects in soil properties that could be beneficial for crop production. In particular, we hypothesised faster decomposition of residue mixtures to result in a higher soil respiration rate in the short term, as well as the release of greater levels of soil available nutrients (N, P, K, Mg) and SOM compared to individual residues (hypothesis 1). An increase in SOM will likely change soil physical properties, which we expected to observe as an increase in soil aggregate stability and a decrease in soil bulk density (hypothesis 2). These changes in soil physicochemical properties were subsequently expected to lead to a higher crop yield (hypothesis 3).

5.2. Methodology

5.2.1. Study site and experimental design

A field experiment was set up in an intensively managed horticultural area of lowland fen on an organic farm near Ely in Cambridgeshire, United Kingdom (52° 21' N; 0° 17' E). During the experiment, between 11 June 2018 and 26 July 2018, the field site was used for growing gem lettuce crops (*Lactuca sativa* L. var. longifolia, commercial variety 'Xamena'), following a year of celery crop in 2017, conversion to organic in 2016 (grass ley), winter wheat in 2015, and beetroot in 2014. The typical crop rotation followed by the farm is celery, followed by beetroot, celery or onion, followed by lettuce, followed by a break crop of perennial ryegrass and white clover or a cereal. The experimental plots were located on clay loam, on a roddon, a dried raised bed formed by the deposition of silt and clay from a watercourse which pushed peat to the sides. The mineral part of the soils typically do not perform as well as the surrounding organic soils

because they require more fertiliser, so we expected they would respond more quickly to residue amendments.

Table 5.1. Treatment structure composed of the factors *residue* and *compost*.

compost → *residue* ↓	**compost**	**no compost**
straw	straw-compost	straw
woodchips	woodchip-comp	woodchip
none	compost	control

Four replicates of six treatments, within a full-factorial randomised complete block design of the factors *compost* and *residue* (Table 5.1) were applied to 2 m × 6 m experimental plots within a 6 m × 48 m field site consisting of 3 × 8 = 24 plots situated between the tire tracks of farm machinery. All samples were taken from the inner 2 m × 2 m of each plot to incorporate a 4-metre long buffer zone between plots along the same strip.

Figure 5.1. Photographs of the preparation of the mixed compost **(a)**, the final compost product **(b)**, the treatments applied on the experimental plots **(c)**, and the lettuce at time of harvest **(d)**.

The residue amendment treatments were prepared on 17 May 2018. Application rates of the different amendments were 20 t ha^{-1} fresh compost (equivalent to 7 t ha^{-1} dry matter), 13.3 t ha^{-1} woodchips (equivalent to 8.7 t ha^{-1} dry matter) and 10±0.8 t ha^{-1} straw (equivalent to 9.2±0.8 t ha^{-1} dry matter). These are within the range of application rates that are common in intensive arable cropping systems in Europe (Recous *et al.*, 1995; S. Gardner, 2018, pers. comm.), and were chosen to obtain similar amounts of dry matter for each residue. These rates were consistently applied in both individual amendment treatments and mixtures, so residue-compost treatments contained roughly twice as much dry matter compared to individual amendments. Applications were spread out evenly over the plots by hand on 12 June 2018 (Figure 5.1c), followed by power-harrowing to incorporate the residues in the soil profile. Gem lettuce plugs were sown the following day.

5.2.2. Soil and residue characterisation

Baseline soil samples were collected on 11 June 2018 (before organic amendments were applied). For each plot, soil samples were collected as the combination of five 30 mm diameter soil cores taken to 20 cm depth. These 24 composite samples were air-dried, disaggregated with the aid of a mortar and pestle, sieved to 2 mm and analysed for soil moisture (at 105 ºC overnight), SOM by loss on ignition (LOI) (at 500 ºC overnight), pH (after 2 hrs shaking 2.5 ±0.005 g soil with 25 ml Ultrapure water [> 18.2 Ω/cm]), and soil texture by laser granulometry (Malvern Mastersizer 3). A portion of each soil sample was ball milled and analysed for total C and N (Flash 2000, Thermo Fisher Scientific, Cambridge, U.K., calibrated with aspartic acid, 104% N and 100% C recovery rates of in-house reference soil material traceable to GBW 07412). There were no significant treatment differences for any of these baseline soil variables, tested with a one-way analysis of variance (ANOVA) of treatments or a two-way ANOVA of the factors *residue* and *compost* (Table 5.2).

Table 5.2. Baseline soil data for each treatment (SEM indicated in parentheses, n = 4).

	Soil LOI (%)	**Soil C:N**	**Soil pH**	**Clay content (%)**
compost	7.94 (0.45)	10.77 (0.25)	8.30 (0.03)	23.3 (0.75)
straw	6.84 (0.03)	10.45 (0.09)	8.30 (0.05)	23.8 (1.18)
straw-compost	7.78 (0.51)	10.76 (0.20)	8.32 (0.04)	26.0 (1.08)
woodchip	8.03 (0.51)	10.64 (0.39)	8.27 (0.03)	24.3 (0.75)
woodchip-compost	8.29 (0.47)	10.95 (0.22)	8.32 (0.03)	26.5 (1.26)
control	8.14 (0.32)	10.79 (0.13)	8.21 (0.02)	24.5 (1.50)

All amendments were provided by the farm and sourced and prepared on-site. The compost amendment was composed of the following vegetable residues from the farm: spinach, celery,

several lettuce varieties, carrots, leeks, spring onions, onions and shallots, cabbage, bell peppers, beetroots, and mushrooms (Figure 5.1a-b). Due to the high water content of these residues, the farm co-composts with straw to provide sufficient dry matter content in the compost mixture. The straw amendment used in the treatments containing straw was winter wheat straw available on-site, and the woodchip amendment was from poplar trees commonly grown as a wind break in the local area. Dried and milled residues were analysed for total C and N (Flash 2000 as aforementioned, 109% recovery rate of both C and N of in-house reference rapeseed material, traceable to certified reference material GBW 07412). The total concentrations of P, K and Mg were determined by ICP-OES (inductively coupled plasma optical emission spectroscopy, Perkin Elmer Optima 7300 Dual View, 99%, 94% and 102% recovery rate of P, K and Mg, respectively, of in-house hay reference material traceable to certified reference NCSDC 73349) analysis of 0.5 g residues samples digested in 8 ml of nitric acid (trace metal grade) using MARS 6 microwave digestion system (Table 5.3).

Table 5.3. Residue characterisation (SEM indicated in parentheses, n = 3).

Nutrient	compost	straw	woodchip
C (g/kg)	322.3 (0.433)	459.0 (1.012)	485.3 (1.121)
N (g/kg)	25.3 (0.167)	11.2 (0.083)	7.6 (0.105)
C:N	*12.7 (0.084)*	*40.9 (0.368)*	*63.6 (0.760)*
P (g/kg)	5.5 (0.076)	1.0 (0.025)	0.9 (0.024)
K (g/kg)	20.6 (0.31)	13.1 (0.22)	5.1 (0.10)
Mg (g/kg)	4.3 (0.014)	0.7 (0.015)	1.3 (0.040)

The amounts of C, N and other nutrients applied in each treatment were calculated based on the chemical characterisation of the residues and their application rates (Table 5.4).

Table 5.4. Amount of C, N and other nutrients applied in each treatment (g/plot).

	straw	woodchip	compost	straw-compost	woodchip-compost
C	4645	5047	2707	8197	7754
N	114	79	213	347	292
C:N ratio	*41*	*64*	*13*	*24*	*27*
P	11	9	46	59	55
K	133	53	173	330	226
Mg	7	14	37	45	50

5.2.3. Assessment of yield

Lettuce crops were planted on 14 June 2018 and harvested from the inner 2 m × 2 m of each plot on 20 and 21 July 2018, i.e. 38 days after residue application and 36 days after planting. Each lettuce head was harvested whole and weighed to calculate the total biomass produced per

treatment. Meanwhile lettuce crops were qualitatively assessed, which included screening for chlorosis, caterpillar damage, tip burn, and rotting. In some cases dried out mushrooms were found on the outer leaves, which was also noted.

5.2.4. Assessment of soil biogeochemical properties

All soil samples were taken from the inner 2 m × 2 m of each plot on 26 July 2018, i.e. 44 days after residue application. From each plot a 10 cm deep bulk density core of 9.8 cm diameter was collected. A series of six 30 mm diameter soil cores to 20 cm depth were collected, combined and homogenised in a zip-lock bag, and used for a suite of analyses. A sub-sample of the fresh soil was sieved to 2 mm for analysis of available N (i.e. sum of NO_3^- and NH_4^+) by 1 M KCl extraction before and after a 4-week incubation at 70% of the water-holding capacity (WHC). Extracts were filtered through a Whatman no. 2 filter and analysed colorimetrically for NO_3^- and NH_4^+ on a Skalar San^{++} continuous flow analyser. Available N was taken as the sum of the NO_3^- and NH_4^+ measured in the first extract. Potentially mineralisable N was calculated as the difference in NO_3^- and NH_4^+ measured before and after the 4-week incubation period. A sub-sample of the fresh soil was sent to NRM laboratories (Bracknell, UK), where it was air-dried and sieved to 2 mm for measurement of available P by extraction with 0.5 M $NaHCO_3$, available K and Mg by extraction with 1 M NH_4NO_3, soil particle size distribution by laser granulometry, SOM based on LOI at 430 ºC, and the Solvita CO_2 burst test measuring the concentration of CO_2 produced by soils moistened to 50% of their WHC.

Earthworm and mesofauna sampling was performed, but only a few juvenile earthworms were found, which made identification difficult. The endogeic species *A. chlorotica* was identified in at least three of the 24 plots. The abundance of mesofauna (Collembola and mites) extracted from the soils using Tüllgen funnels was null. Some Collembola were observed while harvesting the lettuce crop, so their absence from the samples is probably due to the removal of plants that provided some shelter from the hot and dry weather conditions.

Wet aggregate stability was assessed as per Nimmo and Perkins (2002) using soil samples that were collected into tubs (to prevent soil compression) from the top 10 cm of each plot, and subsequently air-dried. A 4 g subsample from each plot was slowly pre-wetted on moistened filter paper. The wet sieving procedure involved a wet-sieving apparatus composed of vertically moving 250 µm sieves to hold the soil samples sitting inside a can. The cans were filled up with water such that the soil was submerged, causing the unstable soil aggregates to break apart and pass through the sieve into the can. First, the soils were wet-sieved for 3 minutes in deionised

water to collect unstable soil particles and subsequently in a solution of 2 g/L $(NaPO_3)_6$ to disperse the water-stable aggregates. The stable fraction of soil (i.e. wet aggregate stability) was then calculated as the weight of soil caught by the dispersing solution divided by the sum of the weights of soil caught by both water and dispersant. Any particles larger than 250 μm did not pass the sieve and were not included in the calculation.

5.2.5. Data analyses

We observed a gradient in the soil %C and a similar gradient in the %N content of the baseline soil samples that was not well captured by our original blocking design, so the data were retrospectively blocked accordingly (Appendix C.1). This was necessary because the calculation of non-additive effects, described below, relies on paired samples within blocks rather than treatment averages across blocks.

Statistical analyses were performed in R 3.5.1 (R Foundation for Statistical Computing) using RStudio 1.1.456 (RStudio, Inc.). To determine effects of treatments and/or factors on individual soil parameters, a two-way ANOVA, including interactions, with the factors *compost* (compost or no compost) and *residue* (straw, woodchips or no residue) was performed. If a factor had a significant effect ($p < 0.05$), a post-hoc Tukey HSD test was run to determine which treatments were significantly different from each other. Taking into account that four replicates per treatment is a limited number of data points, assumptions of the ANOVA test were assessed both visually and via the relevant statistical tests: homoscedasticity was evaluated with a Q-Q plot of the ANOVA residuals plotted against the fitted data of the ANOVA, as well as a Levene test of the data set. Normal distribution of the residuals was evaluated with a residuals-versus-fitted plot and a Shapiro-Wilk test of the residuals of the ANOVA. Pearson correlations were performed to investigate relationships between different variables.

Properties indicative of soil structure and nutrient cycling were used to assess non-additive effects from residue mixtures compared to individual residues, including lettuce crop yield, soil respiration, soil aggregate stability and bulk density, SOM, available and potentially mineralisable N, and available P, K and Mg. The % effect of each measurement of the treatment effects was first determined by adjusting to the measured effect of the control:

$$\%\, effect = \frac{\text{treatment} - \text{control}}{\text{control}}\ 100\%$$

Next, the % non-additive effects of the residue mixtures were calculated as the difference between the % effect of the mixture and the % effect of the sum of the parts:

$$\%\, non - additive\ effect_{mixture} = \%\, effect_{mixture} - (\%\, effect_{compost} + \%\, effect_{residue}),$$

where *residue* refers to straw or woodchips. A one-sided T-test of the % non-additive effects was performed with an alternate hypothesis (H_1) of $\mu > 0$ for yield, available N, potentially mineralisable N, available P, K, Mg, soil respiration, SOM, aggregate stability, and an alternate hypothesis of $\mu < 0$ for bulk density and pH. Normality was tested with a Shapiro-Wilk test.

5.3. Results

5.3.1 Non-additive effects

Non-additive effects measured 44 days after application of the treatments were mostly synergistic (i.e. mixture > sum of the parts), although the majority of effects were not statistically significant (Figure 5.2). The magnitude and direction of deviation from additivity were usually similar for both the woodchip-compost and straw-compost mixtures, although non-additive effects from the woodchip-compost mixture were sometimes less pronounced than those from the straw-compost mixture.

Both compost-residue mixtures resulted in a non-additive increase in lettuce yield, available and potentially mineralisable N, available Mg, SOM, and soil respiration, but not in available K (hypothesis 1), some of which were statistically significant (Table 5.5). Most notably, we observed greater available N and SOM levels in soils to which a mixture of residues was applied, compared to the available N and SOM levels in treatments receiving only individual residue amendments. The straw-compost mixture resulted in a significant ($T = 4.022$, $p = 0.014$) non-additive increase in SOM of 13.10%, and while the woodchip-compost mixture did not result in statistically significant non-additivity ($T = 0.954$, $p = 0.205$), it did result in a positive non-additive increase in SOM of 6.73%.

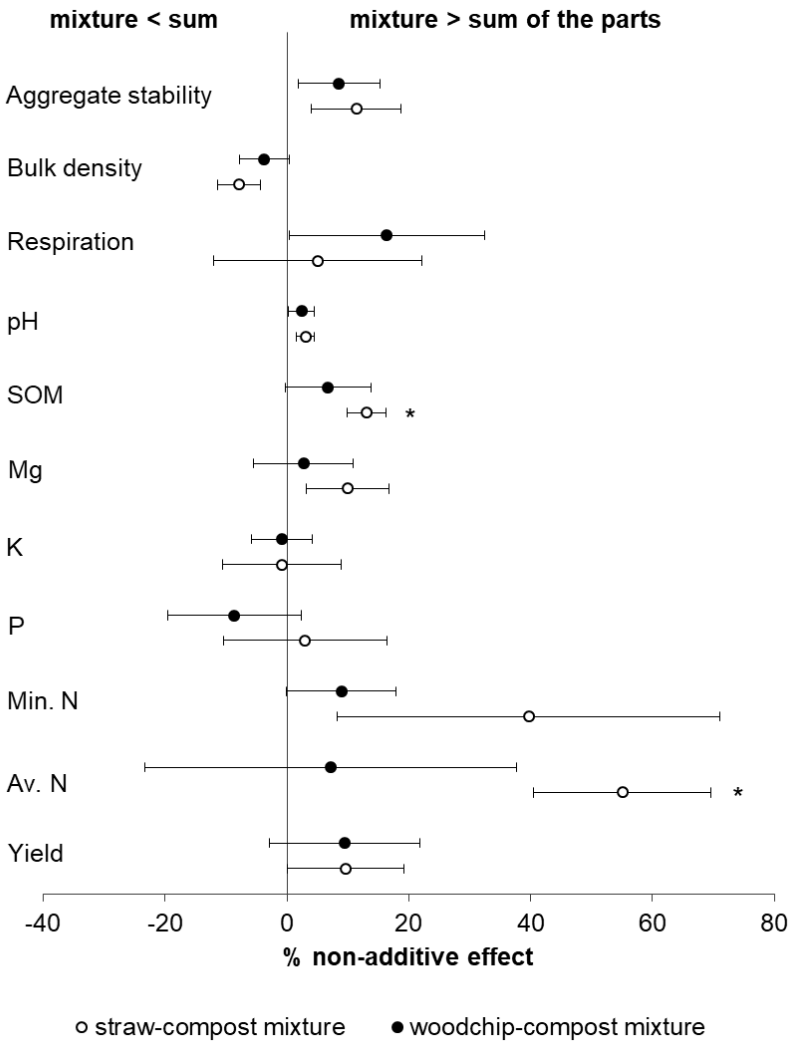


Figure 5.2. Non-additive effects of crop-residue mixtures on soil properties. The % non-additive effect is the difference in % effect between the mixture and the sum of the parts. Positive % non-additive effects mean that the effect of the mixture is greater than the sum of the parts, and vice versa. Yield is total lettuce biomass produced per plot, Av. N is available N, Min. N is potentially mineralisable N, soil P, K, and Mg are soil available nutrients, SOM measured as LOI, soil respiration assessed by CO_2 burst. Error bars represent SEM (n = 4). Significant difference from zero (where 0 = no significant non-additivity) is indicated by * (one-tailed T-test, $p < 0.05$).

Likewise, amendment with the straw-compost mixture led to significantly ($T = 3.789$, $p = 0.016$) greater available N levels that were 55.06% higher on average than would have been expected from the available N levels in treatments receiving individual amendments of straw or compost only. The positive non-additive effect on available N observed in soils that received the woodchip-compost mixture was, however, smaller (7.16% increase on average) and not statistically significant ($T = 0.235$, $p = 0.415$). A non-significant non-additive increase in available P was only observed after application of the straw-compost mixture, but not after application of the woodchip-compost mixture (hypothesis 1). Contrary to our hypothesis, there was a non-

additive increase in pH from the mixtures relative to individual amendments (hypothesis 1), although this was not significant (Table 5.5) and per-treatment results (discussed in next section) show that the pH decreased in all treatments relative to the control ($F = 2.238$; $p = 0.095$; one-way ANOVA; Appendix C.2). We also observed non-additive effects from both compost-residue mixtures on the soil structure, i.e. a decrease in bulk density and an increase in aggregate stability (hypothesis 2), and a non-additive increase of about 10% was found for crop yield from both crop-residue mixtures (hypothesis 3). Although the effects on soil structure and yield were mostly non-significant, the decrease in bulk density after amendment with the straw-compost mixture was borderline significant ($F = -2.232$, $p = 0.056$) (Table 5.5).

Table 5.5. Statistical outputs of one-tailed T-tests of non-additive effects. Significance of deviation from additivity (0) is indicated as **$p < 0.05$** and $p < 0.1$.

	straw-compost mixture			**woodchip-compost mixture**		
	Mean % non-additivity	T	p	Mean % non-additivity	T	p
Yield	9.66	1.004	0.195	9.54	0.771	0.249
Available N	55.06	3.789	**0.016**	7.16	0.235	0.415
Mineralisable N	39.67	1.265	0.147	8.93	0.990	0.198
P	3.01	0.226	0.417	-8.60	-0.788	0.756
K	-0.79	-0.082	0.530	-0.86	-0.171	0.562
Mg	9.95	1.475	0.118	2.73	0.335	0.380
SOM	13.10	4.022	**0.014**	6.73	0.954	0.205
pH	3.04	2.006	0.931	2.41	1.118	0.828
Respiration	5.12	0.300	0.392	16.41	1.023	0.191
Bulk density	-7.80	-2.232	0.056	-3.73	-0.919	0.213
Aggregate stability	11.41	1.555	0.109	8.57	1.291	0.144

The following sections contain the per-treatment results of the soil physical and biochemical properties measured in this experiment. It should be noted that application rates of the mixtures were about twice as high as individual amendments to enable calculation of non-additivity, so measurements from residue-mixture treatments cannot be directly compared to individual-residue treatments.

5.3.2. Per-treatment results

Yield assessed by total biomass of gem lettuce produced per plot seemed to be somewhat reduced by the straw-only treatment but was not significantly affected by any of the treatments or factors (Figure 5.3a; see Appendix C.4 for statistical outputs).

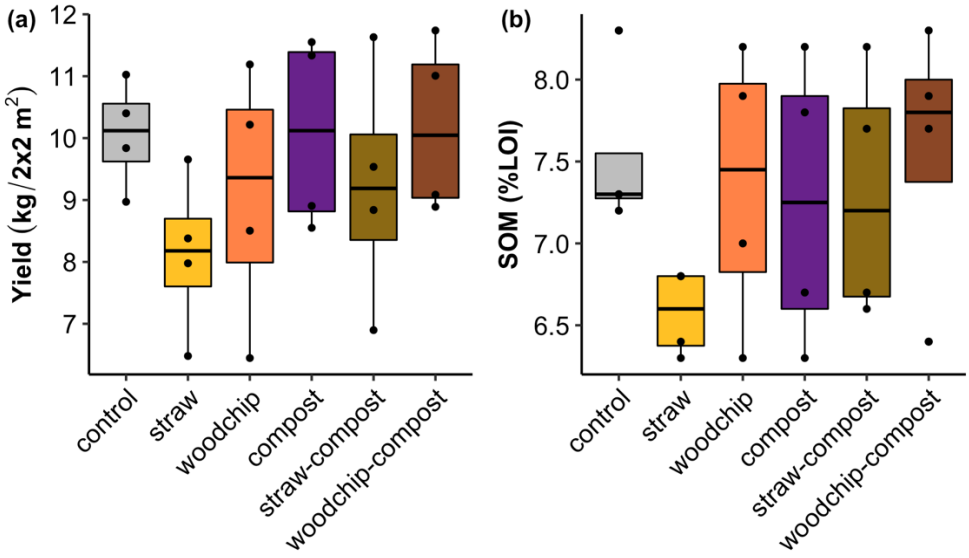


Figure 5.3. (a) Gem lettuce yield as total biomass produced per 2 m × 2 m plot sampled. **(b)** Soil organic matter by percent loss on ignition (% LOI) after each soil amendment treatment. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual data points, occasionally overlapping (n = 4).

Lettuce plants in the straw-only treatments suffered noticeably less damage, particularly from caterpillars, tip burn, and rot (Table 5.6). There was a significant interaction between *residue* and *compost* in terms of the qualities of lettuce plants harvested ($F = 3.568$, $p = 0.050$; two-way ANOVA), with the biggest difference between straw-only and straw-compost ($p = 0.067$; post-hoc Tukey HSD). Mushrooms were observed on the outer leaves of some lettuce heads in plots receiving woodchips, or in two cases in plots neighbouring treatments including woodchips, so fungi may have been introduced and/or promoted by woodchips.

Table 5.6. Qualitative assessment of lettuce plants as the % of lettuce heads per plot affected by each condition. "Overall" quality impairment is the % of lettuce head per plot affected by one or more conditions. Mean values per treatment (n = 4; SEM in parentheses).

Treatment	**Chlorosis**		**Tip burn**	**Rot**	**Overall**
	(All)	(Tips only)			
control	49.1 (16.1)	47.3 (16.9)	15.5 (4.9)	1.7 (1.1)	77.8 (12.3)
straw	31.5 (11.7)	21.7 (7.49)	1.9 (1.3)	0.0 (0.0)	43.1 (15.6)
woodchip	39.3 (9.3)	33.4 (8.5)	12.2 (4.5)	4.3 (2.0)	80.4 (11.6)
compost	40.4 (7.7)	34.3 (6.6)	14.5 (9.0)	0.6 (0.6)	69.4 (10.7)
straw-compost	58.3 (14.9)	56.1 (15.2)	16.9 (8.6)	0.7 (0.7)	93.0 (7.0)
woodchip-compost	61.7 (14.0)	54.1 (16.7)	18.0 (8.7)	0.0 (0.0)	82.2 (11.0)

Levels of SOM and N (available and potentially mineralisable) were negatively affected by the straw-only treatment, while treatments of woodchip-only and compost-only had little effect on SOM and N levels compared to the control (Figures 5.3b and 5.4). Residue mixtures increased

SOM and N in most cases, with the exception of the effect of the straw-compost treatment on SOM. Nonetheless, there was a non-additive effect in SOM and N in the straw-compost treatment, as this non-additivity was in fact a negation of the negative effect on SOM and N of straw applied as an individual residue.

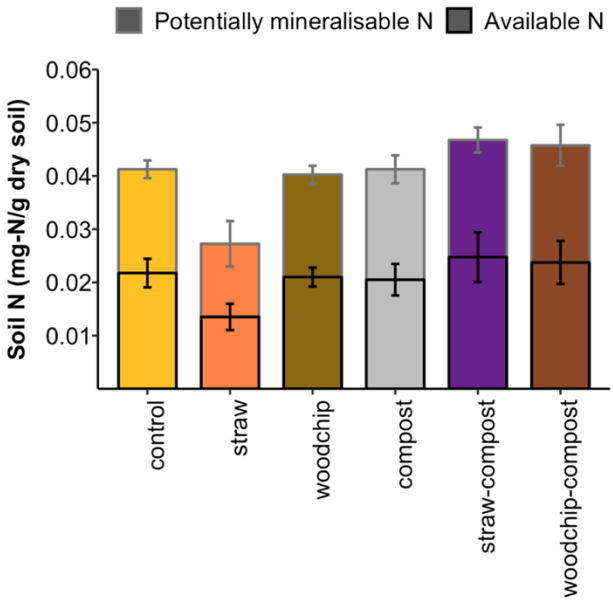


Figure 5.4. Soil available and potentially mineralisable N after each soil amendment treatment. Error bars represent SEM of available and potentially mineralisable N separately (n = 4).

Treatment effects on SOM or N levels were not significantly different between treatments (SOM: $F = 0.981$, $p = 0.456$; N: $F = 1.81$, $p = 0.163$; one-way ANOVA), but the factor *compost* tended to increase soil N ($F = 3.88$; $p = 0.065$; two-way ANOVA). Soil respiration in the different treatments was rather similar in all treatments and none of the treatments caused soil respiration to deviate significantly from the control or from each other ($F = 1.358$, $p = 0.286$; one-way ANOVA; Appendix C.2).

The addition of compost, either as an individual residue or in a mixture, significantly affected soil available K ($F = 7.761$; $p = 0.012$) and Mg ($F = 4.953$; $p = 0.039$) (Figure 5.5a). Akin to soil N and SOM, the lowest levels of nutrients were found in soils amended with the straw-only treatment. The increases in nutrient availability were not consistent with the crop residue amendments and ranged from -242% to 57% of the nutrient added as part of the amendments (Appendix C.3). If there was an increase in nutrients, the contribution of the amendments was relatively small in most cases and exhibited very large error margins. The most notable observations from these data is the consistent immobilisation of nutrients brought about by the straw-only treatment, while amendments including woodchips or compost had a tendency to

modestly increase soil available nutrients. None of the nutrient increases exceeded 100% of the nutrients added, indicating that residue amendments did not result in net mobilisation of nutrients already present in the soil.

We observed no significant effects on the aggregate stability of the differently amended soils, but the soil bulk density tended to be lowered by the *residue* factor, i.e. when a low-quality residue was part of the treatment ($F = 3.28$; $p = 0.062$; two-way ANOVA) (Figure 5.5b).

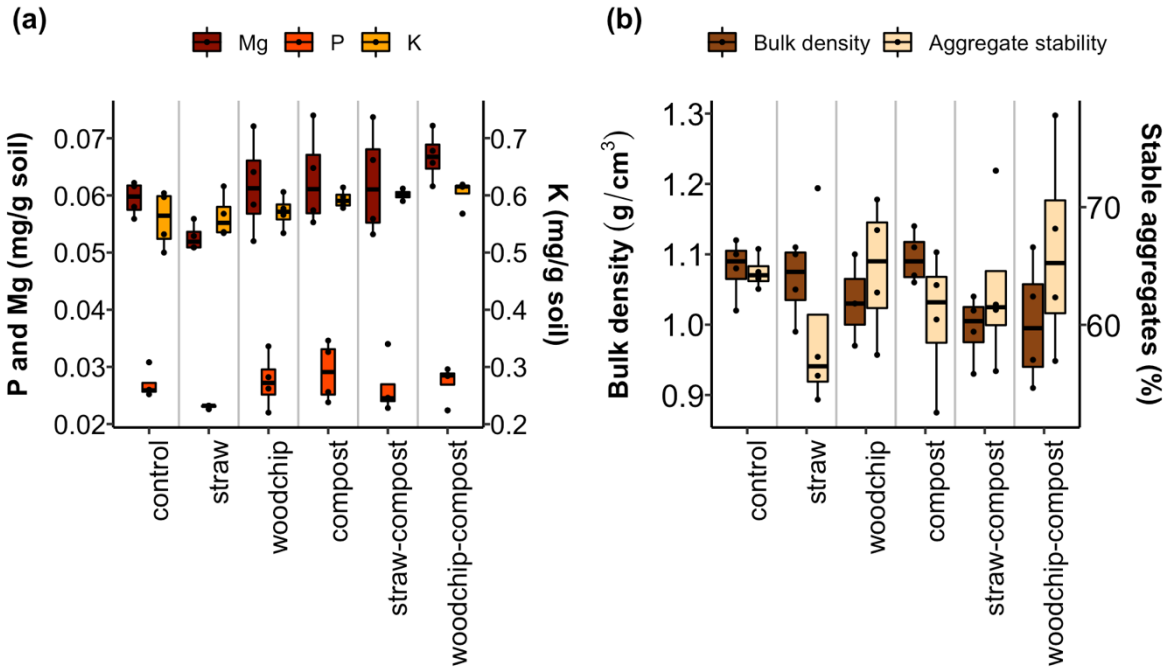


Figure 5.5. (a) Soil available nutrients after each soil amendment treatment. **(b)** Soil physical properties after each treatment. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual data points, occasionally overlapping (n = 4).

5.3.3. Correlations

A number of noteworthy correlations may help explain the data and are summarised in Table 5.7. There were some significant correlations between the amount of nutrients applied and the amount of available K and Mg in the soils at the end of the experiment, which indicates a positive effect of the residue amendments. The amount of C applied via the residue amendments was not correlated with the levels of SOM. Yield was positively and significantly correlated with the sum of available and potentially mineralisable N, available P and Mg, SOM and aggregate stability.

Table 5.7. Selected Pearson correlations (r-values). Significance indicated as **p < 0.05** and p < 0.10.

	Yield	Av N	Av+Min N	P	K	Mg	SOM	Resp
App_C	-0.10	0.17	0.17	-0.07	0.40	0.22	0.00	-0.01
App_N	0.07	0.26	0.30	0.08	**0.54**	0.32	0.06	-0.09
App_P	0.00	0.20	0.23	0.00	**0.49**	0.22	-0.01	-0.17
App_K	0.17	0.30	0.36	0.17	**0.56**	0.39	0.12	-0.05
App_Mg	0.19	0.33	0.38	0.19	**0.56**	**0.45**	0.16	0.02
Yield	-	0.29	**0.45**	**0.75**	0.19	**0.78**	**0.74**	0.36
Av N	0.29	-	**0.91**	**0.42**	0.27	**0.55**	**0.58**	0.36
Av + Min N	**0.45**	**0.91**	-	**0.49**	0.35	**0.61**	**0.65**	0.30
P	**0.75**	**0.42**	**0.49**	-	0.02	**0.83**	**0.86**	**0.51**
K	0.19	0.27	0.35	0.02	-	0.35	0.02	-0.26
Mg	**0.78**	**0.55**	**0.61**	**0.83**	0.35	-	**0.80**	**0.47**
SOM	**0.74**	**0.58**	**0.65**	**0.86**	0.02	**0.80**	-	**0.62**
Agg stab	**0.45**	**0.55**	**0.48**	0.36	0.00	**0.58**	**0.58**	**0.41**
Overall qual.	0.20	**0.51**	**0.46**	0.10	0.34	0.23	0.21	0.14

Abbreviations: App_ = application rate of, Av = available, Min = potentially mineralisable, Agg stab = aggregate stability, Resp = soil respiration, Overall qual. = overall quality impairment.

5.4. Discussion

5.4.1. Non-additive effects

The objective of this study was to find out if greater benefits could be obtained from crop-residue soil amendments in an arable soil by applying them as chemically heterogeneous mixtures of low-C:N vegetable waste compost with high-C:N straw or woodchips, compared to individual residue amendments. Relative benefits of the mixtures were assessed by calculating the non-additivity of a range of effects, including yield and a selection of soil properties that are likely to be beneficial for crop production. We found some degree of non-additivity in the direction (synergy or antagonism) we predicted in most parameters (except available P in the woodchip-compost mixture and available K in both mixtures), and significant non-additive increases in available N and SOM after application of the straw-compost mixture, indicating that even after a short amount of time (44 days) beneficial effects from a mixture of residues can be greater than the sum of its parts.

Examining per-treatment effects can help further explain the non-additivity results. The per-treatment difference in terms of SOM and available N between the woodchip-compost treatment and the straw-compost treatment was relatively small. Yet, only the straw-compost mixture exhibited significant non-additivity. Comparison of the per-treatment effects on SOM and available N reveals that the significant non-additive effects observed after application of the

straw-compost mixture are in fact a negation of the negative (compared to control) effect of the straw-only treatment. As suggested earlier, this indicates that decomposition of single crop residue amendments does not always translate into agronomic benefits, and applying mixtures of crop residues could be a route to improve those benefits.

5.4.2. Decomposition

Although we suggested that non-additive effects might be related to differences in decomposition rates in the mixtures compared to the individual residues, we have no evidence of this in terms of soil respiration measurements. At the time of sampling, high microbial activity may have increased N immobilisation and therefore decreased soil mineral N availability. However, respiration rates were equally low in the straw-only (N immobilisation) and the straw-compost treatments (N mineralisation), and both were lower than the control (Appendix C.2). Likewise, Redin *et al*. (2014), who studied residue mixtures of stems and leaves of 25 different arable crop species, found mostly additive effects for decomposition rates of mixtures, but unlike the results presented here they found no synergistic effects on N mineralisation. Both here and in the study by Redin *et al*. (2014), decomposition was measured in terms of C mineralisation (measured as CO_2 release), which does not account for the possibility of a higher CUE when chemically diverse residue mixtures are applied, and also does not distinguish between mineralisation of residues or organic matter already present in the soil. Moreover, our soil respiration measurements were taken by the Solvita burst method, on soil samples removed from the field and sieved to 4 mm, which may not have been a good representation of the respiration produced *in-situ* by a soil mixed with crop residues at various stages of decomposition.

Another reason for the absence of different soil respiration rates may be the relatively short duration of this experiment, covering the short growing period of gem lettuce. As pointed out by Lecerf *et al*. (2011), niche complementarity effects, in which different groups of decomposing organisms develop a synergistic association in residue breakdown, tend to advance with time, leading to a generally higher number of long-term litter-mixing studies finding non-additive effects. Indeed, Ball *et al*. (2014) only observed a non-additive effect on mass loss in a five-component mixture after 193 days. Therefore an experiment of longer duration may be able to capture more and greater treatment effects and non-additive effects.

5.4.3. Yield

Although yield, assessed by total biomass of gem lettuce produced per plot, was not significantly affected by any of the treatments or factors, there were some notable differences between treatments. Yield appeared to be somewhat depressed by the straw-only treatment, which is not surprising considering the lower concentration of soil available N, SOM, soil nutrients and aggregate stability in this treatment, compared to the control. Crops tend to require most nitrogen during the vegetative growth stage and when this is not available, yield will be affected (Chen *et al*., 2014). The lettuce plants were planted as plugs just after application of the treatments, so when they were introduced to the experimental plots they were already in their vegetative stage. Significant positive correlations of yield with the sum of available and potentially mineralisable N, available P and Mg, SOM, and aggregate stability suggest that these are the main benefits provided by the crop residue amendments from an agronomic perspective.

Overall lettuce quality was least affected in the straw-only treatments, despite the location of these treatments being towards the low soil-C end of the field site (Appendix C.1). Available N levels were positively correlated with overall quality impairment (i.e. % lettuce heads affected by some form of quality impairment) ($p = 0.011$), and in particular with yellow tips ($p = 0.017$) and tip burn ($p = 0.041$), which may indicate the crop was suffering from N deficiency (Table 5.7). Indeed, the N levels were relatively low compared to those recommended for lettuce crops (RB209, 2019), and N deficiency leads to reduced plant size, which would lead to decreased biomass production, as well as chlorosis and outside leaves senescing prematurely and dropping off (Brady and Weil, 2002), all of which were observed.

5.4.4. Nutrient dynamics and transfer

The straw-only treatment led to a notable immobilisation of N, which was unlike the other treatments. Although this could be only a temporary effect (e.g. as in Silgram and Chambers, 2002), it may be unfavourable for lettuce crop productivity and should be taken into account when timing crop residue applications. The notable N immobilisation in the straw-only treatment suggests that straw decomposed differently as an individual residue than in a mixture with compost, which could be explained by the C:N ratio of the treatments. Chen *et al*. (2014) evaluated soil N processing during crop residue decomposition and suggested that residues with a C:N ratio below ~25 result in net mineralisation (increase in soil available N) and those with a C:N ratio above ~30 result in net immobilisation (decrease in soil available N). Therefore, in the present study the woodchip-only (C:N = 64) and straw-only (C:N = 41) treatments are both

expected to result in net N immobilisation. The reason why N immobilisation is only observed in the straw treatment could be due to a lower decomposition rate of the woodchips and therefore lower microbial N-mining requirement at the time of sampling. Straw is likely more decomposable due to a comparatively lower C:N ratio, a higher water-holding capacity (being more friable and having a greater surface area to hold on to moisture) (Hättenschwiler *et al*., 2005; Iqbal *et al*., 2015) and possibly a soil microbial community that is more adapted to decomposing straw because wheat is sometimes grown in these soils.

A slight increase in soil N (available and potentially mineralisable N) observed in the straw-compost treatment and to a lesser extent in the woodchip-compost treatment, compared to the control, could be due to N derived from the compost, the residue, or primed native SOM. Priming of native SOM caused by the amendment seems unlikely in the woodchip-compost treatment, because SOM levels were higher compared to the control treatment. Even in the straw-compost treatment, the SOM level was very close to that of the control treatment, suggesting mineralisation of native SOM was negligible. Compost was the most significant factor related to higher soil N levels, which can be attributed to its low C:N ratio, allowing for easy decomposition with minimal immobilisation of native soil mineral N. In the residue mixtures, it is likely that compost provided nutrients for decomposer microbes to be able to decompose the high-C:N residues (i.e. inter-specific nutrient transfer).

Therefore, the non-additive effects on soil N in the straw-compost treatment can probably be attributed to interspecific net transfer of N from high-N to low-N residues resulting in (1) the retention of compost-derived N by straw or woodchips in the mixture, preventing it from being leached, and (2) a higher nutrient availability in treatments including compost, enabling decomposer organisms to break down and release N contained in the amendment mixture more readily. The transfer of N can occur by a combination of uptake and release by microbes on the high-N residue as they produce enzymes for decomposition, and diffusion along a gradient of high N to low N (Schimel and Hättenschwiler, 2007). The woodchips likely had a higher lignin content than straw. Ligninolytic enzyme production can be inhibited by elevated N concentrations (Carreiro *et al*., 2000; Knorr *et al*., 2005), resulting in a relatively greater inhibition of decomposition of the woodchips.

The transfer of N in litter mixtures appears to go hand in hand with a C transfer. In a microcosm experiment by Berglund *et al*. (2013) on pine and maize litters inoculated with both forest and arable soils, mixing residues mostly increased C loss from the lower quality litter, while C released from the higher quality litter was equivalent to decomposing as an individual litter.

Therefore, the non-additively higher SOM in the straw-compost treatment is likely to be the result of enhanced C release from the straw due to the addition of compost. This phenomenon could be explained by a bidirectional transfer of C and N between high- and low-quality residues – e.g. via transport of amino acids by fungal mycelia (Tlalka *et al*., 2007) – where increased N availability near the low-quality residue enhances its decomposition and subsequent C release, while increased C in the presence of the high-quality residue has little effect on its decomposition (Berglund *et al*., 2013).

5.4.5. Soil physical structure

Increased SOM positively affects aggregate stability because soil microbes feeding on organic substrates enhance soil aggregate formation and stability by biofilm formation and the production of extracellular polymeric substances that increase cohesion between soil particles (Martens, 2000; Totsche *et al*., 2018). Aggregate stability, in turn, is involved in the protection of mineral-associated SOM (Angst *et al*., 2017). Therefore, with an increase in SOM, an increase in aggregate stability would be expected, and we did indeed observe a positive correlation between these variables ($p = 0.028$). We also observed a positive correlation between aggregate stability and soil available N ($p = 0.005$). This is contrary to the observation that high-quality residues and/or addition of N fertilisers result in higher aggregate turnover (formation and breakdown) compared to a greater aggregate stability when low-quality residues are applied (Chivenge *et al*., 2011).

Because we observed positive effects on both soil N and SOM from crop residue mixtures, an increased non-additive effect on the soil physical structure from application of the right residue mixtures can therefore be anticipated over time. However, in many arable cropping systems tillage may undermine the emergence of this benefit by destroying soil aggregates and exposing the SOM contained within (Nath and Lal, 2017). Furthermore, bulk density was lowered by the addition of the low-quality residues (straw and woodchips; $p = 0.062$), especially when combined with compost. This could be partially due to increases in the aggregate stability in most of these treatments, although some residues (with a lower density than soil) may have also been included in the bulk density ring when sampling.

5.4.6. Potential of residue mixing to obtain more benefits from low-quality residues

Our study provides some evidence that chemically heterogeneous crop residue mixtures can provide agronomically beneficial non-additive effects. We found prevention of N immobilisation

to be the most prominent effect in the short term. Positive non-additivity in SOM levels and other soil nutrients may develop over time, but a longer term experiment is necessary to investigate this.

Other authors have also found beneficial effects on soil N levels from mixed residue amendments. For instance, Kaewpradit *et al*. (2009) mixed groundnut residues (high N) and rice straw (low N), which slowed down N loss by mineralisation during the phase between two different crops, i.e. a beneficial temporary N immobilisation. McDaniel *et al*. (2016) found that non-additive effects of soil C and N dynamics after application of residue mixtures depend on the diversity in cropping history, with non-additive effects primarily observed in monoculture soils rather than diverse crop rotations. The authors attribute this to the low respiration rates from monoculture soils after application of low-quality residues, while soil response to high-quality residues is similar in both monoculture and crop rotation soils (McDaniel *et al*., 2016). These studies indicate that potential benefits from residue-mixing are dependent on the arable cropping system.

Manipulation of the number of component residues, the mixing ratio, and the quantity applied can be used to optimise timing and amount of nutrient release for a better synchrony with crop demand (Myers *et al*., 1997). For instance Kuo and Sainju (1998) demonstrated that the timing of N mineralisation can be manipulated by the proportion of leguminous cover crop residues in the mixture, while Mao and Zeng (2012) found that both the number of residue components and their mixing ratio affected non-additivity. Furthermore, the quantity of residues applied can impact on microbial CUE: while microbial CUE is often unaffected at low substrate additions, applications of high amounts of the same material can lead to diminishing CUE levels (Jones *et al*., 2018), e.g. as shown by Roberts *et al*. (2007) with glucose and glucosamine additions to various foraging soil types in a microcosm experiment.

The interplay of environmental factors and amendment properties affect microbial CUE and the mechanisms involved in non-additivity of decomposing residue mixtures on soil properties (Kuebbing and Bradford, 2019), which need to be accounted for to be able to create a methodology for optimised benefits from crop residues as soil amendments in arable cropping systems. Therefore, future research on residue mixtures should incorporate not only substrate quality, but also application rate (quantity), diversity (number of residue species) and mixing ratio and how these interact with different arable soil types.

5.5. Conclusions

This experiment tested agronomic benefits obtained from multi-component and chemically heterogeneous residue mixtures compared to the individual residues. Significant positive non-additive effects on available N and SOM were measured after application of a straw-compost mixture, so we can partially accept our first hypothesis predicting greater levels of soil available nutrients and SOM in mixtures compared to individual residues. However, due to variation in the total %C contents across the experimental field site, we have some reservations about this result. Nevertheless, this study provides some evidence for the potential of crop residue mixtures to provide greater agronomic benefits than single high-C residue amendments of straw or woodchips, at least in terms of preventing N immobilisation during crop growth.

Chapter 6 – General discussion

6.1. Literature review

The accumulation of soil organic matter (SOM) in arable and horticultural soils is desirable for many reasons: (1) for greenhouse gas mitigation purposes, by maximising microbial carbon use efficiency (CUE) to increase the amount of soil organic C formed relative to the amount of CO_2 respired (2) for biodiversity purposes, by nourishing a more diverse soil food web, and (3) for food security purposes, because SOM contains nutrients and is involved in soil aggregation, which can improve the soil chemical fertility and its physical structure for increased crop production (Oldfield *et al*., 2019). Crop residues contain valuable ingredients conductive to these ends: carbon to build SOM, calories to feed members of the soil food web, and nutrients that can be fed back into plant tissue. However, decomposition of crop residue amendments does not always translate into greater SOM levels, increased nutrient availability, or improved soil structure, and instead may enhance greenhouse-gas emissions as crop residue-derived and/or native soil C is mineralised by the decomposer community. Therefore, a better understanding of the decomposition processes involved are needed to improve the management of crop residues in arable and horticultural cropping systems.

The literature review in this thesis demonstrated that there is a considerable amount of ecological understanding on topics including SOM dynamics, decomposition processes and how these may be affected by legacy effects, and interactions between plant residues and the soil food web. However, most of the research underpinning this understanding has been carried out in natural ecosystems, and the underlying mechanisms are not fully understood. Therefore, it is difficult to determine how to apply this knowledge to better use crop residues in horticultural and arable settings, which are highly managed unnatural systems, subject to physical (e.g. ploughing, drilling), chemical (e.g. application of fertilisers and pesticides), and biological (e.g. forced monocultures, weed suppression, suboptimal conditions for soil biota) soil disturbance. We need to better understand crop residue-soil interactions in an arable context to inform decisions about crop residue management aimed at maximising SOM accumulation.

6.2. Experiments

The experiments presented in this thesis are an attempt to be able to better determine what crop residues should be selected, where on the farm, when during the season and how they should be

applied in order to maximise SOM accumulation, nutrient availability, and soil structure in arable soils. Most farmers who apply crop-residue amendments simply leave and incorporate residues in the same field as where they were grown. But there might be benefits to employing a different approach that involves removal and re-application of crop residues. The home-field advantage (HFA) experiment (Chapter 4), the set-up of which was supported by observations of effects of crop identity on belowground biota (Chapter 3), was a first test to determine the potential of an amendment approach of applying crop residues to a different field from where they were grown, effectively devising a "crop-residue rotation" within a common crop rotation. The residue-mixing experiment (Chapter 5) tested the potential of an amendment approach of applying crop residues as a chemically heterogeneous mixture.

The first experiment (Chapter 3) demonstrated that aboveground botanical diversity in crop rotations did not affect belowground microbial community structure or the abundance of soil faunal groups, but instead different stages in a simple crop rotation significantly affected the abundance of some soil mesofauna. The HFA experiment subsequently presented in Chapter 4, was performed in soils at three different stages of this same crop rotation. However, no HFA effect on crop residue decomposition was found in these soils. This may have had something to do with the mesh size selected for this experiment, which, at 500 μm, excluded mesofauna, although the pros and cons of larger and smaller mesh sizes were considered (Appendix B.4). Mesh bags form a partial physical separation between the soil and the residue that can limit the impact imparted by the soil microbial community on the decomposition of residues contained within the mesh bag. Curtin *et al*. (2008) compared different methods of measuring decomposition of wheat and barley straw and found that mass loss was about two times higher in soil-mixed straw compared to straw in mesh-bags (4 mm mesh). Soil is heterogeneous and, as opposed to simply incorporating the crop residue into soil, mesh bags were in contact with only a fraction of the soil surface, and microbial communities involved in HFA effects may have been excluded due to dispersal limitations (Eisenlord *et al*., 2012). A three-way interaction may have also been prevented, whereby larger species of soil fauna were unable to act as microbial vehicles because they were excluded due to the mesh size being too small for many faunal species. Therefore, mesh bags might be an inherently suboptimal method for detecting a HFA, although most of the HFA experiments in forest systems have been performed using mesh bags. If future experiments do establish the existence of HFA effects in some arable contexts, this could be exploited to manipulate decomposition rates and the accumulation of SOM from decomposing crop residues.

The next strategy that was tested involved combinations of crop residues as chemically heterogeneous mixtures (Chapter 5). This experiment was performed at a commercial farm, which

presented some challenges. The field selected for my experiment was not confirmed until immediately prior to establishing the experiment because the farm was responding to weather conditions and customer demand in their timing of lettuce planting. The establishment of my experimental treatments needed to coincide well with the timing of lettuce planting. Since the field site was not situated locally, this prevented me from seeing the field until the day I started establishing the experiment, which is when I observed a colour gradient in the soil across the field. I decided to change the blocking design of the experiment to account for this gradient, but after characterisation of the initial soil samples, the new blocking structure did not capture the realised gradient in soil properties (C, N, SOM). Nonetheless, the residue-mixing experiment (Chapter 5) yielded promising results in terms of beneficial non-additive effects in soil available nutrients and SOM levels, albeit with some caveats resulting from the suboptimal blocking design.

A second challenge presented itself during the set-up of the experiment, which was that the pegs marking the corners of each plot were removed. Fortunately I had taken GPS coordinates of each peg with the help of one of the G's employees. While working in the field, some field workers were usually around and spotted me as a stranger in the field. They kindly helped me weigh and apply crop residues. While assessing the yield in each plot, harvesting of the neighbouring plots took place, which gave me an insight in how food is produced on a large commercial scale. Generally, being more integrated in the farm operations goes hand in hand with an understanding of their routine and perspective, and enables a smoother establishment of a scientific experiment within a commercial organisation. I would therefore recommend others considering the establishment of an experiment on a commercial farm to embed themselves within the organisation prior to establishing the experiment.

Application of chemically heterogeneous crop-residue mixtures may be the more promising idea arising from the experiments in this thesis for making better use of crop residues. This approach is applicable to highly managed farming systems, although some practical implications may arise. At the farm where I performed my experiment, a modest amount of wheat straw and poplar woodchips was available, but this would not have been sufficient to be able to supply all the land with chemically heterogeneous soil amendments. For horticultural and livestock farms, sourcing organic inputs with a high C content needed to create a chemically heterogeneous mixture with vegetable waste or animal manure might be a challenge. Conversely, for arable farms producing a lot of wheat, sourcing organic matter inputs with a high N content might be a challenge. Farms could trade resources with each other, but that may involve substantial transportation costs and associated environmental impacts. Meanwhile, the use of crop residues as soil amendments may be outcompeted by the financial value of wheat straw and other high-C:N crop residues for

purposes of bioenergy and animal feed and fodder. A life cycle analysis would be necessary to weigh the missed financial opportunity of selling residues and the costs incurred in transportation against the benefits gained from SOM accumulation and reduced need for fertilisers and other chemical inputs.

Moreover, the implementation of any strategy involving the translocation of crop residues would be challenged by restrictions preventing the movement of some crop residues to different fields, due to concerns of transferring pests and diseases. Yet, removing and re-applying crop residues as mixtures comes with the added benefit of increasing biodiversity within an otherwise monocultural system, and greater biodiversity in arable systems can increase natural pest suppression, as well as decrease soil erosion, decrease eutrophication of water ways, increase crop yields, and increase resilience, among other beneficial effects (Gurr *et al*., 2003; Lin, 2011). Although increasing biodiversity, and the benefits arising from it, does not necessarily suppress pests, increasing biodiversity in arable systems can be achieved in many ways (Lin, 2011), and it would be interesting to further investigate the effect of increased biodiversity by means of diverse crop-residue amendments.

6.3. Future work

To be able to better assess existence, or not, of an HFA effect in arable soils, we need to better comprehend the underlying mechanisms. If I had been funded for a fourth year, I would have performed a mechanistic experiment to test for the relative legacy effects of previous residue applications and previous plant growth on the presence of an HFA, as outlined in Appendix D. Residue application could select for a microbial community by their physical and chemical (molecular make-up) characteristics, or even by emitting plant litter volatiles (Austin *et al*., 2014). Living plants could select for a soil microbial community via root exudates and interactions in the rhizosphere. Both residues and living plants could further affect the decomposer community via “green-leaf hitchhikers” that persist from green leaves/stalks to the litter stage (Austin *et al*., 2014).

Future studies on soil amendment with residue mixtures need to determine the long-term effects and interactions with different arable soil types. Other areas that could be explored include changing the number of component residues, the mixing ratio, and the quantity and timing of residues application.

6.4. Conclusions

The literature (Chapter 2) and an investigation of the link between aboveground botanical diversity in crop rotations and belowground soil faunal diversity and microbial community structure (Chapter 3) led me to test two methods for making better use of crop residues:

(1) Manipulation of the decomposition rate by attempting to realise an "away-field disadvantage," based on the assumption that a slower more constant decomposition rate can increase the CUE of soil biota over time by decreasing microbial transitions in and out of dormancy and by promoting K-selected species. However, this approach was undermined by the finding that no HFA could be detected via the mesh-bag experiment performed within a typical arable crop rotation (Chapter 4).

(2) Manipulation of the microbial CUE by applying crop residues as chemically heterogenous mixtures, based on the assumption that the inherent CUE windows of the range of species that make up the soil microbial community is maximised when resources with a range of C:N ratios are supplied. An experiment on the application of chemically heterogeneous crop-residue mixtures yielded promising effects on SOM and available N within a short time frame (Chapter 5).

In conclusion, from the experiments conducted during my PhD, optimisation of substrate chemistry by soil amendment with chemically heterogeneous mixtures of crop residues appears to be the most promising route for making better use of this abundant resource, although additional research is required. Implementation of this method on a large scale would involve some practical implication, particularly in terms of crop-residue removal from the field, transportation of high-C:N residues to farms producing low-C:N residues and vice versa, and subsequent re-application of residues in a mixed form. Further investigation into the mechanisms involved in HFA effects is necessary to be able to better determine if this ecological phenomenon in natural ecosystems also applies to arable systems (Appendix D) and could potentially be exploited to accumulate more SOM in agricultural soils.

References

Addison, J.., Trofymow, J.., Marshall, V.., 2003. Functional role of Collembola in successional coastal temperate forests on Vancouver Island, Canada. Applied Soil Ecology 24, 247–261. https://doi.org/10.1016/S0929-1393(03)00089-1

Adejuyigbe, C.O., Tian, G., Adeoye, G.O., 2006. Microcosmic study of soil microarthropod and earthworm interaction in litter decomposition and nutrient turnover. Nutrient Cycling in Agroecosystems 75, 47–55. https://doi.org/10.1007/s10705-006-9010-5

Adl, S.M., 2003. The Ecology of Soil Decomposition. CABI Publishing.

Adl, S.M., Alastair, G.B., Simpson, M.A., Farmer, R.A., Andersen, R.A., Aderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, M.F.J.R., Mann, D.G., McCourt, R.M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A. V., Spiegel, F.W., Taylor, M.F.J.R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. The Journal of Eukaryotic Microbiology 52, 399–451. https://doi.org/10.1111/j.1550-7408.2005.00053.x

Adl, S.M., Simpson, A.G.B., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., le Gall, L., Lynn, D.H., McManus, H., Mitchell, E.A.D., Mozley-Stanridge, S.E., Parfrey, L.W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C.L., Smirnov, A., Spiegel, F.W., 2012. The revised classification of eukaryotes. Journal of Eukaryotic Microbiology 59, 429–514. https://doi.org/10.1111/j.1550-7408.2012.00644.x

Aira, M., Bybee, S., Pérez-Losada, M., Domínguez, J., 2015. Feeding on microbiomes: Effects of detritivory on the taxonomic and phylogenetic bacterial composition of animal manures. FEMS Microbiology Ecology 91, 1–10. https://doi.org/10.1093/femsec/fiv117

Al-Maliki, S., Scullion, J., 2013. Interactions between earthworms and residues of differing quality affecting aggregate stability and microbial dynamics. Applied Soil Ecology 64, 56–62. https://doi.org/10.1016/j.apsoil.2012.10.008

Allesina, S., Levine, J.M., 2011. A competitive network theory of species diversity. Proceedings of the National Academy of Sciences of the United States of America 108, 5638–42. https://doi.org/10.1073/pnas.1014428108

Allison, S.D., 2014. Modeling adaptation of carbon use efficiency in microbial communities. Frontiers in Microbiology 5. https://doi.org/10.3389/fmicb.2014.00571

Allison, S.D., 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Letters 8, 626–635. https://doi.org/10.1111/j.1461-0248.2005.00756.x

Allison, S.D., 2006. Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. Biogeochemistry 81, 361–373. https://doi.org/10.1007/s10533-006-9046-2

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340. https://doi.org/10.1038/ngeo846

Anderson, J.M., Leonard, M.A., Ineson, P., Huish, S., 1985. Faunal biomass: A key component of a general model of nitrogen mineralization. Soil Biology and Biochemistry 17, 735–737. https://doi.org/10.1016/0038-0717(85)90057-4

Angst, G., Mueller, K.E., Kögel-Knabner, I., Freeman, K.H., Mueller, C.W., 2017. Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter. Biogeochemistry 132, 307–324. https://doi.org/10.1007/s10533-017-0304-2

Armbrecht, I., Perfecto, I., Vandermeer, J., 2004. Enigmatic biodiversity correlations: ant diversity responds to diverse resources. Science 304, 284–6. https://doi.org/10.1126/science.1094981

Austin, A.T., Vivanco, L., González-Arzac, A., Pérez, L.I., 2014. There's no place like home? An exploration of the mechanisms behind plant litter – decomposer affinity in terrestrial ecosystems. New Phytologist 204, 307–314.

Averill, C., Waring, B., 2017. Nitrogen limitation of decomposition and decay: How can it occur? Global Change Biology. https://doi.org/10.1111/gcb.13980

Ayres, E., Dromph, K.M., Bardgett, R.D., 2006. Do plant species encourage soil biota that specialise in the rapid decomposition of their litter? Soil Biology and Biochemistry 38, 183–186. https://doi.org/10.1016/j.soilbio.2005.04.018

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology and Biochemistry 41, 606–610. https://doi.org/10.1016/j.soilbio.2008.12.022

Bååth, E., Anderson, T.-H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35, 955–963. https://doi.org/10.1016/S0038-0717(03)00154-8

Badejo, M.A., Tian, G., Badejo, M.A., Tian, G., 1999. Abundance of soil mites under four agroforestry tree species with contrasting litter quality. Biology and Fertility of Soils 30, 107–112. https://doi.org/10.1007/s003740050595

Baermann, G., 1917. Eine einfache methode zur auffindung von Ankylostomum - (Nematoden) - larven in erdproben. Geneeskundig Tijdschrift voor Nederlandsch-Indië 57, 131–137.

Bailey, V.., Smith, J.., Bolton, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry 34, 997–1007. https://doi.org/10.1016/S0038-0717(02)00033-0

Ball, B.A., Carrillo, Y., Molina, M., 2014. The influence of litter composition across the litter–soil interface on mass loss, nitrogen dynamics and the decomposer community. Soil Biology and Biochemistry 69, 71–82. https://doi.org/10.1016/j.soilbio.2013.10.048

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., Davies, J., 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology 13, 650–660.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511. https://doi.org/10.1038/nature13855

Barea, J.M., Pozo, M.J., Azcón, R., Azcón-Aguilar, C., 2013. Microbial interactions in the rhizosphere. Molecular Microbial Ecology of the Rhizosphere 1, 29–44. https://doi.org/10.1002/9781118297674.ch4

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. The ISME Journal 7, 2229–2241. https://doi.org/10.1038/ismej.2013.104

Barois, I., Lavelle, P., 1986. Changes in respiration rate and some physicochemical properties of a tropical soil during transit through Pontoscolex corethrurus (Glossoscolecidae, Oligochaeta). Soil Biology and Biochemistry 18, 539–541.

Barral, M.T., Arias, M., Guérif, J., 1998. Effects of iron and organic matter on the porosity and structural stability of soil aggregates. Soil and Tillage Research 46, 261–272. https://doi.org/10.1016/S0167-1987(98)00092-0

Barré, P., Quénéa, K., Vidal, A., Cécillon, L., Christensen, B.T., Kätterer, T., Macdonald, A., Petit, L., Plante, A.F., van Oort, F., Chenu, C., 2018. Microbial and plant-derived compounds both contribute to persistent soil organic carbon in temperate soils. Biogeochemistry 140, 81–92. https://doi.org/10.1007/s10533-018-0475-5

Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biology and Biochemistry 41, 262–275. https://doi.org/10.1016/j.soilbio.2008.10.024

Bavage, A., Davies, I.G., Robbins, M.P., Morris, P., 1997. Progress and potential for genetic manipulation of plant quality, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB INTERNATIONAL, Wallingford, UK, pp. 201–211.

Beare, M.H., Reddy, M. V., Tian, G., Srivastava, S.C., 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of decomposer biota. Applied Soil Ecology 6, 87–108. https://doi.org/10.1016/S0929-1393(96)00153-9

Berg, B., Steffen, K.T., McClaugherty, C., 2007. Litter decomposition rate is dependent on litter Mn concentrations. Biogeochemistry 82, 29–39. https://doi.org/10.1007/s10533-006-9050-6

Berg, B., Erhagen, B., Johansson, M.-B., Nilsson, M., Stendahl, J., Trum, F., Vesterdal, L., 2015. Manganese in the litter fall-forest floor continuum of boreal and temperate pine and spruce forest ecosystems – A review. Forest Ecology and Management 358, 248–260. https://doi.org/10.1016/j.foreco.2015.09.021

Berg, B., Johansson, M.-B., Ekbohm, G., McClaugherty, C., Rutigliano, F., Santo, A.V. De, 1996. Maximum decomposition limits of forest litter types: a synthesis. Canadian Journal of Botany 74, 659–672. https://doi.org/10.1139/b96-084

Berglund, S.L., Ågren, G.I., Ekblad, A., 2013. Carbon and nitrogen transfer in leaf litter mixtures. Soil Biology and Biochemistry 57, 341–348. https://doi.org/10.1016/j.soilbio.2012.09.015

Beugnon, R., Steinauer, K., Barnes, A.D., Ebeling, A., Roscher, C., 2019. Plant functional trait identity and diversity effects on soil meso- and macrofauna in an experimental grassland. Advances in Ecological Research 61, 163–184. https://doi.org/10.1016/bs.aecr.2019.06.004

Beugnon, R., Steinauer, K., Barnes, A.D., Ebeling, A., Roscher, C., 2019. Plant functional trait identity and diversity effects on soil meso- and macrofauna in an experimental grassland, in: Eisenhauer, N., Bohan, D.A., Dumbrell, A.J. (Eds.), Mechanisms Underlying the Relationship between Biodiversity and Ecosystem Function. Academic Press, pp. 163–184. https://doi.org/10.1016/bs.aecr.2019.06.004

Bhadauria, T., Ramakrishnan, P.S., 1989. Earthworm population dynamics and contribution to nutrient cycling during cropping and fallow phases of shifting agriculture (Jhum) in North-East India. Journal of Applied Ecology 26, 505–520.

Bhadauria, T., Saxena, K.G., 2010. Role of earthworms in soil fertility maintenance through the production of biogenic structures. Applied and Environmental Soil Science 2010, 1–7. https://doi.org/10.1155/2010/816073

Bianchi, F.J.J., Booij, C.J.., Tscharntke, T., 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proceedings of the Royal Society B: Biological Sciences 273, 1715–1727. https://doi.org/10.1098/rspb.2006.3530

Bilde, T., Axelsen, Jo.A., Toft, So., 2000. The value of Collembola from agricultural soils as food for a generalist predator. Journal of Applied Ecology 37, 672–683. https://doi.org/10.1046/j.1365-2664.2000.00527.x

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115–131. https://doi.org/10.1007/s00374-008-0334-y

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science 64, 161–182. https://doi.org/10.1111/ejss.12025

Bonkowski, M., Cheng, W., Griffiths, B.S., Alphei, J., Scheu, S., 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. European Journal of Soil Biology 36, 135–147. https://doi.org/10.1016/S1164-5563(00)01059-1

Bornebusch, C.H., 1930. The fauna of forest soil. Det Forstlige Forsøgsvaesen 11, 1–224.

Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. Soil Biology and Biochemistry 37, 251–258. https://doi.org/10.1016/j.soilbio.2004.07.035

Bradford, M.A., Crowther, T.W., 2013. Carbon use efficiency and storage in terrestrial ecosystems. New Phytologist 199, 7–9. https://doi.org/10.1111/nph.12334

Bradford, M.A., Tordoff, G.M., Eggers, T., Jones, T.H., Newington, J.E., 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99, 317–323. https://doi.org/10.1034/j.1600-0706.2002.990212.x

Brady, N.C., Weil, R.R., 2002. The Nature and Properties of Soils, 13th ed. Prentice Hall, Upper Saddle River, New Jersey.

Briones, M.J.I., Ineson, P., 1996. Decomposition of eucalyptus leaves in litter mixtures. Soil Biology and Biochemistry 28, 1381–1388. https://doi.org/10.1016/S0038-0717(96)00158-7

Briones, M.J., 2014. Soil fauna and soil functions: A jigsaw puzzle. Frontiers in Environmental Science 2, 1–22. https://doi.org/10.3389/fenvs.2014.00007

Brookes, P.C., Lauber, C.L., Rousk, J., Ba, E., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340–1351. https://doi.org/10.1038/ismej.2010.58

Brown, P.D., Morra, M.J., 1997. Control of soil-borne plant pests using glucosinolate-containing plants. Advances in Agronomy 61, 167–231. https://doi.org/10.1016/S0065-2113(08)60664-1

Brussaard, L.; Bouwman, A.; Geurs, M.; Hassink, J.; Zwart, K.B., 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. Netherlands Journal of Agricultural Science 38, 283–302.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry 58, 216–234. https://doi.org/10.1016/j.soilbio.2012.11.009

Butenschoen, O., Marhan, S., Langel, R., Scheu, S., 2009. Carbon and nitrogen mobilisation by earthworms of different functional groups as affected by soil sand content. Pedobiologia 52, 263–272. https://doi.org/10.1016/j.pedobi.2008.11.001

Cagnarini, C., Blyth, E., Emmett, B.A., Evans, C.D., Griffiths, R.I., Keith, A., Jones, L., Lebron, I., McNamara, N.P., Puissant, J., Reinsch, S., Robinson, D.A., Rowe, E.C., Thomas, A.R.C., Smart, S.M., Whitaker, J., Cosby, B.J., 2019. Zones of influence for soil organic matter dynamics: A conceptual framework for data and models. Global Change Biology 25, 3996–4007. https://doi.org/10.1111/gcb.14787

Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49, 637–644. https://doi.org/10.1016/j.pedobi.2005.06.003

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365. https://doi.org/10.1890/0012-9658(2000)081[2359:MESELD]2.0.CO;2

Carrillo, Y., Ball, B.A., Bradford, M.A., Jordan, C.F., Molina, M., 2011. Soil fauna alter the effects of litter composition on nitrogen cycling in a mineral soil. Soil Biology and Biochemistry 43, 1440–1449. https://doi.org/10.1016/j.soilbio.2011.03.011

Carter, D.O., Yellowlees, D., Tibbett, M., 2007. Autoclaving kills soil microbes yet soil enzymes remain active. Pedobiologia 51, 295–299. https://doi.org/10.1016/j.pedobi.2007.05.002

Catt, J.A., Howse, K.R., Christian, D.G., Lane, P.W., Harris, G.L., Goss, M.J., 1998. Strategies to decrease nitrate leaching in the Brimstone Farm Experiment, Oxfordshire, UK, 1988–93: the effect of straw incorporation. The Journal of Agricultural Science 131, 309–320. https://doi.org/10.1017/S0021859698005905

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biology and Biochemistry 37, 1718–1725. https://doi.org/10.1016/j.soilbio.2005.02.006

Chen, B., Liu, E., Tian, Q., Yan, C., Zhang, Y., 2014. Soil nitrogen dynamics and crop residues. A review. Agronomy for Sustainable Development 34, 429–442. https://doi.org/10.1007/s13593-014-0207-8

Chen, C., Zhang, J., Lu, M., Qin, C., Chen, Y., Yang, L., Huang, Q., Wang, J., Shen, Z., Shen, Q., 2016. Microbial communities of an arable soil treated for 8 years with organic and inorganic fertilizers. Biology and Fertility of Soils 52, 455–467. https://doi.org/10.1007/s00374-016-1089-5

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biology and Biochemistry 43, 657–666. https://doi.org/10.1016/j.soilbio.2010.12.002

Clarholm, M., Bonkowski, M., Griffiths, B., 2007. Protozoa and other protista in soil, in: Modern Soil Microbiology.

Coleman, A.D.C., Cole, C. V, Anderson, R. V, Blaha, M., Campion, M.K., Elliott, E.T., Hunt, H.W., Shaefer, B., Sinclair, J., Schaefer, B., 1977. An analysis of rhizosphere-saprophage interactions in terrestrial ecosystems. Ecological Bulletins 25, 299–309.

Coleman, D.C., 2011. Understanding soil processes: One of the last frontiers in biological and ecological research. Australasian Plant Pathology 40, 207–214. https://doi.org/10.1007/s13313-011-0041-2

Coleman, D. C., Crossley Jr., D. A., Hendrix, P.F., 2004. Fundamentals of Soil Ecology, 2nd ed. Elsevier Academic Press.

Coleman, D.C., Wall, D.H., 2015. Soil fauna: Occurrence, biodiversity, and roles in ecosystem function, in: Paul, E.A. (Ed.), Soil Microbiology, Ecology and Biochemistry. Academic Press, pp. 111–149.

Coleman, D.C., Andrews, R., Ellis, J.E., Singh, J.S., 1976. Energy flow and partitioning in selected man-managed and natural ecosystems. Agro-Ecosystems 3, 45–54. https://doi.org/10.1016/0304-3746(76)90099-8

Coleman, D.C., Reid, C.P.P., Cole, C.V., 1983. Biological strategies of nutrient cycling in soil systems. Advances in Ecological Research 13, 1–55. https://doi.org/10.1016/S0065-2504(08)60107-5

Cong, W., Ruijven, J. Van, Werf, W. Van Der, Deyn, G.B. De, Mommer, L., Berendse, F., Hof, E., van Ruijven, J., van der Werf, W., De Deyn, G.B., Mommer, L., Berendse, F., Hoffland, E., 2015. Plant species richness leaves a legacy of enhanced root litter-induced decomposition in soil. Soil Biology and Biochemistry 80, 341–348. https://doi.org/10.1016/j.soilbio.2014.10.017

Cong, W.-F., van Ruijven, J., Mommer, L., De Deyn, G.B., Berendse, F., Hoffland, E., 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. Journal of Ecology 102, 1163–1170. https://doi.org/10.1111/1365-2745.12280

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38, 2053–2062. https://doi.org/10.1016/j.soilbio.2005.12.022

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995. https://doi.org/10.1111/gcb.12113

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1

Crotty, F.V. V., Fychan, R., Scullion, J., Sanderson, R., Marley, C.L.L., 2015. Assessing the impact of agricultural forage crops on soil biodiversity and abundance. Soil Biology and Biochemistry 91, 119–126. https://doi.org/10.1016/j.soilbio.2015.08.036

Cutler, D.W., Crump, L.M., Sandon, H., 1923. A quantitative investigation of the bacterial and protozoan population of the soil, with an account of the protozoan fauna. Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character 211, 317–350.

Curtin, D., Francis, G.S., McCallum, F.M., 2008. Decomposition rate of cereal straw as affected by soil placement. Soil Research 46, 152. https://doi.org/10.1071/sr07085

van Dam, N.M., Heil, M., 2011. Multitrophic interactions below and above ground: En route to the next level. Journal of Ecology 99, 77–88. https://doi.org/10.1111/j.1365-2745.2010.01761.x

Degani, E., 2018. Novel crop rotations to enhance the provision of multiple ecosystem services underpinning arable production. University of Reading.

Degani, E., Leigh, S.G., Barber, H.M., Jones, H.E., Lukac, M., Sutton, P., Potts, S.G., 2019. Crop rotations in a climate change scenario: short-term effects of crop diversity on resilience and ecosystem service provision under drought. Agriculture, Ecosystems & Environment 285, 106625. https://doi.org/10.1016/j.agee.2019.106625

Deibert, E.J., Utter, R.A., 1994. Earthworm populations related to soil and fertilizer management practices. Better Crops 78, 9–11.

Detheridge, A.P., Brand, G., Fychan, R., Crotty, F. V., Sanderson, R., Griffith, G.W., Marley, C.L., 2016. The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agriculture, Ecosystems and Environment 228, 49–61. https://doi.org/10.1016/j.agee.2016.04.022

de Deyn, G.B., Quirk, H., Oakley, S., Ostle, N., Bardgett, R.D., 2011. Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. Biogeosciences 8, 1131–1139. https://doi.org/10.5194/bg-8-1131-2011

de Deyn, G.B., Raaijmakers, C.E., van Ruijven, J., Berendse, F., van der Putten, W.H., 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106, 576–586. https://doi.org/10.1111/j.0030-1299.2004.13265.x

de Deyn, G.B., Van der Putten, W.H., 2005. Linking aboveground and belowground diversity. Trends in Ecology & Evolution 20, 625–633. https://doi.org/10.1016/j.tree.2005.08.009

Di Lonardo, D.P., De Boer, W., Klein Gunnewiek, P.J.A., Hannula, S.E., Van der Wal, A., 2017. Priming of soil organic matter: Chemical structure of added compounds is more important than the energy content. Soil Biology and Biochemistry 108, 41–54. https://doi.org/10.1016/j.soilbio.2017.01.017

Di Lonardo, D.P., Manrubia, M., De Boer, W., Zweers, H., Veen, G.F., Van der Wal, A., 2018. Relationship between home-field advantage of litter decomposition and priming of soil organic matter. Soil Biology and Biochemistry 126, 49–56. https://doi.org/10.1016/j.soilbio.2018.07.025

Domínguez, J., Aira, M., Gómez-Brandón, M., 2010. Vermicomposting: Earthworms enhance the work of microbes, in: Inslam, H., Franke-Whittle, I., Goberna, M. (Ed.), Microbes at Work: From Wastes to Resources. Springer-Verlag, pp. 93–114. https://doi.org/10.1007/978-3-642-04043-6

Dowling, N.J.E., Widdel, F., White, D.C., 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. Microbiology 132, 1815–1825. https://doi.org/10.1099/00221287-132-7-1815

Dreccer, M.F., Schapendonk, A.H.C.M., van Oijen, M., Pot, C.S., Rabbinge, R., 2000. Radiation and nitrogen use at the leaf and canopy level by wheat and oilseed rape during the critical period for grain number definition. Functional Plant Biology 27, 899. https://doi.org/10.1071/pp00019

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18, 1781–1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x

Eisenhauer, N., Dobies, T., Cesarz, S., Hobbie, S.E., Meyer, R.J., Worm, K., Reich, P.B., 2013. Plant diversity effects on soil food webs are stronger than those of elevated CO_2 and N deposition in a long-term grassland experiment. Proceedings of the National Academy of Sciences of the United States of America 110, 6889–94. https://doi.org/10.1073/pnas.1217382110

Eisenlord, S.D., Zak, D.R., Upchurch, R.A., 2012. Dispersal limitation and the assembly of soil *Actinobacteria* communities in a long-term chronosequence. Ecology and Evolution 2, 538–549. https://doi.org/10.1002/ece3.210

Ekschmitt, K., Liu, M., Vetter, S., Fox, O., Wolters, V., 2005. Strategies used by soil biota to overcome soil organic matter stability: Why is dead organic matter left over in the soil? Geoderma 128, 167–176. https://doi.org/10.1016/j.geoderma.2004.12.024

Ellerbrock, R.H., Gerke, H.H., 2004. Characterizing organic matter of soil aggregate coatings and biopores by Fourier transform infrared spectroscopy. European Journal of Soil Science 55, 219–228. https://doi.org/10.1046/j.1365-2389.2004.00593.x

Emmerling, C., 2001. Response of earthworm communities to different types of soil tillage. Applied Soil Ecology 17, 91–96.

Esteban, G. F., Clarke, K. J., Olmo, J. L., Finlay, B.J., 2006. Soil protozoa - An intensive study of population dynamics and community structure in an upland grassland. Applied Soil Ecology 33, 137–151.

Fanin, N., Kardol, P., Farrell, M., Nilsson, M.-C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biology and Biochemistry 128, 111–114. https://doi.org/10.1016/j.soilbio.2018.10.010

Fenn, P., Choi, S., Kirk, T.K., 1981. Ligninolytic activity of Phanerochaete chrysosporium: Physiology of suppression by NH4+ and L-Glutamate. Archives of Microbiolgy 130, 66–71.

Ferlian, O., Klarner, B., Langeneckert, A.E., Scheu, S., 2015. Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. Soil Biology and Biochemistry 82, 28–35. https://doi.org/10.1016/j.soilbio.2014.12.012

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364. https://doi.org/10.1890/05-1839

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6, 1007–1017. https://doi.org/10.1038/ismej.2011.159

Finn, D., Page, K., Catton, K., Strounina, E., Kienzle, M., Robertson, F., Armstrong, R., Dalal, R., 2015. Effect of added nitrogen on plant litter decomposition depends on initial soil carbon and nitrogen stoichiometry. Soil Biology and Biochemistry 91, 160–168. https://doi.org/10.1016/j.soilbio.2015.09.001

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462. https://doi.org/10.1111/j.1469-185X.1988.tb00725.x

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: A question of microbial competition? Soil Biology and Biochemistry 35, 837–843. https://doi.org/10.1016/S0038-0717(03)00123-8

Forney, L.J., Reddy, C.A., Tien, M., Aust, S.D., 1982. The involvement of hydroxyl radical derived from hydrogen peroxide in lignin degradation by the white rot fungus Phanerochaete chrysosporium. The Journal of Biological Chemistry 257, 11455–11462.

Fraser, P.M., Williams, P.H., Haynes, R.J., 1996. Earthworm species, population size and biomass under different cropping systems across the Canterbury Plains, New Zealand. Applied Soil Ecology. https://doi.org/10.1016/0929-1393(95)00062-3

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B.A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P.H., Wickings, K., 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121, 305–316. https://doi.org/10.1007/s10533-014-0004-0

Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723–730. https://doi.org/10.1016/0038-0717(93)90113-P

Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151–163. https://doi.org/10.1016/0167-7012(91)90018-L

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65. https://doi.org/10.1007/bf00384433

Gadd, G.M., 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156, 609–643. https://doi.org/10.1099/mic.0.037143-0

Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R.L., Zak, D.R., 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microbial Ecology 48, 218–229. https://doi.org/10.1007/s00248-003-9001-x

García-Palacios, P., Maestre, F.T., Kattge, J., Wall, D.H., 2013. Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecology Letters 16, 1045–1053. https://doi.org/10.1111/ele.12137

Garnier, E., Navas, M.-L., 2012. A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agronomy for Sustainable Development 32, 365–399. https://doi.org/10.1007/s13593-011-0036-y

Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104, 230–246. https://doi.org/10.1111/j.0030-1299.2004.12738.x

Gastine, A., Scherer-Lorenzen, M., Leadley, P.., 2003. No consistent effects of plant diversity on root biomass, soil biota and soil abiotic conditions in temperate grassland communities. Applied Soil Ecology 24, 101–111. https://doi.org/10.1016/S0929-1393(02)00137-3

Geisen, S., Mitchell, E.A.D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., Fernández, L.D., Jousset, A., Krashevska, V., Singer, D., Spiegel, F.W., Walochnik, J., Lara, E., 2018. Soil protists: a fertile frontier in soil biology research. FEMS Microbiology Reviews 42, 293–323. https://doi.org/10.1093/femsre/fuy006

Geyer, K.M., Dijkstra, P., Sinsabaugh, R., Frey, S.D., 2019. Clarifying the interpretation of carbon use efficiency in soil through methods comparison. Soil Biology and Biochemistry 128, 79–88. https://doi.org/10.1016/j.soilbio.2018.09.036

Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry 127, 173–188. https://doi.org/10.1007/s10533-016-0191-y

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: Toward a global model of decomposition. Global Change Biology 6, 751–765. https://doi.org/10.1046/j.1365-2486.2000.00349.x

Gießelmann, U.C., Martins, K.G., Brändle, M., Schädler, M., Marques, R., Brandl, R., 2011. Lack of home-field advantage in the decomposition of leaf litter in the Atlantic Rainforest of Brazil. Applied Soil Ecology 49, 5–10. https://doi.org/10.1016/j.apsoil.2011.07.010

Gonzalez, G., Seastedt, T., 2001. Soil fauna and plant litter decomposition in tropical and subalpine forests. Ecology 82, 955–964.

Grandy, A.S., Wieder, W.R., Wickings, K., Kyker-Snowman, E., 2016. Beyond microbes: Are fauna the next frontier in soil biogeochemical models? Soil Biology and Biochemistry 102, 40–44. https://doi.org/10.1016/j.soilbio.2016.08.008

Gregory, A.S., Watts, C.W., Griffiths, B.S., Hallett, P.D., Kuan, H.L., Whitmore, A.P., 2009. The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153, 172–185. https://doi.org/10.1016/j.geoderma.2009.08.002

Guggenberger, G., Thomas, R.J., Zech, W., 1996. Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community, Colombia. Applied Soil Ecology 3, 263–274. https://doi.org/10.1016/0929-1393(95)00081-X

Gupta, V.V.S.R., Germida, J.J., 1989. Influence of bacterial-amoebal interactions on sulfur transformations in soil. Soil Biology and Biochemistry 21, 921–930.

Gurr, G.M., Wratten, S.D., Luna, J.M., 2003. Multi-function agricultural biodiversity: pest management and other benefits. Basic and Applied Ecology 4, 107–116. https://doi.org/10.1078/1439-1791-00122

Hamilton, W.E., Sillman, D.Y., 1989. Influence of earthworm middens on the distribution of soil microarthropods. Biology and Fertility of Soils 8, 279–284.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., van Ruijven, J., Vos, V.C.A., Hättenschwiler, S., 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509, 218–221. https://doi.org/10.1038/nature13247

Handayanto, E., Cadisch, G., Giller, K.E., 1995. Manipulation of quality and mineralization of tropical legume tree prunings by varying nitrogen supply. Plant and Soil 176, 149–160. https://doi.org/10.1007/bf00017685

Hanlon, R.D.G., Anderson, J.M., 1979. The effects of Collembola grazing on microbial activity in decomposing leaf litter. Oecologia 38, 93–99.

Hassink, J., Bouwman, L.A., Zwart, K.B., Brussaard, L., 1993. Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. Soil Biology and Biochemistry 25, 47–55. https://doi.org/10.1016/0038-0717(93)90240-C

Hättenschwiler, S., Tiunov, A., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology and Evolution 36, 191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

Heger, T. J., Straub, F., Mitchell, E.A.D., 2012. Impact of farming practices on soil diatoms and testate amoebae : A pilot study in the DOK-trial at Therwil, Switzerland. European Journal of Soil Biology 49, 31–36. https://doi.org/10.1016/j.ejsobi.2011.08.007

Hernández, D.L., Hobbie, S.E., 2010. The effects of substrate composition, quantity, and diversity on microbial activity. Plant and Soil 335, 397–411. https://doi.org/10.1007/s11104-010-0428-9

Hiiesalu, I., Öpik, M., Metsis, M., Lilje, L., Davison, J., Vasar, M., Moora, M., Zobel, M., Wilson, S. d., Pärtel, M., 2012. Plant species richness belowground: higher richness and new patterns revealed by next-generation sequencing. Molecular Ecology 21, 2004–2016. https://doi.org/10.1111/j.1365-294X.2011.05390.x

Ho, A., Lonardo, D.P. Di, Bodelier, P.L.E., 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiology Ecology 93, fix006. https://doi.org/10.1093/femsec/fix006

Hobbie, S.E., 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends in Ecology & Evolution 30, 357–363. https://doi.org/10.1016/j.tree.2015.03.015

Hobbie, S.E., 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. Ecosystems 8, 644–656. https://doi.org/10.1007/s10021-003-0110-7

Holland, J., Fahrig, L., 2000. Effect of woody borders on insect density and diversity in crop fields: a landscape-scale analysis. Agriculture, Ecosystems & Environment 78, 115–122. https://doi.org/10.1016/S0167-8809(99)00123-1

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75, 3–35. https://doi.org/10.1890/04-0922

Hooper, D.U., Bignell, D.E., Brown, V.K., Brussard, L., Dangerfield, J.M., Wall, D.H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., Van Der Putten, W.H., De Ruiter, P.C., Rusek, J., Silver, W.L., Tiedje, J.M., Wolters, V., 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. BioScience 50, 1049–1061. https://doi.org/10.1641/0006-3568(2000)050[1049:ibaabb]2.0.co;2

Hoorman, J.J., 2011. The role of soil bacteria. Fact sheet: Agriculture and Natural Resources. https://doi.org/10.2323/jgam.7.128

Houghton, R. A. and Nassikas, A. A., 2017. Global and regional fluxes of carbon from land use and land cover change 1850-2015, Global Biogeochemical Cycles, 31(3), 456–472, doi:10.1002/2016GB005546

Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., Rose, S.L., Reid, C.P.P., Morley, C.R., 1987. The detrital food web in a shortgrass prairie. Biology and Fertility of Soils 3, 57–68. https://doi.org/10.1007/bf00260580

Ineson, P., Leonard, M.A., Anderson, J.M., 1982. Effect of collembolan grazing upon nitrogen and cation leaching from decomposing leaf litter. Soil Biology and Biochemistry 14, 601–605. https://doi.org/10.1016/0038-0717(82)90094-3

Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecological Monographs 55, 119–140.

Iqbal, A., Aslam, S., Alavoine, G., Benoit, P., Garnier, P., Recous, S., 2015. Rain regime and soil type affect the C and N dynamics in soil columns that are covered with mixed-species mulches. Plant and Soil 393, 319–334. https://doi.org/10.1007/s11104-015-2501-x

Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., Piñeiro, G., 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. Annual Review of Ecology, Evolution, and Systematics 48. https://doi.org/10.1146/annurev-ecolsys-112414-054234

Jenkinson, D.S., 1977. Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from 14C labelled ryegrass decomposing under field conditions. Journal of Soil Science 28, 424–434. https://doi.org/10.1111/j.1365-2389.1977.tb02250.x

Jenkinson, D.S., Fox, R.H., Rayner, J.H., 1985. Interactions between fertilizer nitrogen and soil nitrogen-the so-called 'priming' effect. Journal of Soil Science 36, 425–444. https://doi.org/10.1111/j.1365-2389.1985.tb00348.x

Johnson, D., Vandenkoornhuyse, P.J., Leake, J.R., Gilbert, L., Booth, R.E., Grime, J.P., Young, J.P.W., Read, D.J., 2004. Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytologist 161, 503–515. https://doi.org/10.1046/j.1469-8137.2003.00938.x

Jones, D.L., Hill, P.W., Smith, A.R., Farrell, M., Ge, T., Banning, N.C., Murphy, D.V., 2018. Role of substrate supply on microbial carbon use efficiency and its role in interpreting soil microbial community-level physiological profiles (CLPP). Soil Biology and Biochemistry 123, 1–6. https://doi.org/10.1016/j.soilbio.2018.04.014

Jones, D.L., Cooledge, E.C., Hoyle, F.C., Griffiths, R.I., Murphy, D. V., 2019. pH and exchangeable aluminum are major regulators of microbial energy flow and carbon use efficiency in soil microbial communities. Soil Biology and Biochemistry 138, 107584. https://doi.org/10.1016/j.soilbio.2019.107584

Jones, S.M., van Dyk, J.S., Pletschke, B.I., 2012. Bacillus subtilis SJ01 produces hemicellulose degrading multi-enzyme complexes. BioResources 7, 1294–1309. https://doi.org/10.1111/j.1365-2958.2009.06893.x

Kaewpradit, W., Toomsan, B., Cadisch, G., Vityakon, P., Limpinuntana, V., Saenjan, P., Jogloy, S., Patanothai, A., 2009. Mixing groundnut residues and rice straw to improve rice yield and N use efficiency. Field Crops Research 110, 130–138. https://doi.org/10.1016/j.fcr.2008.07.011

Kallenbach, C.M., Wallenstein, M.D., Schipanksi, M.E., Grandy, A.S., 2019. Managing agroecosystems for soil microbial carbon use efficiency: ecological unknowns, potential outcomes, and a path forward. Frontiers in Microbiology 10. https://doi.org/10.3389/fmicb.2019.01146

Kampichler, C., Bruckner, A., 2009. The role of microarthropods in terrestrial decomposition: A meta-analysis of 40 years of litterbag studies. Biological Reviews. https://doi.org/10.1111/j.1469-185X.2009.00078.x

Kaur, Amrit, Chaudhary, A., Kaur, Amarjeet, Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89, 1103–1112.

Keiluweit, M., Nico, P., Harmon, M.E., Mao, J., Pett-Ridge, J., Kleber, M., 2015. Long-term litter decomposition controlled by manganese redox cycling. Proceedings of the National Academy of Sciences of the United States of America 112, E5253-60. https://doi.org/10.1073/pnas.1508945112

Keiser, A.D., Strickland, M.S., Fierer, N., Bradford, M.A., 2011. The effect of resource history on the functioning of soil microbial communities is maintained across time. Biogeosciences 8, 1477–1486. https://doi.org/10.5194/bg-8-1477-2011

Keiser, A.D., Keiser, D.A., Strickland, M.S., Bradford, M.A., 2014. Disentangling the mechanisms underlying functional differences among decomposer communities. Journal of Ecology 1–7. https://doi.org/10.1111/1365-2745.12220

Kelley, R.L., Reddy, C.A., 1982. Ethylene production from a-oxo-y-methylthiobutyric acid is a sensitive measure of ligninolytic activity by Phanerochaete chrysosporium. Biochemistry Journal 206, 423–425.

Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, T., Sarneel, J.M., Hefting, M.M., 2013. Tea Bag Index: A novel approach to collect uniform decomposition data across ecosystems. Methods in Ecology and Evolution 4, 1070–1075. https://doi.org/10.1111/2041-210X.12097

Keyser, P., Kirk, T.K., Zeikus, J.G., 1978. Ligninolytic enzyme system of Phanerochaete chrysosporium: synthesized in the absence of lignin in response to nitrogen starvation. Journal of Bacteriology 135, 790–797.

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philosophical Transactions of the Royal Society of London. Series B. 363, 685–701. https://doi.org/10.1098/rstb.2007.2178

Killham, K., 1994. Soil Ecology. Cambridge University Press.

Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2ω6,9. Soil Biology and Biochemistry 36, 57–65. https://doi.org/10.1016/j.soilbio.2003.08.019

Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85, 9–24. https://doi.org/10.1007/s10533-007-9103-5

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86, 3252–3257. https://doi.org/10.1890/05-0150

Kominoski, J.S., Hoellein, T.J., Kelly, J.J., Pringle, C.M., 2009. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? Oikos 118, 457–463. https://doi.org/10.1111/j.1600-0706.2008.17222.x

Korobushkin, DI, I., Gongalsky, K.B., Tiunov, A. V, 2014. Isotopic niche (δ13C and δ15N values) of soil macrofauna in temperate forests. Rapid Communications in Mass Spectrometry 28, 1303–11. https://doi.org/10.1002/rcm.6903

Kostenko, O., Duyts, H., Grootemaat, S., De Deyn, G.B., Bezemer, T.M., 2015. Plant diversity and identity effects on predatory nematodes and their prey. Ecology and evolution 5, 836–47. https://doi.org/10.1002/ece3.1337

Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., Kandeler, E., 2016. Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. Frontiers in Microbiology 7, 1–12. https://doi.org/10.3389/fmicb.2016.01524

Kremen, C., Iles, A., Bacon, C., 2012. Diversified farming systems: An agroecological, systems-based alternative to modern industrial agriculture. Ecology and Society 17, art44. https://doi.org/10.5751/ES-05103-170444

Kuebbing, S.E., Bradford, M.A., 2019. The potential for mass ratio and trait divergence effects to explain idiosyncratic impacts of non-native invasive plants on carbon mineralization of decomposing leaf litter. Functional Ecology 33, 1365-2435.13316. https://doi.org/10.1111/1365-2435.13316

Kumar, K., Goh, K.M., 1999. Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. Advances in Agronomy 68, 197–319. https://doi.org/10.1016/S0065-2113(08)60846-9

Kumar, K., Goh, K.M., 2003. Nitrogen release from crop residues and organic amendments as affected by biochemical composition. Communications in Soil Science and Plant Analysis 34, 2441–2460. https://doi.org/10.1081/css-120024778

Kuo, S., Sainju, U.M., 1998. Nitrogen mineralization and availability of mixed leguminous and non-leguminous cover crop residues in soil. Biology and Fertility of Soils 26, 346–353. https://doi.org/10.1007/s003740050387

Kuzyakov, Y., Friedel, J.., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32, 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1–22. https://doi.org/10.1016/j.geoderma.2004.01.032

Lal, R., 1997. Residue management, conservation tillage and soil restoration for mitigating greenhouse effect by CO_2-enrichment. Soil & Tillage Research 43, 81–107.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6, 6707. https://doi.org/10.1038/ncomms7707

Lavelle, P., 1988. Earthworm activities and the soil system. Biology and Fertility of Soils 6, 237–251. https://doi.org/10.1007/bf00260820

Lawrence, K.L., Wise, D.H., 2000. Spider predation on forest-floor Collembola and evidence for indirect effects on decomposition. Pedobiologia. https://doi.org/10.1078/S0031-4056(04)70026-8

Layman, C.A., Arrington, D.A., Montana, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88, 42–48. https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Lecerf, A., Marie, G., Kominoski, J.S., LeRoy, C.J., Bernadet, C., Swan, C.M., 2011. Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. Ecology 92, 160–169. https://doi.org/10.1890/10-0315.1

Lemtiri, A.., Colinet, G.., Alabi, T.., Cluzeau, D.., Zirbes, L.., Haubruge, E.., Francis, F.., 2014. Impacts of earthworms on soil components and dynamics. A review. Biotechnology, Agronomy and Society and Environment 18, 121–133.

Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: The ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9, 119–130. https://doi.org/10.1038/nrmicro2504

Li, X.-M., Chen, Q.-L., He, C., Shi, Q., Chen, S.-C., Reid, B.J., Zhu, Y.-G., Sun, G.-X., 2019. Organic carbon amendments affect the chemodiversity of soil dissolved organic matter and its associations with soil microbial communities. Environmental Science & Technology 53, 50–59. https://doi.org/10.1021/acs.est.8b04673

Li, Y.-B., Li, Q., Yang, J.-J., Lü, X.-T., Liang, W.-J., Han, X.-G., Martijn Bezemer, T., 2017. Home-field advantages of litter decomposition increase with increasing N deposition rates: a litter and soil perspective. Functional Ecology 31, 1792–1801. https://doi.org/10.1111/1365-2435.12863

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. https://doi.org/10.1111/gcb.14781

Lin, B.B., 2011. Resilience in agriculture through crop diversification: adaptive management for environmental change. BioScience 61, 183–193. https://doi.org/10.1525/bio.2011.61.3.4

Lin, D., Pang, M., Fanin, N., Wang, H., Qian, S., Zhao, L., Yang, Y., Mi, X., Ma, K., 2018. Fungi participate in driving home-field advantage of litter decomposition in a subtropical forest. Plant and Soil 1–14. https://doi.org/10.1007/s11104-018-3865-5

Lindberg, N., Bengtsson, J., 2005. Population responses of oribatid mites and collembolans after drought. Applied Soil Ecology 28, 163–174. https://doi.org/10.1016/j.apsoil.2004.07.003

Lindeman, R.L., 1942. The trophic-dynamic aspect of ecology. Ecology 23, 399–417. https://doi.org/10.2307/1930126

Lubbers, I.M., Groenigen, K.J. Van, Fonte, S.J., Six, J., Brussaard, L., Groenigen, J.W. Van, 2013. Greenhouse-gas emissions from soils increased by earthworms. Nature Climate Change 3, 187–194. https://doi.org/10.1038/nclimate1692

Lubbers, I.M., Pulleman, M.M., Willem, J., Groenigen, V., 2017. Can earthworms simultaneously enhance decomposition and stabilization of plant residue carbon? Soil Biology and Biochemistry 105, 12–24. https://doi.org/10.1016/j.soilbio.2016.11.008

Ludwig, M., Achtenhagen, J., Miltner, A., Eckhardt, K.-U., Leinweber, P., Emmerling, C., Thiele-Bruhn, S., 2015. Microbial contribution to SOM quantity and quality in density fractions of temperate arable soils. Soil Biology and Biochemistry 81, 311–322. https://doi.org/10.1016/j.soilbio.2014.12.002

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions: A review. European Journal of Soil Science. https://doi.org/10.1111/j.1365-2389.2006.00809.x

von Lützow, M., Kögel-Knabner, I., 2009. Temperature sensitivity of soil organic matter decomposition - what do we know? Biology and Fertility of Soils 46, 1–15. https://doi.org/10.1007/s00374-009-0413-8

Ma, T., Zhu, S., Wang, Z., Chen, D., Dai, G., Feng, B., Su, X., Hu, H., Li, K., Han, W., Liang, C., Bai, Y., Feng, X., 2018. Divergent accumulation of microbial necromass and plant lignin components in grassland soils. Nature Communications 9, 3480. https://doi.org/10.1038/s41467-018-05891-1

Mafongoya, P., Dzowela, B.H., Nair, P.K., 1997. Effect of multipurpose trees, age of cutting and drying method on pruning quality, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB INTERNATIONAL, Wallingford, UK, pp. 167–174.

Makkonen, M., Berg, M.P., van Logtestijn, R.S.P., van Hal, J.R., Aerts, R., 2013. Do physical plant litter traits explain non-additivity in litter mixtures? A test of the improved microenvironmental conditions theory. Oikos 122, 987–997. https://doi.org/10.1111/j.1600-0706.2012.20750.x

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., Tourdonnet, S., Valantin-Morison, M., 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. Agronomy for Sustainable Development 29, 43–62. https://doi.org/10.1051/agro:2007057

Malik, A.A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P.G.M., Jehmlich, N., Bergen, M. Von, Griffiths, R.I., Gleixner, G., 2016. Soil fungal:bacterial ratios are linked to altered carbon cycling. Frontiers in Microbiology 7, 1–11. https://doi.org/10.3389/fmicb.2016.01247

Malik, A.A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P.G.M., Jehmlich, N., von Bergen, M., Griffiths, R.I., Gleixner, G., 2016. Soil fungal:bacterial ratios are linked to altered carbon cycling. Frontiers in Microbiology 7, 1247. https://doi.org/10.3389/fmicb.2016.01247

Mao, R., Zeng, D.-H., 2012. Non-additive effects vary with the number of component residues and their mixing proportions during residue mixture decomposition: A microcosm study. Geoderma 170, 112–117. https://doi.org/10.1016/j.geoderma.2011.11.008

Maraun, M., Erdmann, G., Fischer, B.M., Pollierer, M.M., Norton, R.A., Schneider, K., Scheu, S., 2011. Stable isotopes revisited : Their use and limits for oribatid mite trophic ecology. Soil Biology and Biochemistry 43, 877–882. https://doi.org/10.1016/j.soilbio.2011.01.003

Marinissen, J.C.Y., de Ruiter, P.C., 1993. Contribution of earthworms to carbon and nitrogen cycling in agro-ecosystems. Agriculture, Ecosystems and Environment 47, 59–74. https://doi.org/10.1016/0167-8809(93)90136-D

Marschner, P., Hatam, Z., Cavagnaro, T.R., 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biology and Biochemistry 88, 169–177. https://doi.org/10.1016/j.soilbio.2015.05.023

Martens, R., 1995. Current methods for measuring microbial biomass C in soil: Potentials and limitations, Biology and Fertility of Soils, 19(2–3), 87–99, doi:10.1007/BF00336142

Martens, D.A., 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. Soil Biology and Biochemistry 32, 361–369. https://doi.org/10.1016/S0038-0717(99)00162-5

Martin, A., 1991. Short-and long-term effects of the endogeic earthworm *Millsonia anomala* (Omodeo)(Megascolecidae, Oligochaeta) of tropical savannas, on soil organic matter. Biology and Fertility of Soils 11, 234–238. https://doi.org/10.1007/bf00335774

Martin, J.P., Martin, W.P., Page, J.B., Raney, W.A., de Ment, J.D., 1955. Soil aggregation. Advances in Agronomy 7, 1–37. https://doi.org/10.1016/S0065-2113(08)60333-8

Masuko, K., 1994. Specialized predation on oribatid mites by two species of the ant genus *Myrmecina* (Hymenoptera: Formicidae). Psyche: A Journal of Entomology 101, 159–173. https://doi.org/10.1155/1994/96412

Maynard, D.S., Crowther, T.W., Bradford, M.A., 2017. Competitive network determines the direction of the diversity–function relationship. Proceedings of the National Academy of Sciences 114, 11464–11469. https://doi.org/10.1073/PNAS.1712211114

Mazzoncini, M., Canali, S., Giovannetti, M., Castagnoli, M., Tittarelli, F., Antichi, D., Nannelli, R., Cristani, C., Bàrberi, P., 2010. Comparison of organic and conventional stockless arable systems: A multidisciplinary approach to soil quality evaluation. Applied Soil Ecology. https://doi.org/10.1016/j.apsoil.2009.11.001

Bardgett, R. D. and McAlister, E., 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands, Biol. Fertil. Soils, 29(3), 282–290, doi:10.1007/s003740050554

McBride, M.B., 1994. Environmental Chemistry of Soils. Oxford University Press, New York.

McDaniel, M.D., Grandy, A.S., Tiemann, L.K., Weintraub, M.N., 2016. Eleven years of crop diversification alters decomposition dynamics of litter mixtures incubated with soil. Ecosphere 7, e01426. https://doi.org/10.1002/ecs2.1426

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. https://doi.org/10.1890/13-0616.1

McInerney, M., Bolger, T., 2000. Decomposition of *Quercus petraea* litter: Influence of burial, comminution and earthworms. Soil Biology and Biochemistry 32, 1989–2000. https://doi.org/10.1016/S0038-0717(00)00097-3

McKinney, M.L., 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. Annual Review of Ecology and Systematics 28, 495–516. https://doi.org/10.1146/annurev.ecolsys.28.1.495

Medina, J., Monreal, C., Barea, J.M., Arriagada, C., Borie, F., Cornejo, P., 2015. Crop residue stabilization and application to agricultural and degraded soils: A review. Waste Management. https://doi.org/10.1016/j.wasman.2015.04.002

Melody, C., Griffiths, B., Dyckmans, J., Schmidt, O., 2016. Stable isotope analysis (δ13C and δ15N) of soil nematodes from four feeding groups. PeerJ 1–19. https://doi.org/10.7717/peerj.2372

Menta, C., 2012. Soil Fauna Diversity – Function, Soil Degradation, Biological Indices, Soil Restoration, in: Lameed, G.A. (Ed.), Biodiversity Conservation and Utilization in a Diverse World. IntechOpen, pp. 59–94. https://doi.org/http://dx.doi.org/10.5772/51091

Mondini, C., Cayuela, M.L., Sanchez-Monedero, M.A., Roig, A., Brookes, P.C., 2006. Soil microbial biomass activation by trace amounts of readily available substrate. Biology and Fertility of Soils 42, 542–549. https://doi.org/10.1007/s00374-005-0049-2

Moore, J.C., Hunt, H.W., 1988. Resource compartmentation and the stability of real ecosystems. Nature 336, 261–263. https://doi.org/10.1038/332141a0

Moore, J.C., Walter, D.E., Hunt, H.W., 1988. Arthropod regulation of micro-and mesobiota in below-ground detrital food webs. Annual Review of Entomology 33, 419–439.

Mouginot, C., Kawamura, R., Matulich, K.L., Berlemont, R., Allison, S.D., Amend, A.S., Martiny, A.C., 2014. Elemental stoichiometry of fungi and bacteria strains from grassland leaf litter. Soil Biology and Biochemistry 76, 278–285. https://doi.org/10.1016/j.soilbio.2014.05.011

Myers, R.J.K., Noordwijk, M. van, Vityakon, P., 1997. Synchrony of nutrient release and plant demand: plant litter quality, soil environment and farmer management options., in: Cadish, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. pp. 215–229.

Nath, A.J., Lal, R., 2017. Effects of tillage practices and land use management on soil aggregates and soil organic carbon in the north Appalachian region, USA. Pedosphere 27, 172–176. https://doi.org/10.1016/S1002-0160(17)60301-1

Nicolardot, B., Recous, S., Mary, B., 2001. Simulation of C and N mineralisation during crop residue decomposition: a simple dynamic model based on the C:N ratio of the residues. Plant and Soil 228, 83–103.

Nielsen, U.N., Osler, G.H.R., Campbell, C.D., Neilson, R., Burslem, D.F.R.P., van der Wal, R., 2010. The enigma of soil animal species diversity revisited: The role of small-scale heterogeneity. PLoS ONE 5, e11567. https://doi.org/10.1371/journal.pone.0011567

Nielsen, U.N., Osler, G.H.R., van der Wal, R., Campbell, C.D., Burslem, D.F.R.P., 2008. Soil pore volume and the abundance of soil mites in two contrasting habitats. Soil Biology and Biochemistry 40, 1538–1541. https://doi.org/10.1016/j.soilbio.2007.12.029

Nilsson, M.-C., Wardle, D.A., DeLuca, T.H., 2008. Belowground and aboveground consequences of interactions between live plant species mixtures and dead organic substrate mixtures. Oikos 117, 439–449. https://doi.org/10.1111/j.2007.0030-1299.16265.x

Nimmo, J.R., Perkins, K.S., 2002. Aggregate stability and size distribution, in: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis, Part 4: Physical Methods. Soil Science Society of America, Madison, WI.

Ojha, R.B., Devkota, D., 2014. Earthworms: "Soil and ecosystem engineers" – a review. World Journal of Agricultural Research 2, 257–260. https://doi.org/10.12691/wjar-2-6-1

Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. SOIL 5, 15–32. https://doi.org/10.5194/soil-5-15-2019

Öquist, M.G., Erhagen, B., Haei, M., Sparrman, T., Ilstedt, U., Schleucher, J., Nilsson, M.B., 2017. The effect of temperature and substrate quality on the carbon use efficiency of saprotrophic decomposition. Plant and Soil 414, 113–125. https://doi.org/10.1007/s11104-016-3104-x

Östman, Ö., Ekbom, B., Bengtsson, J., Weibull, A.-C., 2001. Landscape complexity and farming practice influence the condition of polyphagous carabid beetles. Ecological Applications 11, 480–488. https://doi.org/10.1890/1051-0761(2001)011[0480:LCAFPI]2.0.CO;2

Ostrowska, A., Porębska, G., 2015. Assessment of the C/N ratio as an indicator of the decomposability of organic matter in forest soils. Ecological Indicators 49, 104–109. https://doi.org/10.1016/j.ecolind.2014.09.044

Otsing, E., Barantal, S., Anslan, S., Koricheva, J., Tedersoo, L., 2018. Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. Soil Biology and Biochemistry 125, 328–339. https://doi.org/10.1016/j.soilbio.2018.08.006

Paoletti, M.G., Sommaggio, D., Favretto, M.R., 1998. Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. Applied Soil Ecology 10, 137–150.

Paoletti, M.G., Schweigl, U., Favretto, M.R., 1995. Soil macroinvertebrates, heavy metals and organochlorines in low and high input apple orchards and a coppiced woodland. Pedobiologia 39.

Paoletti, M.G., 1999. The role of earthworms for assessment of sustainability and as bioindicators. Agriculture, Ecosystems and Environment 74, 137–155. https://doi.org/http://dx.doi.org/10.1016/B978-0-444-50019-9.50011-X

Parker, L.W., Santos, P.F., Phillips, J., Whitford, W.G., 1984. Carbon and nitrogen dynamics during the decomposition of litter an roots of a chihuahuan desert annual, Lepidium lasiocarpum. Ecological Monographs 54, 339–360.

Paul, E.A., 2015. Soil Microbiology, Ecology, and Biogeochemistry, 4th ed. Elsevier.

Paustian, K., Ågren, G.I., Bosatta, E., 1997. Modelling litter quality effects on decomposition and soil organic matter dynamics, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB INTERNATIONAL, Wallingford, UK, pp. 313–335.

Peach, W.J., Lovett, L.J., Wotton, S.R., Jeffs, C., 2001. Countryside stewardship delivers cirl buntings (*Emberiza cirlus*) in Devon, UK. Biological Conservation 101, 361–373. https://doi.org/10.1016/S0006-3207(01)00083-0

Pérez Harguindeguy, N., Blundo, C.M., Gurvich, D.E., Díaz, S., Cuevas, E., 2008. More than the sum of its parts? Assessing litter heterogeneity effects on the decomposition of litter mixtures through leaf chemistry. Plant and Soil 303, 151–159. https://doi.org/10.1007/s11104-007-9495-y

Petersen, H., Luxton, M., 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39, 287–388.

Pfeiffer, T., Schuster, S., Bonhoeffer, S., 2001. Cooperation and competition in the evolution of ATP-producing pathways. Science 292, 504–507. https://doi.org/10.1126/science.1058079

Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: A central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124–134. https://doi.org/10.1016/j.soilbio.2012.07.026

Pokarzhevskii, A.D., Zaboyev, D.P., Ganin, G.N., Gordienko, S.A., 1997. Amino acids in earthworms: Are earthworms ecosystemivorous? Soil Biology and Biochemistry 29, 559–567. https://doi.org/10.1016/S0038-0717(96)00180-0

Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. Ecology Letters 10, 729–736. https://doi.org/10.1111/j.1461-0248.2007.01064.x

Pollierer, M.M., Langel, R., Scheu, S., Maraun, M., 2009. Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios (15N/14N and13C/12C). Soil Biology and Biochemistry 41, 1221–1226. https://doi.org/10.1016/j.soilbio.2009.03.002

Ponge, J.-F., 2000. Vertical distribution of Collembola (Hexapoda) and their food resources in organic horizons of beech forests. Biology and Fertility of Soils 32, 508–522.

Ponsard, S., Arditi, R., 2000. What can stable isotopes (δ15N and δ13C) tell about the food web of soil macro-invertebrates? Ecology 81, 852–864.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. . Ecology 83, 703–718. https://doi.org/Doi 10.2307/3071875

Potapov, A.A., Semenina, E.E., Yu, A., Kuznetsova, N.A., Tiunov, A. V, 2016. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry 101, 20–31. https://doi.org/10.1016/j.soilbio.2016.07.002

Powlson, D.S., Bhogal, A., Chambers, B.J., Coleman, K., Macdonald, A.J., Goulding, K.W.T., Whitmore, A.P., 2012. The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: A case study. Agriculture, Ecosystems & Environment 146, 23–33. https://doi.org/10.1016/j.agee.2011.10.004

Powlson, D.S., Riche, A.B., Coleman, K., Glendining, M.J., Whitmore, A.P., 2008. Carbon sequestration in European soils through straw incorporation: Limitations and alternatives. Waste Management 28, 741–746. https://doi.org/10.1016/j.wasman.2007.09.024

Powlson, D.S., Glendining, M.J., Coleman, K., Whitmore, A.P., 2011. Implications for soil properties of removing cereal straw: Results from long-term studies. Agronomy Journal 103, 279–287. https://doi.org/10.2134/agronj2010.0146s

Powlson, D.S., Stirling, C.M., Jat, M.L., Gerard, B.G., Palm, C.A., Sanchez, P.A., Cassman, K.G., 2014. Limited potential of no-till agriculture for climate change mitigation. Nature Climate Change 4, 678–683. https://doi.org/10.1038/nclimate2292

Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y., Jongmans, A.G., 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. Applied Soil Ecology 29, 1–15. https://doi.org/10.1016/j.apsoil.2004.10.003

Quemada, M., Cabrera, M.L., 1995. Carbon and nitrogen mineralized from leaves and stems of four cover crops. Soil Science Society of America Journal 59, 471. https://doi.org/10.2136/sssaj1995.03615995005900020029x

Rabbi, S.M.F., Linser, R., Hook, J.M., Wilson, B.R., Lockwood, P. V., Daniel, H., Young, I.M., 2014. Characterization of soil organic matter in aggregates and size-density fractions by solid state 13C CPMAS NMR spectroscopy. Communications in Soil Science and Plant Analysis 45, 1523–1537. https://doi.org/10.1080/00103624.2014.904335

Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18, 1918–1927. https://doi.org/10.1111/j.1365-2486.2012.02639.x

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91, 3463–3470. https://doi.org/10.1890/10-0426.1

Rashid, M.I., de Goede, R.G.M., Brussaard, L., Lantinga, E.A., 2013. Home field advantage of cattle manure decomposition affects the apparent nitrogen recovery in production grasslands. Soil Biology and Biochemistry 57, 320–326. https://doi.org/10.1016/j.soilbio.2012.10.005

RB209, 2019. Nutrient Management Guide (RB209). Section 6: Vegetable and bulbs.

Recous, S., Robin, D., Darwis, D., Mary, B., 1995. Soil inorganic N availability: Effect on maize residue decomposition. Soil Biology and Biochemistry 27, 1529–1538. https://doi.org/10.1016/0038-0717(95)00096-W

Redin, M., Recous, S., Aita, C., Dietrich, G., Skolaude, A.C., Ludke, W.H., Schmatz, R., Giacomini, S.J., 2014. How the chemical composition and heterogeneity of crop residue mixtures decomposing at the soil surface affects C and N mineralization. Soil Biology and Biochemistry 78, 65–75. https://doi.org/10.1016/j.soilbio.2014.07.014

Roberts, P., Bol, R., Jones, D.L., 2007. Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. Soil Biology and Biochemistry 39, 3081–3092. https://doi.org/10.1016/j.soilbio.2007.07.001

Robinson, R.A., Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology 39, 157–176. https://doi.org/10.1046/j.1365-2664.2002.00695.x

Roller, B.R., Schmidt, T.M., 2015. The physiology and ecological implications of efficient growth. The ISME Journal 9, 1481–1487. https://doi.org/10.1038/ismej.2014.235

Rousk, J., 2016. Biomass or growth? How to measure soil food webs to understand structure and function. Soil Biology and Biochemistry 102, 1–3. https://doi.org/10.1016/j.soilbio.2016.07.001

Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiology Ecology 62, 258–267. https://doi.org/10.1111/j.1574-6941.2007.00398.x

Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75, 1589–96. https://doi.org/10.1128/AEM.02775-08

Ruess, L., Ferris, H., 2004. Decomposition pathways and successional changes. Nematology Monographs & Perspectives 2, 547–556.

de Ruiter, P.C. De, Moore, J.C., Zwart, K.B., Bouwman, L.A., Hassink, J., Bloem, J., Vos, J.A. De, Marinissen, J.C.Y., Didden, W.A.M., Lebrink, G., Brussaard, L., 1993. Simulation of nitrogen mineralization in the below-ground food webs of two winter wheat fields. The Journal of Applied Ecology 30, 95. https://doi.org/10.2307/2404274

de Ruiter, P.C., Neutel, A., Moore, J.C., 1994. Modelling food webs and nutrient cychg in agro-ecosystems. Tree 9, 378–383.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G., 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32, 305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Salamon, J.-A., Wissuwa, J., Moder, K., Frank, T., 2011. Effects of *Medicago sativa*, *Taraxacum officinale* and *Bromus sterilis* on the density and diversity of Collembola in grassy arable fallows of different ages. Pedobiologia 54, 63–70. https://doi.org/10.1016/j.pedobi.2010.08.007

Sanderman, J., Amundson, R., 2014. Biogeochemistry of decomposition and detrital processing, in: Treatise on Geochemistry. Elsevier Ltd., pp. 217–272. https://doi.org/10.1016/B978-0-08-095975-7.00807-X

Santonja, M., Foucault, Q., Rancon, A., Gauquelin, T., Fernandez, C., Baldy, V., Mirleau, P., 2018. Contrasting responses of bacterial and fungal communities to plant litter diversity in a Mediterranean oak forest. Soil Biology and Biochemistry 125, 27–36. https://doi.org/10.1016/j.soilbio.2018.06.020

Scheu, S., Simmerling, F., 2004. Growth and reproduction of fungal feeding Collembola as affected by fungal species, melanin and mixed diets. Oecologia 139, 347–353. https://doi.org/10.1007/s00442-004-1513-7

Scheu, S., Ruess, L., Bonkowski, M., 2005. Interactions between microorganisms and soil micro- and mesofauna, in: Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag, Berlin/Heidelberg, pp. 253–275. https://doi.org/10.1007/3-540-26609-7_12

Schimel, J.P., Hättenschwiler, S., 2007. Nitrogen transfer between decomposing leaves of different N status. Soil Biology and Biochemistry 39, 1428–1436. https://doi.org/10.1016/j.soilbio.2006.12.037

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 348. https://doi.org/10.3389/fmicb.2012.00348

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35, 549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394. https://doi.org/10.1890/06-0219

Schneider, K., Migge, S., Norton, R.A., Scheu, S., Langel, R., Reineking, A., Maraun, M., 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): Evidence from stable isotope ratios (15N/14N). Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2004.04.033

Seastedt, T.R., 1984. The role of microarthropods in decomposition and mineralization processes. Annual Review of Entomology 29, 25–46.

Sharma, N., Parwez, H., 2017. Population density and diversity of soil mites (Order: Acarina) in agroforestry habitat: relationship to soil temperature and soil moisture. International Journal of Applied Environmental Sciences 12, 1449–1460.

Shipitalo, M.J., Protz, R., 1989. Chemistry and micromorphology of aggregation in earthworm casts. Geoderma 45, 357–374. https://doi.org/10.1016/0016-7061(89)90016-5

Siepel, H., Ruiter-Dijkman, E.M. de, 1993. Feeding guilds of oribatid mites based on their carbohydrase activities. Soil Biology and Biochemistry 25, 1491–1497. https://doi.org/10.1016/0038-0717(93)90004-U

Silgram, M., Chambers, B.J., 2002. Effects of long-term straw management and fertilizer nitrogen additions on soil nitrogen supply and crop yields at two sites in eastern England. Journal of Agricultural Science 139, 115–127. https://doi.org/10.1017/S0021859602002435

Simpson, A.J., Simpson, M.J., Smith, E., Kelleher, B.P., 2007. Microbially derived inputs to soil organic matter: are current estimates too low? Environmental Science & Technology 41, 8070–8076. https://doi.org/10.1021/es071217x

Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nature Reviews Microbiology 8, 779–790. https://doi.org/10.1038/nrmicro2439

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16, 930–939. https://doi.org/10.1111/ele.12113

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Follstad Shah, J.J., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecological Monographs 86, 172–189. https://doi.org/10.1890/15-2110.1

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367. https://doi.org/10.2136/sssaj1998.03615995006200050032x

Six, J., Elliott, E.., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099–2103. https://doi.org/10.1016/S0038-0717(00)00179-6

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555. https://doi.org/10.2136/sssaj2004.0347

Sizmur, T., Martin, E., Wagner, K., Parmentier, E., Watts, C., Whitmore, A.P., 2017. Milled cereal straw accelerates earthworm (*Lumbricus terrestris*) growth more than selected organic amendments. Applied Soil Ecology 113, 166–177. https://doi.org/10.1016/j.apsoil.2016.12.006

Sizmur, T., Tilston, E.L., Charnock, J., Palumbo-Roe, B., Watts, M.J., Hodson, M.E., 2011. Impacts of epigeic, anecic and endogeic earthworms on metal and metalloid mobility and availability. Journal of Environmental Monitoring 13, 266–273. https://doi.org/10.1039/c0em00519C

Smil, V., 1999. Crop residues : Agriculture's largest harvest. BioScience 49, 299–308.

Sollins, P., Swanston, C., Kramer, M., 2007. Stabilization and destabilization of soil organic matter: A new focus. Biogeochemistry 85, 1–7. https://doi.org/10.1007/s10533-007-9099-x

Soong, J.L., Nielsen, U.N., 2016. The role of microarthropods in emerging models of soil organic matter. Soil Biology and Biochemistry 102, 37–39. https://doi.org/10.1016/j.soilbio.2016.06.020

Spehn, E.M., Joshi, J., Schmid, B., Alphei, J., Körner, C., 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant and Soil 224, 217–230. https://doi.org/10.1023/A:1004891807664

Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth – Implications for carbon cycling. Soil Biology and Biochemistry 96, 74–81. https://doi.org/10.1016/j.soilbio.2016.01.016

St. John, M.G., Orwin, K.H., Dickie, I.A., 2011. No 'home' versus 'away' effects of decomposition found in a grassland–forest reciprocal litter transplant study. Soil Biology and Biochemistry 43, 1482–1489. https://doi.org/10.1016/j.soilbio.2011.03.022

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2009. Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. Soil Biology and Biochemistry 41, 357–366. https://doi.org/10.1016/j.soilbio.2008.11.011

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Strickland, M.S., Osburn, E., Lauber, C., Fierer, N., Bradford, M.A., 2009. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology 23, 627–636. https://doi.org/10.1111/j.1365-2435.2008.01515.x

Stursova, M., Sinsabaugh, R.L., 2008. Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. Soil Biology and Biochemistry 40, 550–553. https://doi.org/10.1016/j.soilbio.2007.09.002

Summerhayes, V.S., Elton, C.S., 1923. Contributions to the ecology of Spitsbergen and Bear Island. Journal of Ecology 11, 214–216.

Sun, T., Cui, Y., Berg, B., Zhang, Q., Dong, L., Wu, Z., Zhang, L., 2019. A test of manganese effects on decomposition in forest and cropland sites. Soil Biology and Biochemistry 129, 178–183. https://doi.org/10.1016/j.soilbio.2018.11.018

Swift, M. J., Heal, O. W., Anderson, J.M., 1979. Decomposition in terrestrial ecosystems. University of California Press.

Thomsen, I.K., Christensen, B.T., 2006. Yields of wheat and soil carbon and nitrogen contents following long-term incorporation of barley straw and ryegrass catch crops. Soil Use and Management 20, 432–438. https://doi.org/10.1111/j.1475-2743.2004.tb00393.x

Tian, G., Brussaard, L., Kang, B.T., 1995. An index for assessing the quality of plant residues and evaluating their effects on soil and crop in the (sub-) humid tropics. Applied Soil Ecology 2, 25–32. https://doi.org/10.1016/0929-1393(94)00033-4

Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., Mcdaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecology Letters 18, 761–771. https://doi.org/10.1111/ele.12453

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677. https://doi.org/10.1038/nature01014

Tilman, D., Reich, P.B., Knops, J.M.H., 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441, 629–632. https://doi.org/10.1038/nature04742

Tisdale, S.L., Nelson, W.L., Beaton, J.D., 1985. Soil Fertility and Fertilizers, Fourth ed. ed. Macmillan Publishing Company, New York.

Tiunov, A. V., 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. Biology Bulletin 34, 395–407. https://doi.org/10.1134/S1062359007040127

Tlalka, M., Bebber, D.P., Darrah, P.R., Watkinson, S.C., Fricker, M.D., 2007. Emergence of self-organised oscillatory domains in fungal mycelia. Fungal Genetics and Biology 44, 1085–1095. https://doi.org/10.1016/J.FGB.2007.02.013

Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018. Microaggregates in soils. Journal of Plant Nutrition and Soil Science 181, 104–136. https://doi.org/10.1002/jpln.201600451

Tresch, S., Frey, D., Bayon, R.-C. Le, Mäder, P., Stehle, B., Fliessbach, A., Moretti, M., 2019. Direct and indirect effects of urban gardening on aboveground and belowground diversity influencing soil multifunctionality. Scientific Reports 9, 9769. https://doi.org/10.1038/s41598-019-46024-y

Tunlid, A., White, D., 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil, in: Stotzky, G., Bollag, J.-M. (Eds.), Soil Biochemistry. Marcel Dekker, Inc., pp. 229–262. https://doi.org/10.1002/jpln.19911540118

Vance, E.D., Brooks, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vanlauwe, B., Diels, J., Sanginga, N., Merckx, R., 1997. Residue quality and decomposition: an unsteady relationship?, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB INTERNATIONAL, Wallingford, UK, pp. 157–166.

Veen, G.F.C., Freschet, G.T., Ordonez, A., Wardle, D.A., 2015. Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos 124, 187–195. https://doi.org/10.1111/oik.01374

Veen, G.F.C., Keiser, A.D., van der Putten, W.H., Wardle, D.A., 2018. Variation in home-field advantage and ability in leaf litter decomposition across successional gradients. Functional Ecology. https://doi.org/10.1111/1365-2435.13107

Verhoef, H. a., Brussaard, L., 1990. Decomposition and nitrogen mineralization in natural agro-ecosytems: The contribution of soil animals. Biogeochemistry 11, 175–211.

Verhulst, P.-F., 1838. Notice sur la loi que la population suit dans son accroissement. Correspondance mathématique et physique de l'Observatoire de Bruxelles 10, 113–121.

Viketoft, M., Bengtsson, J., Sohlenius, B., Berg, M.P., Petchey, O., Palmborg, C., Huss-Danell, K., 2009. Long-term effects of plant diversity and composition on soil nematode communities in model grasslands. Ecology 90, 90–99. https://doi.org/10.1890/08-0382.1

Vivanco, L., Austin, A.T., 2008. Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. Journal of Ecology 96, 727–736. https://doi.org/10.1111/j.1365-2745.2008.01393.x

Vos, V.C.A., van Ruijven, J., Berg, M.P., Peeters, E.T.H.M., Berendse, F., 2013. Leaf litter quality drives litter mixing effects through complementary resource use among detritivores. Oecologia 173, 269–280. https://doi.org/10.1007/s00442-012-2588-1

de Vries, F.T. De, Willem, J., Groenigen, V., Hof, E., Bloem, J., 2011. Nitrogen losses from two grassland soils with different fungal biomass. Soil Biology and Biochemistry 43, 997–1005. https://doi.org/10.1016/j.soilbio.2011.01.016

de Vries, F.T., Bardgett, R.D., 2012. Plant-microbial linkages and ecosystem nitrogen retention: Lessons for sustainable agriculture. Frontiers in Ecology and the Environment 10, 425–432. https://doi.org/10.1890/110162

de Vries, F.T., Caruso, T., 2016. Eating from the same plate? Revisiting the role of labile carbon inputs in the soil food web. Soil Biology and Biochemistry 102, 4–9. https://doi.org/10.1016/j.soilbio.2016.06.023

Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America 111, 5266–70. https://doi.org/10.1073/pnas.1320054111

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G.A., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nature Communications 10, 4841. https://doi.org/10.1038/s41467-019-12798-y

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecological Applications 14, 1172–1177. https://doi.org/10.1890/03-5120

Walter, D.E., Ikonen, E.K., 1989. Species, guilds and functional groups: taxonomy and behaviour in nematophagous arthropods. Journal of Nematology 21, 315–327.

Wardle, D.A., Bonner, K.I., Nicholson, K.S., 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. Oikos 79, 247–258.

Wardle, D.A., 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9, 870–886. https://doi.org/10.1111/j.1461-0248.2006.00931.x

Wardle, D.A., Nilsson, M.C., Zackrisson, O., Gallet, C., 2003. Determinants of litter mixing effects in a Swedish boreal forest. Soil Biology and Biochemistry 35, 827–835. https://doi.org/10.1016/S0038-0717(03)00118-4

Wardle, D.A., Yeates, G.W., Williamson, W., Bonner, K.I., 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos 102, 45–56. https://doi.org/10.1034/j.1600-0706.2003.12481.x

Weibull, A.-C., Bengtsson, J., Nohlgren, E., 2000. Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. Ecography 23, 743–750.

White, C.A., Sylvester-Bradley, R., Berry, P.M., 2015. Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield. Journal of Experimental Botany 66, 2293–2303. https://doi.org/10.1093/jxb/erv077

Whitford, W.G., Freckman, D.W., Parker, L.W., Shaefer, D., Snatos, P.F., Steinberger, Y., 1983. The contributions of soil fauna to nutrient cycles in desert systems, in: Lebrun, P., André, H.M., Medts, A. de, Grégoire-Wibo, C., Wauty, G. (Eds.), New Trends in Soil Biology. Washington, D. C., pp. 49–59.

Wickings, K., Grandy, A.S., Reed, S.C., Cleveland, C.C., 2012. The origin of litter chemical complexity during decomposition. Ecology Letters 15, 1180–1188. https://doi.org/10.1111/j.1461-0248.2012.01837.x

Wilkinson, D.M., Creevy, A.L., Valentine, J., 2012. The past, present and future of soil protist ecology. Acta Protozoologica 51, 189–199. https://doi.org/10.4467/16890027AP.12.015.0761

Wilson, E.O., 2005. Oribatid mite predation by small ants of the genus *Pheidole*. Insectes Sociaux 52, 263–265. https://doi.org/10.1007/s00040-005-0802-4

Wink, M., 2008. Plant secondary metabolism: Diversity, function and its evolution, Nat. Prod. Commun., 3(8), 1205–1216, doi:10.1177/1934578x0800300801

Wissuwa, J., Salamon, J.-A., Frank, T., 2012. Effects of habitat age and plant species on predatory mites (Acari, Mesostigmata) in grassy arable fallows in Eastern Austria. Soil Biology and Biochemistry 50, 96–107. https://doi.org/10.1016/j.soilbio.2012.02.025

Wu, J., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biology and Biochemistry 25, 1435–1441. https://doi.org/10.1016/0038-0717(93)90058-J

Wu, T., Ayres, E., Bardgett, R.D., Wall, D.H., Garey, J.R., 2011. Molecular study of worldwide distribution and diversity of soil animals. Proceedings of the National Academy of Sciences of the United States of America 108, 17720–5. https://doi.org/10.1073/pnas.1103824108

Yeates, G.W., Bongers, T., De Goede, R.G., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera: An outline for soil ecologists. Journal of nematology 25, 315–331.

Zar, J.H., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, New Jersey.

Zheng, B., Marschner, P., 2017. Previous residue addition rate and C/N ratio influence nutrient availability and respiration rate after the second residue addition. Geoderma 285, 217–224. https://doi.org/10.1016/j.geoderma.2016.10.007

Appendix A – Supplement to Chapter 3

A.1. Plot designations in each year of the experiment

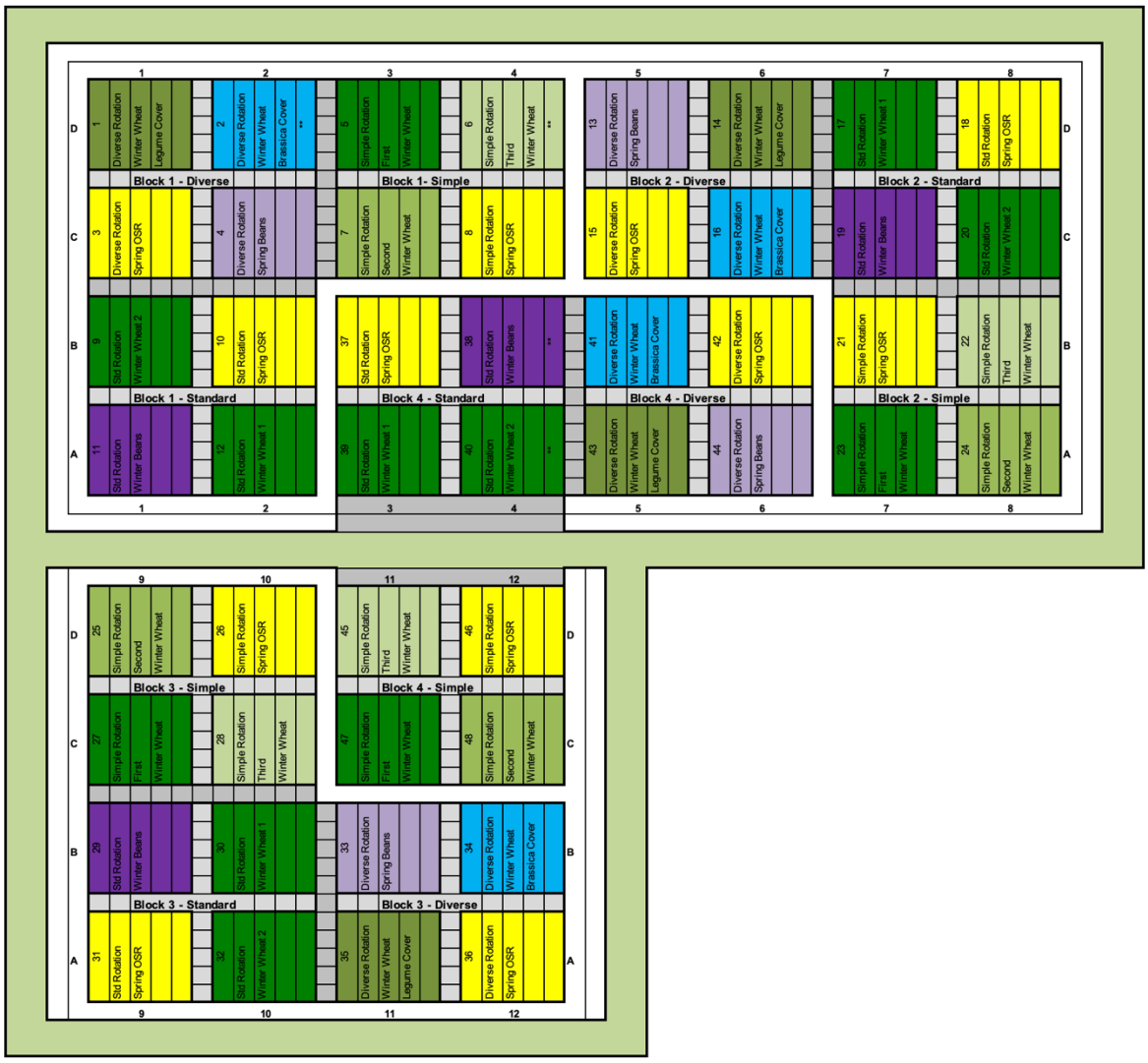


Figure A.1. Map of the Liberation field site illustrating its design and indicating the crop stage of the rotation represented by each plot in 2013-2014.

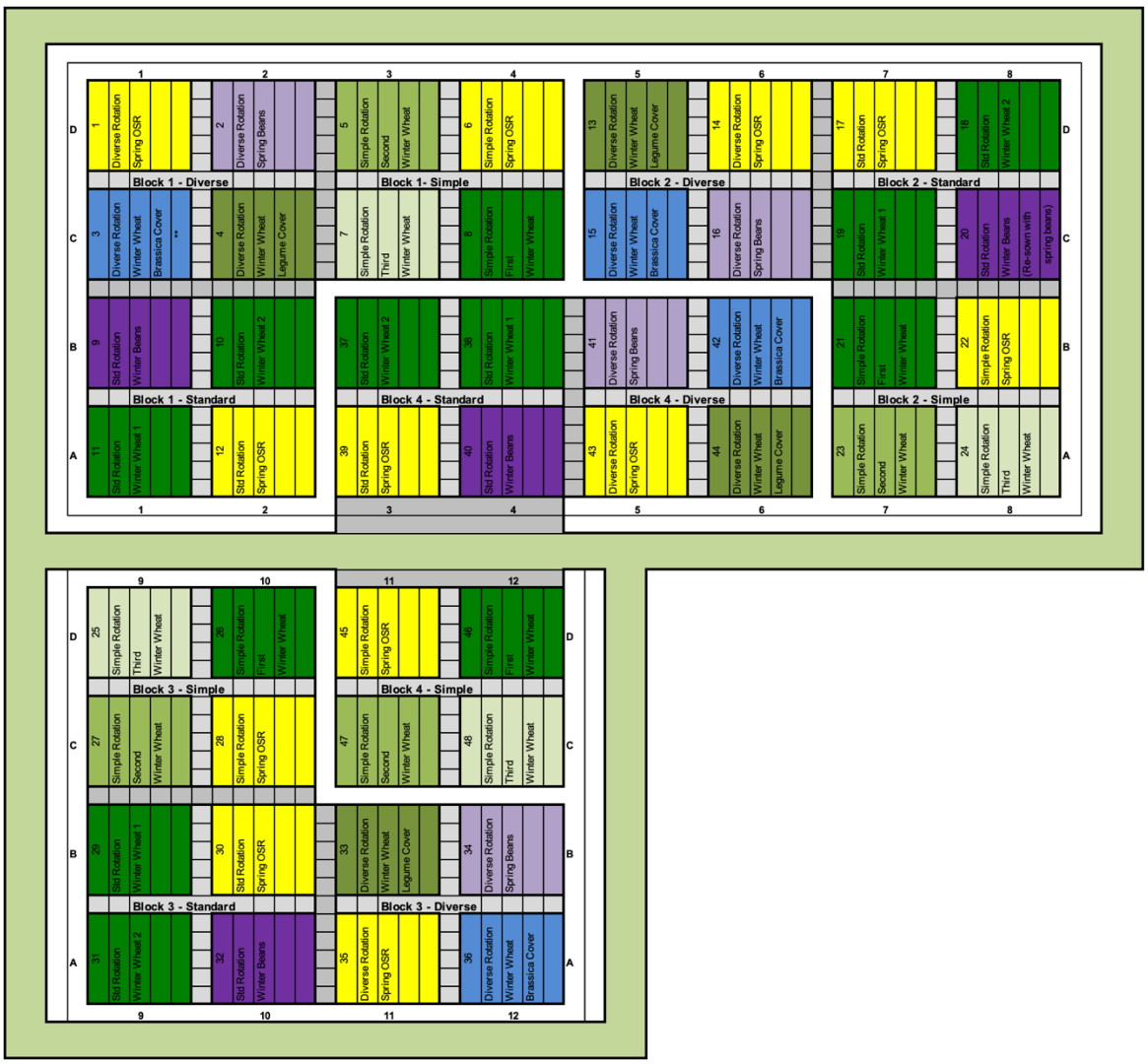


Figure A.2. Map of the Liberation field site illustrating its design and indicating the crop stage of the rotation represented by each plot in 2014-2015.

Figure A.3. Map of the Liberation field site illustrating its design and indicating the crop stage of the rotation represented by each plot in 2015-2016.

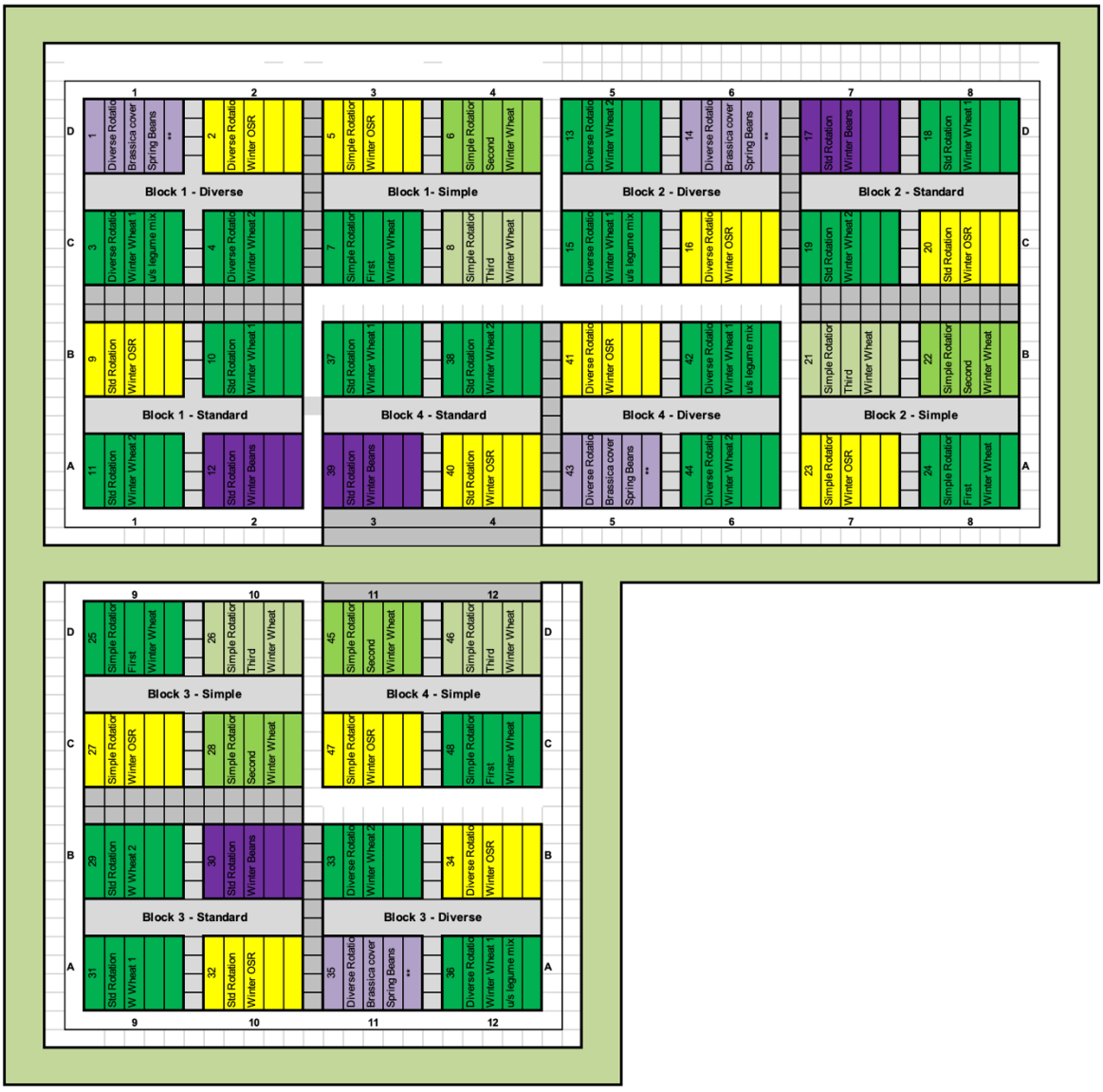


Figure A.4. Map of the Liberation field site illustrating its design and indicating the crop stage of the rotation represented by each plot in 2016-2017.

A.2. Statistical outputs

Table A.1. Results of one-way ANOVA of the effect of rotation on selected fatty acids (shown if $p < 0.1$), and other variables obtained from PLFA data. Significance indicated as **$p < 0.05$** and $\underline{p < 0.1}$.

	Mean±SD	**F**	**p**	**Biomarker**
C16:1ω7t (nmol/g soil)	35.18±10.30	4.159	<u>0.058</u>	G– bacteria[1]
C17:0brα (nmol/g soil)	37.22±10.37	3.214	<u>0.095</u>	Bacteria[2]
C17:0brβ (nmol/g soil)	74.99±17.36	4.188	<u>0.057</u>	Bacteria[2]
C18:1ω10or11 (nmol/g soil)	52.81±11.94	3.901	<u>0.066</u>	
C20:4ω6 (nmol/g soil)	45.20±15.98	3.431	<u>0.084</u>	Protists[3]
C20:5ω3 (nmol/g soil)	16.05±7.29	4.405	<u>0.051</u>	
Sum of above FAs (nmol/g soil)	239.7±95.6	2.702	0.120	
F:B	3.40±0.46	0.573	0.586	n/a
G+:G–	2.44±0.60	1.451	0.290	n/a
Total PLFA biomass (nmol/g soil)	3865±1081	2.426	0.150	n/a
Actinomycetes (nmol/g soil)	122.5±31.3	2.999	0.107	n/a

[1] Frostegård and Bååth, 1996
[2] Harwood and Russell, 1984; mostly G+ bacteria
[3] Myers *et al.*, 2001

Table A.2. Pearson correlations (r-values) between soil biochemical and PLFA parameters. Significance indicated as **$p < 0.05$** and $\underline{p < 0.1}$.

	Soil C	Soil N	pH
PLFA biomass	**0.71**	0.52	0.44
Fungal biomass	**0.64**	0.44	0.32
Bacterial biomass	**0.76**	**0.60**	0.52
Actinomycetes	**0.69**	**0.67**	<u>0.53</u>
G+ biomass	**0.63**	0.43	0.31
G– biomass	**0.75**	<u>0.58</u>	0.48

Table A.3. Statistical output of ANOVA with treatment structure: Rotation/(Diverse + Moderate + Simple). Data were transformed by cube root if $p < 0.07$ (indicated by *). Significance indicated as **$p < 0.05$** and $p < 0.1$.

Variable	**Rotation/**		**(Simple +**		**Moderate +**		**Diverse)**	
	F	p	F	p	F	p	F	p
Collembolan abundance*	0.14	0.872	3.16	**0.041**	0.06	0.982	0.72	0.552
Poduromorpha	1.50	0.296	0.80	0.503	0.55	0.651	0.43	0.736
*Entomobryomorpha**	0.06	0.938	4.21	**0.014**	0.21	0.892	1.42	0.260
Mite abundance*	0.16	0.855	4.61	**0.010**	0.98	0.419	0.98	0.416
Mesostigmata	1.60	0.278	0.19	0.905	1.49	0.240	0.00	1.000
*Oribatida**	0.09	0.911	10.56	**<0.001**	0.31	0.817	0.91	0.447
Larvae	2.92	0.130	0.14	0.938	1.27	0.304	0.97	0.423
Nematode abundance	0.20	0.833	0.59	0.636	0.04	0.990	2.51	0.125
Bacterial	0.63	0.612	0.70	0.576	0.47	0.713	0.6	0.628
Plant	0.68	0.596	0.40	0.753	0.24	0.868	0.51	0.684
Predator	5.25	0.160	0.29	0.830	2.20	0.158	0.37	0.779
Earthworm abundance	0.34	0.725	0.62	0.609	2.00	0.138	2.02	0.135
Earthworm biomass*	0.76	0.509	1.58	0.216	1.59	0.215	4.91	**0.008**
Total soil C (2016)	4.59	0.062	0.27	0.845	0.47	0.708	0.47	0.704
Total soil N (2016)	5.02	0.052	0.21	0.890	0.64	0.594	0.42	0.741
pH (2016)	27.95	**<0.001**	0.63	0.601	0.68	0.571	0.04	0.988

Table A.4. Selected Pearson correlations (r-values) of the abundances of different soil faunal groups. Significance indicated as **$p < 0.05$** and $p < 0.1$.

	Collembola	**Mites**	**Nematodes**	**Earthworms**
Collembola	-	**0.33**	-0.08	0.05
Mites	**0.33**	-	-0.28	0.20
Mesostigmata	0.15		-0.07	0.20
Oribatida	0.25		-0.13	0.18
Nematodes	-0.08	-0.28	-	-0.28
Bacterivorous	0.02	-0.03		**-0.48**
Plant parasite	0.00	0.02		**0.46**
Predatory	-0.07	0.01		0.03
Earthworms	0.05	0.20	-0.28	-

A.3. Field records

2015	
10/9	OSR and Brassica cover areas power harrowed.
11/9	Brassica covers broadcast with hege drill, coulters raised, covering harrow lowered.
11/9	OSR and Brassica cover areas rolled with Cambridge roller.
11/9	Drilled Amalie WOSR at 75 seeds/m^2 using Moore zero till drill.
12/9	OSR - Sprayed pre emergence herbicide Katamaran Turbo at 2.5 L/ha in 230 L water.
30/9	OSR - Applied slug pellets, Carakol 3 at 11.5 kg/ha. Applied by hand.
8/10	Sprayed insecticide against flea beetle; Hallmark Zeon at 75 mL/ha in 200 L water.
30/09	Sprayed stubbles with glyphosate (Roundup Biactive) at 4 L/ha in 230 L water.
14/10	Ploughed non OSR/cover areas.
16/10	Power harrowed and drilled wheat and beans plots.
19/10	Applied pre-emergence herbicide to wheat and beans plots. Stomp Aqua at 2.9 L/ha + Defy at 5 L/ha, in 220 L water.
26/11	OSR – Sprayed with Graminicide, Fusilade Max intended dose, 1.5 L/ha, Proline 0.5 L/ ha, Hallmark 0.75 mL/ha. However sprayer applied all at $^2/_3$ rate, so 1 L, 0.33 L and 0.5 mL respectively, in 147 L water per hectare.
14/12	OSR: Applied slug pellets; Ferric Phosphate 'Sluxx HP' (29.7 g/kg ferric phosphate) at approx. 40 kg/ha. Applied by hand.
2016	
4/2	Re-drilled discards with rye at approx. 250 seeds/m^2.
27/2	Topped off spring bean cover crop.
11/3	Sprayed spring bean cover crops and WOSR plots with Roundup Biactive at 4 L/ha in 220 L water.
18/3	Cultivated spring bean plots with power harrow.
18/3	Drilled spring beans at 30 seeds/m^2. Rolled with flat roller.
21/3	Sprayed spring bean plots with Stomp Aqua at 2.5 L/ha in 240 L water.
22/3	Sprayed all wheat plots except diverse rotation legume plots with Harmony M SX at 125 g/ha in 228 L water.
7/4	Power harrowed OSR plots.
8/4	Applied fertiliser to wheat plots; 50 kg/ha N + 50 kg/ha SO_3, applied as ammonium nitrate 34.5% N and ammonium sulphate nitrate 26% N, 37% SO_3.
8/4	Applied fertiliser to bean plots; 50kg S per ha as K_2SO_4 (Sulphate of Potash) 45% S, 50% K_2O.
13/4	Drilled Tamarin spring OSR on old WOSR plots; 75 seeds/m^2, 27 cm spacing. Rolled with flat roller.
18/4	Applied slug pellets to SOSR : Carakol 3 at 11.5 kg/ha using Hege drill.
19/4	Sprayed wheat plots with T1 fungicide; Amistar Opti at 1.25 L/ha + Proline at 0.4 L/ha, in 220 L water.
20/4	Applied fertiliser to SOSR plots; 50 kg/ha N + 50 kg/ha SO3, applied as ammonium nitrate 34.5% N and ammonium sulphate nitrate 26% N, 37% SO3.
29/4	Covered SOSR plots with fleece.
4/5	Hand planted pre-soaked beans (Fuego) in spring bean plots at approximately 15 seeds/m2.
5/5	Applied fertiliser to wheat and OSR plots; 50 kg/ha N as ammonium nitrate 34.5% N.
23/5	Removed fleece from OSR plots.
26/5	Sprayed T2 fungicide on all wheat; Seguris (isopyrazam (SDHi) + epoxiconazole) at 0.5 L/ha in 228 litres water. Tunnels and rows with tunnels in, sprayed with knapsack sprayer in 220 L water per ha.
26/5	Sprayed OSR plots with Hallmark Zeon (lambda-cyhalothrin and 1,2-benzisothiazolin-3-one) at 75 mL/ha in 287 L water. Against pollen beetle.
3/6	Sprayed OSR plots with Plenum WG (50% w/w pymetrozine) at 0.15 kg/ha in 260 L water. Against pollen beetle.
14/8	Sprayed OSR and Spring Bean plots with glyphosate; Roundup Biactive at 4 L/ha + Podstik at 1 L/ha.

2016 – next season

24/8	Sprayed new season WOSR and cover crop plots with Roundup Biactive at 4 L/ ha.
7/9	Sprayed remaining plots and discards with Roundup Biactive at 4 litres per ha in 220 L water.
12/9	Power harrowed WOSR and cover crop plots. Rolled WOSR plots with Cambridge roller.
14/9	Drilled WOSR plots with Amalie WOSR at 75 seeds per m2 using Moore drill. Variator 28.
15/9	Sprayed WOSR plots with pre-em herbicide Katamaran Turbo at 2.5 L/ ha. in 220 L water.
23/9	Sprayed for flea beetle; Hallmark Zeon at 75 mL/ha in 220 L water.
2/10	Sprayed for flea beetle; Hallmark Zeon at 50 mL/ha + Activator 90 at 0.01% in 220 L water
7/10	Applied slug pellets: Gusto 3 (metaldehyde 3%) at 11.5 kg/ha. Applied using Hege plot drill.
18/10	Ploughed remaining non OSR/cover area to 250mm.
19/10	Power harrowed, drilled wheat (Trinity, Scout, Santiago) at 300 seeds/m2, Beans (Tundra) at 30 s/m^2.
20/10	Rolled wheat and beans plots with Cambridge roller.
20/10	Sprayed bean plots with pre-em herbicide: Stomp Aqua, 2 L/ha + Defy, 5 L/ha. in 220 L water.
29/11	Re-drilled bean plots 12, 30, 39 (destroyed by crows) w/ Tundra at 40 seeds/m^2. Fleeced for protection.

2017

7/3	Sprayed brassica covers with Roundup Biactive at 4 litres per ha in 220 L water.
15/3	Sprayed diverse rotation wheat with spring herbicide; Starane 2 at 2 L/ha in 220 L water.
15/3	Sprayed oat discards with spring herbicide; Starane 2 at 1 L/ha in 220 litres water.
15/3	Sprayed all wheat except diverse rotation with spring herbicide; Harmony M SX at 125 g/ha in 220 L water.
15/3	Sprayed OSR plots with glyphosate; Roundup Biactive at 4 L/ha in 220 L due to crop failure.
23/3	Rotovated OSR and cover/bean plots.
24/3	Power harrowed bean plots. Drilled with Fuego beans at 60 s/m^2. Rolled with Cambridge roller.
24/3	Broadcast clover / trefoil mix into diverse wheat plots. Doubled to 20 g/pass by accident.
27/3	Sprayed spring bean plots with pre-em herbicide; Stomp Aqua at 2.9 L/ha + Defy at 3 l/ha, in 220 L.
29/3	Power harrowed then rolled OSR plots.
30/3	Drilled Tamarin SOSR at 100 seeds/m^2 with hege plot drill. Rolled all with Cambridge roller.
2/4	Sprayed OSR plots with pre-em herbicide; Katamaran Turbo at 2 L/ha in 220 L water.
3/4	Applied fertiliser to Wheat and OSR plots; 50 kg/ha N + 40 kg/ha SO_3 as ammonium nitrate 34.5% N and ammonium sulphate nitrate 26% N, 37% SO_3.
3/4	Applied fertiliser to bean plots; 40kg SO_3 per ha as K_2SO_4 (Sulphate of Potash) 45% S, 50% K_2O.
11/4	Sprayed OSR plots with Hallmark at 75 mL/ha in 220 L water.
28/4	Sprayed OSR plots with Hallmark at 75 mL/ha in 220 L water.
28/4	Sprayed Wheat and Bean plots with fungicide (half rate): Proline 0.4 L/ha + Amistar Opti L/ha in 220 L water.
4/5	Applied fertiliser to all wheat plots; 50 kg/ha N as ammonium nitrate 34.5% N.
18/5	Sprayed T2 fungicide; Aviator Xpro at 0.625 L/ha in 220 L water (half rate).
18/5	Sprayed beans with fungicide; Folicur (Tebuconazole) 0.5 L/ha + Amistar (azoxystrobin) 0.5 L/ha in 220 L water (half rate).
19/5	Sprayed OSR with herbicide; Lentagran at 2 kg/ha in 300 L water.
24/5	Sprayed OSR with insecticide; Hallmark Zeon 75 mL/ha in 220 L. To protect newly emerging OSR against flea beetle.

Appendix B – Supplement to Chapter 4

B.1. Statistical analyses testing HFA hypothesis

Table B.1 details the results of the range of analyses of variance (ANOVAs) performed to determine if any home-field advantage (HFA) effect could be detected. A range of covariates that could reasonably affect decomposition or be representative of the decomposition ability of the soil were also tested to find out if this would improve prediction of the decomposition rate constant by the microsite (treatment).

Table B.1. Results of one-way ANOVAs attempted to test HFA hypothesis. Assumptions of normal distribution of residuals and equal variance tested with Shapiro-Wilk and Levene tests, respectively. Blocking structure is included as error factor. Other factors included in the model are specified. Significance indicated as **$p < 0.05$** and $p < 0.1$.

	Y = wheat *k*		**Y = OSR *k***	
Factors	**F**	**p**	**F**	**p**
Microsite	1.191	0.353	0.524	0.611
Microsite	1.155	0.369	0.964	0.427
Covariate: average available N	0.760	0.412	7.710	**0.027**
Microsite	1.060	0.396	0.493	0.631
Covariate: k_{TBI}	0.124	0.735	0.515	0.496
Microsite	1.050	0.399	0.473	0.642
Covariate: S_{TBI}	0.055	0.822	0.214	0.657
Microsite	1.042	0.402	0.480	0.638
Covariate: PLFA biomass start	0.001	0.971	0.317	0.591
Microsite	1.603	0.267	0.472	0.642
Covariate: PLFA biomass end	3.769	0.093	0.207	0.663
Microsite	0.989	0.435	0.516	0.625
Covariate: F:B start	0.089	0.777	0.027	0.876
Microsite	0.973	0.440	0.651	0.561
Covariate: F:B end	0.004	0.951	1.337	0.300
Microsite	0.987	0.435	0.549	0.609
Covariate: C18:1ω9c start	0.076	0.793	0.343	0.584
Microsite	1.339	0.342	0.646	0.563
Covariate: C18:1ω9c end	1.891	0.228	1.286	0.308
Microsite	1.069	0.411	0.515	0.626
Covariate: C18:2ω6c start	0.499	0.511	0.009	0.929
Microsite	1.064	0.412	0.742	0.522
Covariate: C18:2ω6c end	0.474	0.522	2.223	0.196

Table B.2. Results of two-way ANOVAs attempted to test HFA hypothesis. Assumptions of normal distribution of residuals and equal variance tested with Shapiro-Wilk test and Levene test, respectively. Y-variate is *k*. Blocking structure is included as error factor. Other factors included in the model are specified. Significance indicated as **$p < 0.05$** and $p < 0.1$.

Factors	**F**	**p**
Microsite	1.582	0.238
Residue	18.738	**<0.001**
Microsite × residue	0.194	0.825
Microsite	2.337	0.133
Residue	27.674	**<0.001**
Covariate: average available N	8.153	**0.013**
Microsite × residue	0.287	0.755
Microsite	1.526	0.251
Residue	18.072	**<0.001**
Covariate: k_{TBI}	0.466	0.506
Microsite × residue	0.187	0.931
Microsite	1.479	0.261
Residue	17.513	**<0.001**
Covariate: S_{TBI}	0.019	0.893
Microsite × residue	0.182	0.836
Microsite	1.816	0.195
Residue	21.510	**<0.001**
Covariate: Rooibos mass loss	1.314	0.268
Microsite × residue	0.223	0.803
Microsite	1.699	0.214
Residue	20.119	**<0.001**
Covariate: Rooibos mass loss	0.665	0.665
Microsite × residue	0.814	0.814
Microsite	1.497	0.257
Residue	17.732	**<0.001**
Covariate: PLFA biomass start	0.194	0.666
Microsite × residue	0.184	0.834
Microsite	1.891	0.188
Residue	22.393	**<0.001**
Covariate: PLFA biomass end	3.925	0.068
Microsite × residue	0.232	0.796
Microsite	1.513	0.254
Residue	17.917	**<0.001**
Covariate: Increase in PLFA biomass	0.342	0.568
Microsite × residue	0.186	0.832
Microsite	1.479	0.261
Residue	17.515	**<0.001**
Covariate: F:B start	0.021	0.887
Microsite × residue	0.182	0.836

Table B.2. continued		
Microsite	1.543	0.248
Residue	18.270	**<0.001**
Covariate: F:B end	0.625	0.442
Microsite × residue	0.189	0.829
Microsite	1.542	0.248
Residue	18.259	**<0.001**
Covariate: G+:G– start	0.617	0.445
Microsite × residue	0.189	0.830
Microsite	1.485	0.260
Residue	17.587	**<0.001**
Covariate: G+:G– end	0.078	0.783
Microsite × residue	0.182	0.835

To assess presence of a HFA effect, a range of ANOVAs was performed. Neither of the residues were significantly affected by microsite (OSR: $F = 0.62$, $p = 0.57$; wheat: $F = 1.17$, $p = 0.37$; one-way ANOVA of each residue). For a HFA effect to be present, a significant interaction between the microsite and residue type in a two-way ANOVA is necessary, so this test was chosen as the initial method of detection. Decomposition rates of wheat were significantly higher than oilseed rape (OSR) ($F = 18.74$, $p < 0.001$). However, the microsite had no significant effect on decomposition ($F = 1.58$, $p = 0.24$; two-way ANOVA) and neither did the interaction of *microsite* and *residue*.

Because we expected the fungal:bacterial (F:B) ratio of the soil to be related to the decomposition rate, a two-way ANOVA of *k* and microsite with F:B ratio as a covariate was performed, but this led to no significant results. Because soil available N plays a major factor in the ability of the soil microbial community to decompose residues, the average available N during the experimental period was calculated and taken into account as a covariate of *k*. However, this did not affect the significance of the interactive effect of microsite × residue.

B.2. Alternative microsite designation

It is unlikely but possible that the microsite designation for wheat straw follows a different logic than that of OSR straw, where the home field of OSR residue decomposition is a soil with OSR crop, while the home field of wheat straw is a soil with a wheat crop in the previous year. If this is true, and the microsite designation is adjusted, the results of a two-way ANOVA with and without available N as a covariate are summarised in Table B.3.

Table B.3. Results of two-way ANOVAs if microsite designation is different for decomposing wheat straw. Y-variate is *k*. Blocking structure is included as error factor. Other factors included in the model are specified. Significance indicated as **$p < 0.05$** and $p < 0.1$.

Factors	**F**	**p**
Microsite	0.437	0.654
Residue	18.738	**<0.001**
Microsite × residue	1.339	0.292
Microsite	0.664	0.539
Residue	27.674	**<0.001**
Covariate: average available N	4.866	**0.045**
Microsite × residue	3.621	0.054
Microsite	0.422	0.664
Residue	18.072	**<0.001**
Covariate: k_{TBI}	2.626	0.127
Microsite × residue	0.212	0.812
Microsite	0.522	0.604
Residue	22.393	**<0.001**
Covariate: PLFA biomass end	0.896	0.360
Microsite × residue	3.115	0.076

From these results the presence of an HFA effect is possible with a 90% confidence interval if designation of the microsites in a crop rotation is different for different residues.

This is the only method of data analysis that has yielded an almost significant interactive effect of microsite × residue. However, to assign different microsites to the wheat residue is an artificial, speculative and highly doubtful approach.

B.3. Additional analyses of PLFA data

Table B.4. Fatty acids that were significantly affected by the factors Time and/or Microsite. Significance indicated as **$p < 0.05$** and $p < 0.1$. Biomarker information taken from Bååth and Anderson, 2003; Frostegård and Bååth, 1996; Frostegard *et al*., 1993; Bardgett *et al*., 1999.

Biomarker	Fatty acid	F	p	Factor
	C14:1ω9c	84.37	**<0.001**	Time
Bacteria, G+	C15:0i	21.74	**<0.001**	Time
Bacteria, G+	C15:0ai	26.63	**<0.001**	Time
		4.52	**0.030**	Microsite
Bacteria	C15:0	20.67	**<0.001**	Time
Bacteria, G+	C16:0i	35.89	**<0.001**	Time
		2.80	0.088	Microsite
G–	C16:1ω7c	7.30	**0.015**	Time
Bacteria, G–	C16:1ω7t	31.22	**<0.001**	Time
AMF and bacteria, G+	C16:1ω5	9.97	**0.005**	Time
	C16:0	41.00	**<0.001**	Time
		2.82	0.086	Microsite
	C17:0brα	7.42	**0.014**	Time
Bacteria, G+	C17:0i	40.72	**<0.001**	Time
	C17:0brβ	27.74	**<0.001**	Time
		2.69	0.095	Microsite
	C17:1ω8c	6.86	**0.017**	Time
Bacteria, G–	C17:0cy	34.92	**<0.001**	Time
G–	C17:1ω7	9.41	**0.007**	Time
		3.14	0.067	Microsite
Bacteria	C17:0	54.14	**<0.001**	Time
	C17:0-12me	11.45	**0.003**	Time
Actinomycetes, G+	C17:0-10me	51.86	**<0.001**	Time
	C18:3-5,10,12	15.79	**<0.001**	Time
Fungi	C18:1ω9c	7.18	**0.020**	Time
	C18:1ω9t	3.50	0.080	Time
	C18:1ω13	20.62	**<0.001**	Time
		4.16	**0.033**	Microsite
	C18:1ω10or11	3.81	0.067	Time
	C18:0	48.67	**<0.001**	Time
	C19:1ω6	3.72	0.070	Time
Actinomycetes, G+	C18:0-10me	51.88	**<0.001**	Time
	C19:1ω8	38.56	**<0.001**	Time
	C19:0	9.43	**0.007**	Time
Bacteria	C19:0cy	2.95	0.078	Microsite
Protists	C20:4ω6	12.72	**0.002**	Time
	C20:5ω3	15.86	**<0.001**	Time
	C20:1ω9	13.07	**0.002**	Time
	C20:0	31.89	**<0.001**	Time
		3.33	0.059	Microsite
	Total of all FAs	17.93	**<0.001**	Time

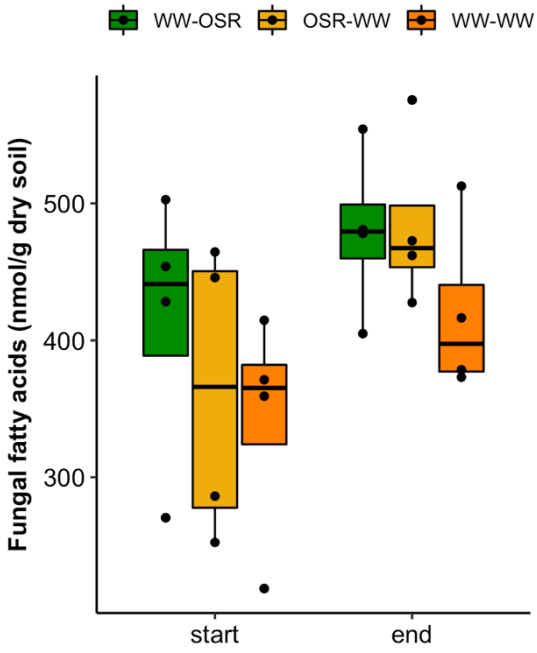


Figure B.1. Biomass of fungal PLFAs per microsite at the start (March 2016) and end (July 2016) of the growing season. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints (n = 4).

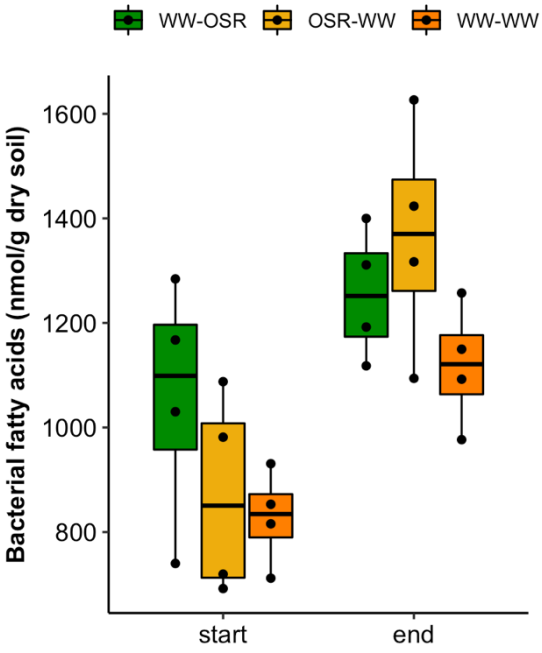


Figure B.2. Biomass of bacterial PLFAs per microsite at the start (March 2016) and end (July 2016) of the growing season. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints (n = 4).

There was a significant difference between the start and the end of the experimental period for both fungal fatty acids ($F = 7.520$; $p = 0.126$) and bacterial fatty acids ($F = 20.551$, $p < 0.001$; two-way ANOVA). There were no significant differences between microsites.

B.4. Mesh-size rationale

In a study by Castro Huerta *et al*. (2015) the following mesh sizes were used to correspond to in/exclusion of the soil biota groups specified:

- 4-mm mesh size to give access to total biota
- 2-mm mesh size excludes macrofauna (access to meso- and microfauna)
- 0.25-mm mesh size excludes macro- and mesofauna (access to microfauna only)

Bradford *et al*. (2002) took the following approach:

- Micromesh of 100 μm, entry of microfauna only
- Mesomesh of 2 mm, entry of both micro- and meso-fauna
- Macromesh of 4.7 mm, entry of all fauna.

Meso- and macrofauna have traditionally been considered to play major – yet unclear – roles in litter decomposition (Berg and McClaugherty, 2003). Relative contributions of soil animals and soil microorganisms are not evident, which has led to a greater number of studies on the more visible soil animals (Berg and McClaugherty, 2003). A meta-analysis in 2009 on the role of microarthropods on decomposition of different substrates (e.g. straw, leaf litter, roots) in different habitats (e.g. forest, agriculture, grassland) was shown to be moderate yet significant (Kampichler and Bruckner, 2009). However, in this meta-analysis they identify several factors that are not accounted for in mesh bag studies that may have enhanced the observed effect, including: (1) a larger mesh-size (7 mm compared to 1 mm and 175 μm) resulting in more leaching and therefore greater mass loss (Anderson, 1973), particularly during the first period of the study (Kampichler and Bruckner, 2009); (2) a finer mesh-size slowing down colonization of the litter by microbes shortly after burial (Wise and Shaefer, 1994 cited in Kampichler and Bruckner, 2009); and (3) microclimatic conditions differ depending on the mesh size (the moisture content was found to decrease with larger mesh sizes (Lousier and Parkinson, 1976)), which may have affected decomposition by microbes and other soil fauna. Fungal strands seem to come mostly from the soil rather than from the litter, and the abundance of vegetative fungal structures in no mesh bag, and in fine- (1.9 mm) and coarse- (3.6 mm) mesh bags was found to be smaller with finer mesh sizes (St. John, 1980).

A trade-off needs to be made between granting access to soil fauna, and particularly fungal strands, and unintended mass loss independent of the presence or absence of these fauna. I suggest to use a mesh size of 500 µm, including all microfauna, although they may experience initial colonization difficulties. This would exclude most mesofauna, but based on the literature presented above, whether they have a significant effect on litter mass loss is contested and we want to prevent overestimations due to leaching or displacement of residues. Unintended mass loss is particularly important if chemical characterization of the residues is to be pursued. To facilitate initial colonization of litter, the litter could initially be applied to the soil directly, before being placed in mesh bags, or alternatively some soil could be added to the litterbag as well. However, neither of these compromises are very practical.

References

Anderson, J.M., 1973. The breakdown and decomposition of sweet chestnut (*Castanea sativa* Mill.) and beech (*Fagus sylvatica* L.) leaf litter in two deciduous woodland soils. Oecologia 12, 251–274. https://doi.org/10.1007/BF00347566

Bååth, E., Anderson, T.-H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35, 955–963. https://doi.org/10.1016/S0038-0717(03)00154-8

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., Davies, J., 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology 13, 650–660.

Berg, B., McClaugherty, C., 2003. Plant Litter: decomposition, humus formation, carbon sequestration., Third edit. Springer-Verlag. https://doi.org/10.1007/978-3-642-38821-7

Bradford, M.A., Tordoff, G.M., Eggers, T., Jones, T.H., Newington, J.E., 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99, 317–323. https://doi.org/10.1034/j.1600-0706.2002.990212.x

Castro-Huerta, R.A., Falco, L.B., Sandler, R. V, Coviella, C.E., 2015. Differential contribution of soil biota groups to plant litter decomposition as mediated by soil use. PeerJ 3, e826. https://doi.org/10.7717/peerj.826

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65. https://doi.org/10.1007/BF00384433

Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723–730. https://doi.org/10.1016/0038-0717(93)90113-P

Kampichler, C., Bruckner, A., 2009. The role of microarthropods in terrestrial decomposition: A meta-analysis of 40 years of litterbag studies. Biological Reviews. https://doi.org/10.1111/j.1469-185X.2009.00078.x

Lousier, J.D., Parkinson, D., 1976. Litter decomposition in a cool temperate deciduous forest. Canadian Journal of Botany 54, 419–436. https://doi.org/10.1139/b76-041

St. John, T. V., 1980. Influence of litterbags on growth of fungal vegetative structures. Oecologia 46, 130–132. https://doi.org/10.1007/BF00346977

Appendix C – Supplement to Chapter 5

C.1. Supporting information for blocking structure

Based on the strong and consistent gradient we observed in %C content of the soils (Figure C.1a) and a similar gradient for the %N content of the soils (Figure C.1b), we applied a retrospective blocking structure to enable a more accurate assessment of non-additive effects. The plots with the highest %C content for each treatment were grouped into one block, the plots with the second highest %C content for each treatment were grouped into another block, etc. (Figure C.1c).

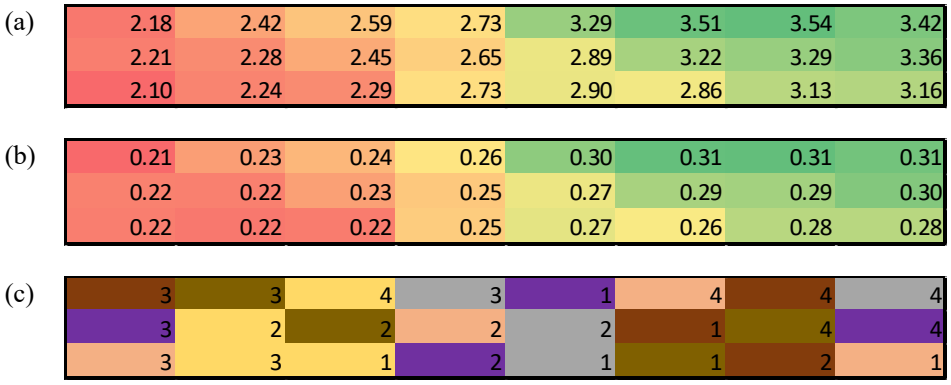


Figure C.1. The gradient in **(a)** %C and **(b)** %N observed in the plots, and **(c)** the retrospective blocking structure we applied, where each box represents a plot, numbers = blocks; and colours = treatments (grey = control, yellow = straw, beige = woodchip, purple = compost, light brown = straw-compost, chestnut brown = woodchip-compost).

C.2. Additional per-treatment results

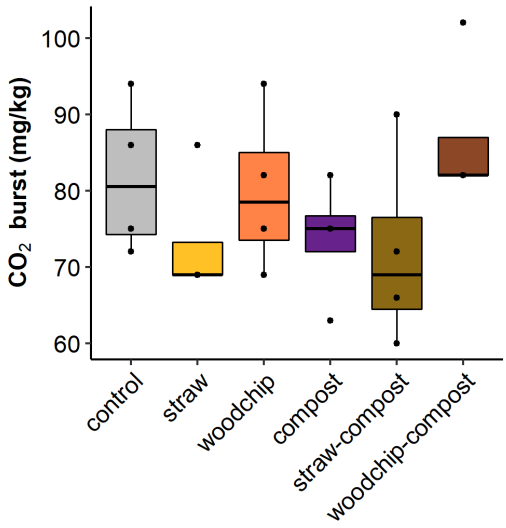


Figure C.2. Soil respiration measured by the Solvita CO_2-burst method. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual data points, occasionally overlapping (n = 4).

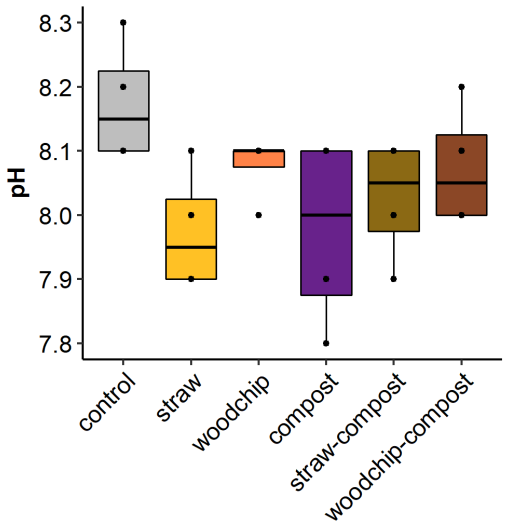


Figure C.3. Soil pH after different treatments. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual data points, occasionally overlapping (n = 4).

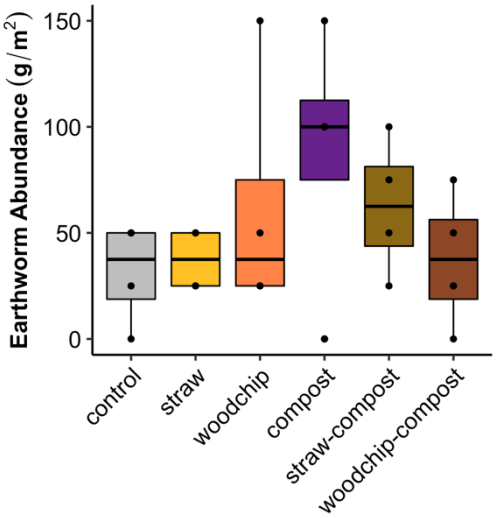


Figure C.4. Earthworm abundance per plot after different treatments. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual data points, occasionally overlapping (n = 4).

C.3. Calculation of amount of nutrients added to the soil via residue mixtures

First the mass of nutrients applied per plot was calculated, using the application rate of each residue (mg nutrient/plot) and the amount of each nutrient in the residues (g nutrient/kg residue). Then, using the bulk density (g/cm^3) and assuming nutrients from the residues applied remained in the top 20 cm of the soil (the sampling depth) resulting in a sampled soil volume of 0.2 m × 6 m × 2 m = 2.4 m^3/plot, the amount of nutrients added to the soil via the residues (mg nutrients/g soil sampled) was calculated as:

(mg nutrient/plot)/(m^3/plot)/(g sampled soil/m^3) = mg nutrient/g sampled soil

Then the difference between the amount of nutrients measured in each plot and the average amount of nutrients measure in the control plots was calculated as:

(mg nutrient/g soil)in plot – (mean mg nutrient/g soil) in control plots = (mg nutrient/g soil) increase relative to control

Then we determined this increase in soil available nutrients (relative to control) as a proportion of the amount of nutrients added to the soil via residue amendments:

(mg nutrient/g soil increase relative to control)/(mg nutrient/g sampled soil added via residue amendment) * 100%

Table C.1. Increase in soil available nutrients (relative to control treatment) as a proportion (%) of the quantity of nutrients added to the soil (assuming nutrients added via residues remained in the top 20 cm of the soil that was sampled). Numbers in bold are significantly different ($p < 0.05$) from 0 (SEM indicated in parentheses).

	straw	**woodchip**	**compost**	**straw-compost**	**woodchip-compost**
P	**-95 (4)**	15(65)	12 (15)	-2 (10)	1 (7)
K	10 (37)	57 (68)	**53 (12)**	**31 (3)**	**49 (13)**
Mg	**-242 (42)**	38 (74)	25 (30)	15 (25)	**35 (10)**
N	**-19 (5)**	-3 (5)	-2 (4)	2 (3)	1 (3)

C.4. Statistical outputs

Table C.2. Statistical outputs of baseline soil properties. Significance indicated as **p < 0.05** and p < 0.1.

Variable	**two-way ANOVA** (per *factor*) (*residue*; *compost*; *residue*compost*)		**Levene**		**Shapiro-Wilk of residuals**	
	F	p	F	p	W	p
SOM (LOI)	2.433; 0.914; 0.938	0.116; 0.352; 0.410	2.092	0.114	0.966	0.578
Soil moisture	0.843; 2.425; 0.315	0.447; 0.137; 0.733	2.911	**0.043**	0.965	0.536
pH	1.142; 3.241; 0.345	0.341; 0.089; 0.713	0.881	0.513	0.932	0.108
C:N	0.427; 1.094; 0.328	0.659; 0.310; 0.725	0.809	0.558	0.948	0.244
Variable	**one-way ANOVA** (per treatment)		**Levene**		**Shapiro-Wilk of residuals**	
	F	p	F	p	W	p
SOM (LOI)	1.206	0.350	1.727	0.175	0.966	0.574
Soil moisture	1.067	0.420	1.598	0.208	0.947	0.228
pH	1.382	0.278	0.735	0.628	0.950	0.275
C:N	0.410	0.862	0.692	0.659	0.948	0.242

Table C.3. Statistical outputs of per-treatment results. Significance indicated as **p < 0.05** and p < 0.1.

Variable	**two-way ANOVA** (per *factor*) (*residues*; *compost*; *residues*compost*)		**Levene**		**Shapiro-Wilk of residuals**	
	F	p	F	p	W	p
Available N	0.509; 2.566; 1.930	0.609; 0.127; 0.174	1.871	0.150	0.950	0.273
Mineralisable N	0.504; 2.936; 0.797	0.612; 0.104; 0.466	1.508	0.237	0.981	0.909
Mineralisable:Available	0.372; 0.597; 0.204	0.695; 0.450; 0.818	0.656	0.661	0.973	0.759
Available+Mineralisable	0.680; 3.877; 1.895	0.519; **0.065**; 0.179	1.313	0.303	0.958	0.391
Total biomass	1.625; 1.306; 0.303	0.225; 0.268; 0.742	0.883	0.513	0.971	0.697
CO_2 Burst	2.289; 0.033; 1.091	0.130; 0.859; 0.357	0.323	0.893	0.906	0.029
Earthworm abundance	0.136; 1.221; 1.945	0.874; 0.284; 0.172	0.449	0.809	0.956	0.361
P (mg/g soil)	1.547; 1.214; 0.440	0.240; 0.285; 0.651	1.300	0.308	0.967	0.586
K (mg/g soil)	0.291; 7.761; 0.009	0.751; **0.012**; 0.991	2.369	**0.081**	0.987	0.918
Mg (mg/g soil)	2.067; 4.953; 0.450	0.156; **0.039**; 0.645	2.573	**0.063**	0.960	0.437
SOM (LOI)	1.219; 0.574; 0.945	0.319; 0.458; 0.407	1.434	0.260	0.954	0.331
pH	1.459; 1.459; 3.405	0.259; 0.243; **0.056**	1.600	0.211	0.902	**0.024**
Bulk density	3.283; 1.269; 0.994	**0.062**; 0.276; 0.391	1.214	0.345	0.966	0.589
Aggregate stability	0.836; 0.022; 0.646	0.449; 0.883; 0.536	0.685	0.641	0.955	0.342
Quality impairment	0.653; 2.294; 3.568	0.532; 0.147; **0.050**	0.466	0.796	0.946	0.233

Appendix D – HFA mechanisms experimental plan

D.1. Introduction

This appendix describes the experimental plan of a mechanistic pot study for which there was, regrettably, not enough time or funding during my PhD due to the unforeseen need for vernalisation of wheat seeds. If I had received four years of funding instead of three, this appendix describes the experiment I would have done. The aim of the experiment would have been to determine the main drivers of a home-field advantage (HFA) effect, according the potential mechanisms specified by Austin *et al*. (2014): plant roots (e.g. root exudates), previous litter application, green leaf hitchhikers and/or litter volatiles. The soil and plant seeds and residues specified in this experiment have been collected from various farms during my PhD.

D.2. Context of the experiment

The HFA hypothesis predicts a faster decomposition of residues derived from home plants compared to away plants, where away plants are assumed to be of a different species. The litter affinity effect described by the HFA hypothesis is typically attributed to adaptation and optimization of the soil microbial population, via differences in metabolic capacities and types of competition, to be able to quickly degrade litter in the home environment (Wickings *et al*., 2012; Austin *et al*., 2014; Ayres *et al*., 2009). However, the mechanisms underlying the formation of this specialized decomposer community are not fully understood (Austin *et al*., 2014). It is also not clear what and how environmental factors affect the HFA (Veen *et al*., 2018).

A list of mechanisms proposed by Austin *et al*. (2014) can be found in the literature review of this thesis (section 2.5.3). Considering an arable cropping system, the first likely mechanism through which a microbial community may be selectively formed is the repeated application of a certain residue type. According to this mechanism *home* is a soil that has repeatedly received the same litter. There is already some evidence for this from a study by Austin *et al*. (2011) on three consecutive sequences of a hundred days of litter decomposition in a series of microcosms, which demonstrated higher decomposition rates in each follow-up sequence. Therefore, the microbial community was more adapted after already having experienced the same litter addition.

Austin *et al*. (2014) further suggested studying the possibility of plant litter volatiles as a repellent or attractant of soil invertebrates, following from the idea that plant volatile compounds are

known to attract or repel other plants and herbivores (e.g. Dicke and Baldwin, 2009). Though many crop residues will emit no or very few volatiles directly, microbes do emit volatiles, which can be olfactory cues to secondary consumers as well as mediate bacterial and fungal interactions (Wheatley, 2002; Garbeva *et al*., 2014). For instance, earthworms preferentially feed on cellulose filter-paper or apple leaf discs inoculated with fungal or bacterial species over uninoculated controls (Cooke and Luxton, 1980; Wright, 1972), and employ olfactory cues to direct their foraging towards the volatiles emitted by microbes (Zirbes *et al*., 2011). Furthermore, Zhao *et al*. (2016) demonstrated that wheat-straw amended soils emitted a distinct mixture of volatile organic carbon compounds that correlated well with the predominating bacterial species present in the soil over time.

Another mechanism through which the HFA could come about is by microbial adaptation to plants that grow at *home*. This may be through root exudates and interactions in the rhizosphere, or through microbes living on the above-ground tissue of the plant somehow becoming part of the decomposer microbes in the soil (e.g. through hitchhiking from leaves/stalks to the litter stage). In a study on the soil bacterium *Collimonas* exposed to different conditions, it appears that the composition of root exudates affects the volatiles produced by the species (Garbeva *et al*., 2014), which may further affect the soil food web via the mechanisms described above.

The aim of this experiment is to distinguish between the role and relative contributions of (1) previous litter (or residue) application to soil and (2) the effect of a plant growing in the soil in bringing about a HFA effect.

Validity of the experiment

Whether a HFA effect is demonstrated in my mesh bag experiment or not, it would be insufficient evidence to assert that it does or does not apply to arable cropping systems in general. Therefore, this experiment must independently test the HFA hypothesis, in a well-controlled pot experiment, as well as test two of the main suspected HFA mechanisms.

The establishment of plants and application of crop residues for only a few months in this experiment, is comparable to that in agricultural crop rotations. Therefore, if priming by means of previous crop residue application on or plant growth in the soils is observed and results in a measurable HFA effect, preliminary recommendations on crop residue management could be made (e.g. crop residue applied to a different soil from where it originated in order to obtain slower decomposition and nutrient release throughout the growing season).

D.3. Hypotheses

1. HFA hypothesis:
    a. Residue A decomposes faster in soils primed with plant A compared to soils primed with plants B and C
    b. Residue A decomposes faster in soils primed with residue A compared to soils primed with residues B and C
    c. The same applies to residue/plant B and C
2. HFA mechanisms:
    a. Both HFA mechanisms tested for will be observed, but to differing degrees.
    b. A crop residue applied to a soil that has previously been primed with the same residue will decompose faster than a crop residue applied to a soil that has previously been primed with the same plant species the residue originates from. i.e. previous crop-residue application is a stronger HFA mechanism than the presence of the same plant.
    c. Stronger HFA with decreasing crop-residue quality
3. Soil food web:

    It follows from hypothesis 2 that:

    a. The microbial population assessed by PLFA between soils primed with different residues is significantly different.
    b. The microbial population assessed by PLFA between soils primed with different plants is not significantly different.
4. If soil is C4, then:
    a. In treatments where HFA applies, more C3-carbon will have been incorporated in microbial biomass.

D.4. Treatment and blocking structure

Treatment structure

Table D.1. Proposed treatment structure of HFA mechanisms experiment, which is a full factorial combination of three factors: *priming* type (with plant or with residue), plant *species*, and *residue* application.

residue → *priming* × *species* ↓	Application of **residue A**	Application of **residue B**	Application of **residue C**
Soil primed with **plant A**	plantA-residueA	plantA-residueB	plantA-residueC
Soil primed with **residue A**	residueA-residueA	residueA-residueB	residueA-residueC
Soil primed with **plant B**	plantB-residueA	plantB-residueB	plantB-residueC
Soil primed with **residue B**	residueB-residueA	residueB-residueB	residueB-residueC
Soil primed with **plant C**	plantC-residueA	plantC-residueB	plantC-residueC
Soil primed with **residue C**	residueC-residueA	residueC-residueB	residueC-residueC
Control (**unprimed** soil)	control-residueA	control-residueB	control-residueC

Plant/residue species aimed for:
A – wheat straw (high C), sourced from Sonning farm, variety Scout
B – oilseed rape (biofumigant), sourced from Penn Croft farm
C – beans (high N), sourced from Penn Croft farm
Soil: C4 soil (continuous maize)

Blocking structure

Replication of 4, so a total of 84 samples, plus blanks if appropriate. Blocking structure depends on the location in the greenhouse where the experiment will be carried out.

D.5. Characterisation and experimental measurements

1. Residue characterisation
    a. C:N (Flash)
    b. Mineral composition/nutrients (MARS 6 microwave digestion)
    c. Isotopic signature (GC-C-IRMS)
    d. Consider FTIR or solid-state NMR
2. Soil characterisation
    a. C:N (Flash)
    b. Texture (laser granulometry)
    c. pH

d. SOM (LOI at 550 °C)
e. Available N on initial soil (KCl extraction)
f. Water-holding capacity
g. Isotopic signature (GC-C-IRMS)

3. Decomposition rate measurement
    a. CO_2 evolution as proxy of decomposition – weekly
4. Soil food web assessment
    a. PLFA – at the end of each phase of the experiment (i.e. 2 times 72 samples)
5. Measurement of C assimilation
    a. Isotopic signature of the microbial biomass by PLFA (samples run on the GC-C-IRMS as well as the GC-FID)

D.6. Specific materials

Apart from general laboratory materials, these are specific materials to obtain beforehand:

1. 90 chambers + septa (84 + blanks)
2. 90 bulk density cores that fit inside the chambers (84 + blanks)
3. Vials and syringes
4. Sufficient reagents for PLFA analysis, as specified in PLFA protocol

D.7. General methodology

Characterisation phase

The soil will be sieved to 4 mm and combined in a big box to homogenise. A portion of the soil will be milled and analysed for C:N and/or weighed into crucibles for measurement of SOM (by LOI), and a portion will be analysed for its isotopic signature.

Residues will be sorted, homogenised. A portion of each residue will be milled to enable C:N analysis by Flash and determination of the isotopic signature. Hopefully this is sufficiently different from the soil. The rest of the residues will be milled for application to the soils.

Preparation phase

Several months (about 3, depending on the length of the growing season of the plants used) before the experiment starts, soils will be primed in the Biology greenhouses by the addition of one of the residues, or the growth of one of the plants. The winter wheat will require a period of vernalisation. Sow the wheat beginning of December, leave to overwinter (in a polytunnel, outside or in a cold store at about 4 ºC until germination), and they should flower around end May or beginning June (Hadley, C., 2019, pers. comm.).

In the plant-primed soil, the plant is harvested and the roots removed so that selection of soil microbes through further decomposition of root litter is avoided. As much soil as possible is shaken off the roots though, to sustain the presence of root-associated organisms in the soil. In the crop-residue-primed soil, large bits of residues are removed. Both soils are sieved to 4 mm to remove remaining bits of roots and residues.

Samples are collected for PLFA analysis: (1) from the initial bulk soil, before the priming phase of the experiment, and (2) from each soil after the priming phase to demonstrate that the priming has altered the soil microbial community. These 12 samples will be stored in the freezer and analysed when there is time at the end of the experimental phase. Sampling should be done in triplicate, or three sub-samples should be taken for the PLFA analysis.

Experimental phase

The microcosms for the experimental phase will be placed in the lab in Russell. On day 1 of the experiment microcosms are prepared as follows:

1. Determine WHC of each soil and prepare microcosms of […] g soil at 60% WHC.
2. Each microcosm receives a crop-residue application of crop residues A, B or C, according to the treatment structure presented above.
3. CO_2 emissions will be measured on a weekly basis to assess the decomposition rate over time.

PLFA analysis is performed on the soil in each microcosm at the end of the experiment.

D.8. Points to consider

4. In plant-primed soils no plant is growing during experimental phase. Maybe root exudates during crop residue decomposition is crucial, which is not tested for. Also the comparison between plant- and crop-residue-primed soils will be biased and the results can only tell if the plant- and/or crop-residue-priming mechanism is present.
5. Maintain constant soil moisture content during experimental phase?
6. Incorporate DOM? Look into Suva index. Include control of no residue application in the treatment structure of the experimental phase (for DOM)
7. Look into substrate induced respiration (Mark Tibbett's lab) as an alternative for PLFA. This is a measure of the functional diversity of soil microbial community.
8. Check quality of maize residues since soil is from a maize field and soil microbes have adjusted to decomposing this.
9. Read about Decomposer Ability Regression Test (DART), proposed by Keiser *et al*. (2014).

D.9. Acknowledgements

Simon Porter from Penn Croft farm for supplying residues for the HFA mechanisms experiment; Jenny Denton for C4 maize soil and Arnaud Duranel for setting up the contact; Val Jaspers for a tour of the greenhouses in Biology; Caroline Hadley for wheat residues and seeds, and an explanation on vernalisation and growing wheat in a greenhouse.

References

Austin, A.T., Vivanco, L., González-Arzac, A., Pérez, L.I., 2014. There's no place like home? An exploration of the mechanisms behind plant litter – decomposer affinity in terrestrial ecosystems. New Phytologist 204, 307–314.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology and Biochemistry 41, 606–610. https://doi.org/10.1016/j.soilbio.2008.12.022

Dicke, M., Baldwin, I.T., 2009. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help .' Trends in Plant Science 15, 167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Garbeva, P., Hordijk, C., Gerards, S., de Boer, W., 2014. Volatiles produced by the mycophagous soil bacterium Collimonas. FEMS Microbiology Ecology 87, 639–649. https://doi.org/10.1111/1574-6941.12252

Garbeva, P., Hordijk, C., Gerards, S., de Boer, W., 2014. Volatile-mediated interactions between phylogenetically different soil bacteria. Frontiers in Microbiology 5, 1–9. https://doi.org/10.3389/fmicb.2014.00289

Keiser, A.D., Strickland, M.S., Fierer, N., Bradford, M.A., 2011. The effect of resource history on the functioning of soil microbial communities is maintained across time. Biogeosciences 8, 1477–1486. https://doi.org/10.5194/bg-8-1477-2011

Wheatley, R.E., 2002. The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie van Leeuwenhoek 81, 357–364.

Wickings, K., Grandy, A.S., Reed, S.C., Cleveland, C.C., 2012. The origin of litter chemical complexity during decomposition. Ecology Letters 15, 1180–1188. https://doi.org/10.1111/j.1461-0248.2012.01837.x

Zhao, J., Wang, Z., Wu, T., Wang, X., Dai, W., Zhang, Yujie, Wang, R., Zhang, Yonggan, Shi, C., 2016. Volatile organic compound emissions from straw-amended agricultural soils and their relations to bacterial communities: A laboratory study. Journal of Environmental Sciences 45, 257–269. https://doi.org/10.1016/J.JES.2015.12.036