Plant microbial interactive effects on soil carbon in relation to soil structure

Hana Mohamed Muslem Husain

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Faculty of Science

THE UNIVERSITY OF SYDNEY

2023

Declaration of originality

I certify that the content of this thesis is my own work. To the best of my knowledge, all materials in this thesis used from previous research have been acknowledged in the text and appropriately cited. This thesis has not been submitted for any other degree at the University of Sydney or any other institution.

Hana Mohamed Muslem Husain

8 August 2023

Statement of authorship

This thesis comprises of six chapters, and chapters 3, 4 and 5 have been written as separate manuscripts. Consequently, there might be some repetition in the methods in these chapters.

Chapter 1 of this thesis consists of introduction and research aims.

Chapter 2 of this thesis is a literature review titled "Factors influencing soil aggregation and associated carbon stabilization with a focus on microbial processes".

Chapter 3 has been published as

Hana Husain, Feike A. Dijkstra, 2023. The influence of plant residues on soil aggregation and carbon content: A meta-analysis. J. Plant Nutr. Soil Sci. 2023; 1-11.

Chapter 4 has been published as in Applied Soil Ecology Journal as

Hana Husain, Claudia Keitel, Feike A. Dijkstra, 2024. Fungi are more important than bacteria for soil C loss through priming effects and C protection through aggregation. Applied Soil Ecology 195 (2024), 105245.

Chapter 5 of this thesis is an experimental report titled "Plant-microbial interactive effects on plant residue decomposition, soil aggregation and soil organic carbon content".

Chapter 6 consists of general discussion, conclusion and future research ideas.

I am the first or main author of all chapters. I am also corresponding author of chapter 3. I co-designed all studies with my co-author, conducted the experiments, analyzed the data, and drafted the manuscripts.

I hereby confirm that the above-mentioned authorship statements are correct.

Hana Mohamed Muslem Husain

As a supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Assoc. Prof. Feike A Dijkstra

Abstract

The plant input of organic carbon (C) into soil is essential for the soil structure, including the formation of soil aggregates. The inclusion of C in soil aggregates ensures protection from microbial decomposers that can move C from the soil -as a C sink- to the atmosphere through CO_2 emissions, thereby raising the concentration of atmospheric greenhouse gases. The plant C sequestration in soil aggregates and its stability in the long-term is dependent on the microbial processes that vary widely according to the soil, climate, plant C quality, and soil microbial communities. The exact mechanisms of the microbial decomposition of plant substrates and role of soil microbes (like bacteria and fungi) in the formation and stability of soil aggregates is still unknown. Plant rhizodeposits in the soil can influence the soil microbial activity.

In this thesis, the factors affecting the soil aggregate formation, the microbial decomposition of plant residue added to the soil, and the plant-microbial interactive effects on the decomposition of added litter to soil, soil structure, and soil C content were explored.

The literature review addressed the key factors influencing soil aggregate formation and destruction. A special focus was given to the microbial processes governing plant residue decomposition in the soil and the related effects on soil aggregation and C sequestration in the soil. A conceptual framework was developed to highlight the microbial processes of plant substrate decomposition and soil aggregation processes.

The effects of plant residue amendments on soil aggregation and soil organic C content were investigated with a meta-analysis study. Overall, the meta-analysis revealed that plant residues increase soil aggregation. Nevertheless, soil amendments with fresh plant residue were more effective in enhancing soil aggregation (although the response varied according to the origin of

plant residue), while charred plant residues were more beneficial in enhancing the soil C content (where initial soil C content was a limiting factor).

In a 16-day laboratory incubation investigated the bacterial and fungal decomposition of labile (glucose) and complex C (wheat root biomass) effect on soil aggregation and soil C using ^{13}C-labelling technique. The results indicated that soil bacteria are more influential for the decomposition of labile C, while both bacteria and fungi are responsible for the decomposition of complex C in the soil. The effects of added C substrates to the soil were more apparent on the soil microbial parameters than the soil aggregation and soil C. Fungi were found to be more influential on soil priming than bacteria.

In a glasshouse study, the effect of rye grass plants (rhizosphere) along with two levels of rye grass litter applications were investigated on soil aggregation and soil C, where a $^{13}CO_2$ pulse labeling was conducted to separate the plant derived C from other C pools in the soil. The presence of rye grass rhizosphere significantly increased the microbial biomass C (MBC) and the rye grass litter addition enhanced the MBC more when coupled with the effect of the rye grass rhizosphere, and thus increasing the soil primed C. There was no significant increase in the macroaggregate formation nor the mean weight diameter (MWD) of the aggregates, likely due to the short duration of this study.

Longer studies are needed to investigate the effects of plant residue soil amendments and rhizosphere on soil aggregation and soil C. Nevertheless, plant residue inputs to the soil should be always considered by farmers and growers to retain depleted nutrients from the soil such as C, despite the inconsistent magnitude of benefits to soil C and aggregation among different residue origins.

Acknowledgements

I am grateful to finish my PhD and for all the support I got from everyone around. First, I sincerely thank my supervisor, Feike Dijkstra, for all the support and professional guidance he provided throughout my PhD candidature. His high expertise in research, patience, and flexibility have been invaluable to me and very encouraging to finish my PhD project and produce this thesis (especially at the stressful times of COVID19). I am also grateful for my auxiliary supervisor, Claudia Keitel for her efforts in analyzing my samples and for the comments she provided for my research.

I also greatly appreciate the support from my friends and colleagues at Sydney University. A special thanks to Milad Bagheri Shirvan, Bahareh Bicharanloo, Iona Gyorgy, Yuxin Huo, Zongtang Yang for technical and laboratory help they provided.

I am lucky to have a supporting family. I am very grateful for all the love and encouragement they had not spared to make sure I sustain to the end of my PhD especially my brother Ali who provided technical support when needed. I thank my husband for financial, emotional, and patience throughout my PhD candidature. To my mother, who passed away before I finish what I started with her blessings and inspiration, where she is the one who paved the way for her sisters to finish school in a society denying the value of girls' education. Rest in peace mother, I am done with what you started.

Finally, I am thankful to the Paulatte Isabel Jones Completion scholarship I received, which supported me financially at the end of my candidature. I also thank the University of Sydney for giving me the opportunity to pursue a PhD research degree.

Contents

List of Figures

List of Tables

Chapter 1. General introduction

A well-structured soil serves an important role in the ecosystem. Soil provides nutrient and habitat for plants and soil microbes. The spaces within soil structure or pores mediate water infiltration and gas exchange for life underground. Intensive farming, especially tillage, can degrade soil structure and result in soil organic carbon (C) loss due to enhanced mineralization, exacerbating climate change impacts (Garcia, Nannipieri & Hernandez, 2018).

Soil particles such as sand, silt, and clay aggregate together around organic matter (*i.e.,* C), potentially sequestering C in soil aggregates for the long term, and protecting C from soil decomposers through various types of organo-mineral associations such as adsorption to clay mineral surfaces (Baldock & Skjemstad, 2000; Tisdall & Oades, 1982). Soil aggregate formation needs organic C which enters the soil as plant litter, root biomass and rhizodeposition or from soil microbes and animals and their by-products (Gunina & Kuzyakov, 2015; Tisdall & Oades, 1982).

Plant root growth and rhizodeposition influence physical, chemical, and biological changes in the soil. For instance, the growth of roots to explore soil for water and nutrients affect the water content of the soil, create wet-dry cycles changing the soil bulk density, creating macropores in the soil structure after the decay of dead roots, while the mass of the roots compacts the soil and restructures soil aggregates (Hinsinger et al., 2009; Lu et al., 2019; Poirier et al., 2018). Rhizodeposition can be a way of plant acclimation in the soil environment, where plants produce rhizodepositions to modify soil pH, signaling and responding to signals from specific soil microbes or react to pathogens (Pantigoso, Newberger & Vivanco, 2022). Nevertheless, plants also leak .The rhizodeposition around plant roots creates a C rich habitat for soil microbes, attracting more soil microbes to reside in the rhizosphere zone (Wang et al., 2020b). However, Plant rhizodeposition

varies in the chemical composition according to species traits and age (Hütsch, Augustin & Merbach, 2002), soil physiochemical conditions or exposure to environmental stresses (such as drought) (Dijkstra & Cheng, 2007).

On the other hand, the plant induced changes in soil structure influence the type and size of the soil microbes that can reside in the rhizosphere area and within the soil aggregate pores. Consequently, microbial activity in the soil can be regulated by plant rhizodeposition chemistry and the plant root traits.

The microbial C use efficiency (CUE) of added C sources to soil (Wang et al., 2021) and the type of added organic matter (Yan et al., 2023) influence the amounts of sequestered C in soil aggregates and the amount of C loss from the soil or soil priming effect. In this thesis, I will address the microbial CUE of different types of plant organic C added to the soil, and how the microbial CUE is correlated to substrate type, microbial community, and soil aggregation.

Soil aggregation is a dynamic process with continuous aggregate formation and destruction due to soil biological, chemical, physical, and environmental factors influencing the soil microbes (such as bacteria and fungi) that govern the C decomposition in the soil (Védère et al., 2022; Wang et al., 2020c). Due to the complexity of factors affecting the soil environment that vary widely spatially and temporally, and the variations in research approaches between soil studies, there is still uncertainty in results from soil studies that may underestimate the role of some important soil factors that can impact soil aggregation and soil organic C transformations by soil microbes. This thesis is an attempt to highlight some of the factors affecting soil aggregation and C content in response to plant rhizosphere and plant residue soil amendments.

In **chapter 2**, I conducted a literature review to address the factors responsible for soil aggregate formation and destruction processes. In this review I focused on the soil microbial processes governing decomposition and formation of soil C, including the microbial decomposition of plant residue added to the soil, the efficiency by which this is converted into microbial products, and the impact on soil aggregation and soil C content. I focused on the role of soil bacteria and fungi in soil aggregation and soil C stabilization, and presented a conceptual framework showing the links between the bacterial and fungal decomposition of plant substrates, their CUE, and aggregate formation and destruction.

Plant residue amendment effects on soil aggregation and soil C varied in the published research due to differences in the type of plant amendment, application rate, soil type and chemistry, study length, and methodology. In **chapter 3**, I conducted a meta-analysis to investigate the effects of plant residue soil amendments on soil aggregation and soil C content. The meta-analysis included data from 50 published studies with 299 pairs of observations. I investigated the effects of soil factors (clay content, initial C content, and soil pH), experimental factors (experiment duration and residue addition rate), residue type (fresh and charred), and fresh residue origin (alfalfa, barley, rice, wheat, and other) on three aggregate size classes (macroaggregates, microaggregates, and silt and clay fraction), soil aggregation expressed as the mean weight diameter (MWD), water aggregate stability (WAS), total soil C (TSC), and C associated with each soil aggregate size fraction.

Despite the confirmed importance of soil microbes for the decomposition of the different types of plant organic matter added to the soil, the exact mechanisms, and the magnitude of effects on soil C content are still not fully understood (Kästner & Miltner, 2018). In **chapter 4**, I conducted a 16-day incubation study to investigate the bacterial and fungal role in the decomposition of a labile C

source (glucose, to represent rhizodeposition) and wheat root litter in the soil using ^{13}C labeled substrates. I used a bactericide (bronopol) and a fungicide (captan) to suppress bacteria and fungi, respectively, in the soil to be able to examine the role of each group of soil microbes. I measured the respiration rate throughout the 16-day incubation period, the microbial biomass C (MBC), the ^{13}C in MBC from added substrates, and calculated the microbial CUE of each added substrate and the soil priming effect. I further separated three soil aggregate size classes (macroaggregates, microaggregates, and silt and clay fraction), and measured the total C and the ^{13}C (from added substrates) content in each aggregate size class, and MWD.

The plant root exudation of organic C to the soil (*i.e.,* in the rhizosphere zone) largely differs between different plant species, and attracts, and activates different soil microbial communities (Jones & Hinsinger, 2008), imposing a challenge to understand the effects on soil native C decomposition (*i.e.,* soil priming), soil aggregation, and the stabilization of added C in soil aggregates. In **chapter 5**, I conducted a glasshouse pot study to investigate the rye grass (*Lolium perenne*) rhizosphere effects on soil C and aggregation in the absence and presence of plant litter. I used a $^{13}CO_2$ pulse labeling method to be able to trace the plant rhizodeposition in soil aggregates, MBC, respiration, and to link the plant rhizodeposition C to the soil C decomposition rate (including added litter to soil). The pulse labeling method allowed me to separate the different sources of respiration (decomposition of rhizodeposition, decomposition of soil native organic matter, and root respiration). In this study, a plant treatment (planted and unplanted) and a rye grass litter treatment (none, low as 1 mg kg^{-1}, and high as 10 mg kg^{-1}) were applied to the soil. The rye grass shoots were trimmed after 30 days of growth and the $^{13}CO_2$ pulse labeling was performed after regrowth for a 2 h period. Three days after the pulse labeling, the soil total respiration was measured, and the soil was sampled for the same measurements that were performed in **chapter 4**

except for the CUE, which was not possible because of the temporal dynamics of the pulse labeling. The concentration of ^{13}C in respiration and the ^{13}C label in soil, shoots, and roots were measured and the contribution of plant rhizodeposition in each component was calculated.

The specific aims of this thesis were:

1. To synthesize the literature regarding factors controlling soil aggregation and C content, with a focus on microbial processes.
2. To investigate the effect of plant residue amendments on soil C and soil structure.
3. To study the role of soil microbes (bacteria vs fungi) on soil organic matter decomposition and aggregation with addition of different plant substrates.
4. To investigate the interactive effects of rhizodeposition and plant litter on soil aggregation and C content.

References

Baldock, JA & Skjemstad, JO 2000, 'Role of the soil matrix and minerals in protecting natural organic materials against biological attack', *Organic Geochemistry,* vol. 31, no. 7, pp. 697-710.

Dijkstra, FA & Cheng, W 2007, 'Moisture modulates rhizosphere effects on C decomposition in two different soil types', *Soil biology & biochemistry,* vol. 39, no. 9, pp. 2264-2274.

Garcia, C, Nannipieri, P & Hernandez, T 2018, 'Chapter 9 - The Future of Soil Carbon', in Garcia, C, Nannipieri, P & Hernandez, T (eds.), *The Future of Soil Carbon*, Academic Press.

Gunina, A & Kuzyakov, Y 2015, 'Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate', *Soil Biology and Biochemistry,* vol. 90, pp. 87-100.

Hinsinger, P, Bengough, A, Vetterlein, D & Young, I 2009, 'Rhizosphere: biophysics, biogeochemistry and ecological relevance', *An International Journal on Plant-Soil Relationships*, Springer Netherlands, Dordrecht.

Hütsch, BW, Augustin, J & Merbach, W 2002, 'Plant rhizodeposition — an important source for carbon turnover in soils', *Journal of Plant Nutrition and Soil Science,* vol. 165, no. 4, pp. 397-407.

Jones, D & Hinsinger, P 2008, 'The rhizosphere: complex by design', *Plant and Soil,* vol. 312, no. 1, pp. 1-6.

Kästner, M & Miltner, A 2018, 'Chapter 5 - SOM and Microbes—What Is Left From Microbial Life', in Garcia, C, Nannipieri, P & Hernandez, T (eds.), *The Future of Soil Carbon*, Academic Press.

Lu, J, Dijkstra, FA, Wang, P & Cheng, W 2019, 'Roots of non-woody perennials accelerated long-term soil organic matter decomposition through biological and physical mechanisms.(Report)', *Soil Biology and Biochemistry,* vol. 134, pp. 42.

Pantigoso, HA, Newberger, D & Vivanco, JM 2022, 'The rhizosphere microbiome: Plant–microbial interactions for resource acquisition', *Journal of Applied Microbiology,* vol. 133, no. 5, pp. 2864-2876.

Poirier, V, Roumet, C, Angers, DA & Munson, AD 2018, 'Species and root traits impact macroaggregation in the rhizospheric soil of a Mediterranean common garden experiment', *Plant and soil,* vol. 424, no. 1/2, pp. 289-302.

Tisdall, JM & Oades, JM 1982, 'Organic matter and water-stable aggregates in soils', *Journal of Soil Science,* vol. 33, no. 2, pp. 141-163.

Védère, C, Lebrun, M, Honvault, N, Aubertin, M-L, Girardin, C, Garnier, P, Dignac, M-F, Houben, D & Rumpel, C 2022, 'How does soil water status influence the fate of soil organic matter? A review of processes across scales', *Earth-Science Reviews,* vol. 234, pp. 104214.

Wang, C, Qu, L, Yang, L, Liu, D, Morrissey, E, Miao, R, Liu, Z, Wang, Q, Fang, Y & Bai, E 2021, 'Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon', *Global change biology,* vol. 27, no. 10, pp. 2039-2048.

Wang, X, Sale, P, Hayden, H, Tang, C, Clark, G & Armstrong, R 2020a, 'Plant roots and deep-banded nutrient-rich amendments influence aggregation and dispersion in a dispersive clay subsoil', *Soil Biology and Biochemistry,* vol. 141, pp. 107664.

Wang, X, Yin, L, Dijkstra, FA, Lu, J, Wang, P & Cheng, W 2020b, 'Rhizosphere priming is tightly associated with root-driven aggregate turnover', *Soil Biology and Biochemistry,* vol. 149, pp. 107964.

Yan, S, Yin, L, Dijkstra, FA, Wang, P & Cheng, W 2023, 'Priming effect on soil carbon decomposition by root exudate surrogates: A meta-analysis', *Soil Biology and Biochemistry,* vol. 178.

Chapter 2: A review: factors influencing soil aggregation and associated carbon stabilization with a focus on microbial processes.

Abstract

The quality of soil structure to function well in the ecosystem depends on soil aggregation processes. Soil aggregate stability depends on carbon (C) stabilization mechanisms governed by soil microbial utilization of organic C added to the soil from primary (plants) and secondary sources (heterotrophs). The microbial processes in the soil structure are influenced by natural (biological, chemical, and environmental) and manmade factors (such as land use change). This review aim is to discuss the factors influencing soil aggregate formation and destruction factors, the bacterial and fungal decomposition of plant inputs to the soil and how they affect the soil aggregation and C stabilization in the soil. A conceptual framework was developed summarizing the microbial and aggregation processes in relation to plant substrate decomposition in the soil.

2.1 Introduction

Soil structure depends on aggregate stability and characteristics. Aggregation is the binding process of soil grains by organic matter in the soil to form the basic units of soil structure. Aggregates are of different sizes (ranging from micrometers to a few millimeters) with spaces within and between aggregate units called pores. This architecture of clumps of aggregates and various-sized pores gives the soil the ability to hold/filter water, exchange/hold gases (such as CO_2 and O_2) and provide a habitat for soil microorganisms, soil fauna and life resources for roots to grow (Davet, 2004; Rabot et al., 2018; Tisdall & Oades, 1982). Furthermore, soil aggregates can be considered as a terrestrial sink for carbon (C) sequestration in the soil (Rabot et al., 2018; Six et al., 2006). Indeed, sequestration of C in soil aggregates can result in the stabilization of C for the long-term. Practically, that means C in the aggregates is protected from mineralization by soil

microbes. While soil aggregation prevents the exposure of C to the surrounding decomposers, formation, and destruction of small (micro) and large (macro) aggregates are continuous processes. At the macroaggregate scale of aggregate formation and destruction, plant roots and hyphae can physically influence both processes. While at the microaggregate scale, soil fauna, fungi, bacteria and plant roots secretions into soil can influence binding soil particles that favor aggregate formation or influence the destruction of aggregates by using the binding materials that keep soil particles together as an energy source (Oades, 1993). Consequently, soil C sequestration is dependent on aggregate formation and destruction rates. Therefore, studying the dynamics of soil microbe interactions along with labile C and soil organic matter inputs from plants is needed to understand C-cycling and its global implications (Juarez et al., 2013). In this review I will first describe factors influencing soil aggregate formation and destruction, and then discuss the interactive role of bacteria and fungi and various types of plant inputs on soil aggregation and C stabilization in the soil.

2.2 Soil aggregation

Aggregates can be divided into two main categories in terms of size. The large aggregates (greater than 0.25 mm in size) are called macroaggregates. Whereas the small aggregates (less than or equal to 0.25 mm in size) are called microaggregates (Tisdall & Oades, 1982). The latter ones are believed to be more stable than macroaggregates although they form the basic units for macroaggregates formation. Microaggregates can be divided into smaller soil fractions depending on experimental design, methods or aims of the studies (Bossuyt et al., 2001; Helfrich et al., 2008; Mikha, Rice & Milliken, 2005; Tisdall & Oades, 1982). Particles of less than 0.002 mm in size (clay fraction) can consist of extremely stable bonding between minerals and organic materials (Field, Minasny & Gaggin, 2006). Mean weight diameter (MWD) is another way that has been

used repeatedly to describe soil aggregation as a whole instead of focusing on one soil aggregate fraction to get more representative results for soil structure investigations (Franzluebbers, 2022). The MWD can be calculated from soil aggregate fractions using equation (1):

$$\text{MWD} = \sum_{i=1}^{n} \bar{x}_i \, w_i \quad (1)$$

where $\bar{x}_i$ is the mean diameter of each size fraction, $i = 1,\ldots, n$, w_i is the fraction of that aggregate size class, n is the number of size fractions (Hernandez, Hernandez & Garcia, 2017).

The importance of MWD has influenced the emergence of new identification methods using machine learning approaches for predicting MWD by using the more "easier to measure" and aggregate-formation related parameters such as soil texture, organic C content and bulk density (Bhattacharya et al., 2021). Aggregate fractionation has been done using various methods such as wet sieving and dry sieving. For instance, wet sieving, adapted from Elliott (1986), can be done by slaking the soil in deionized water before placing the soil sample on the larger mesh size sieve as part of a set of nested sieves of the desired aggregate sizes according to the experimental aims (Xiao et al., 2020a). The dry sieving method works by applying a mechanical stress on soil structure particles compared to the hydraulic applied stress in the wet sieving. Applying the mechanical pressure to separate aggregates can be done by placing the air-dried soil sample on an automatic vibratory sieve shaker after gently crushing the big soil particles by pestle with a rubber tip (Felde et al., 2021; Fomin et al., 2019).

The impact of each sieving method (wet or dry) on the amount of recovered aggregate size classes is still not clear as results from studies vary. Results from a study by Hu, Chen and Zhu (2020) indicated that dry sieving resulted in a higher recovery of macroaggregates (> 0.25 mm) than microaggregates (< 0.25 mm), while another study showed a better recovery of microaggregates

using the dry sieving or “crushing” method, with less affected aggregate stability compared to wet sieving (Felde et al., 2021). However, a meta-analysis study by Islam et al. (2021) confirmed that the wet sieving method is 12% more efficient than the dry sieving method for assessing aggregate stability after biochar amendments .

Aggregate formation and destruction depend on many factors, including physical and biological factors in the soil environment (Tisdall & Oades, 1982), land management (Nandan et al., 2019) and climate factors affecting soil microbial activity (Qiao et al., 2019) and soil structure stability (Römkens, Helming & Prasad, 2002). Physical factors include soil texture (Angst et al., 2021), mineralogy (Baldock & Skjemstad, 2000; Sarkar et al., 2018), and water availability (Materechera et al., 1994).

Biological factors include microbial and plant community composition (Naga Raju, Golla & Vengatampalli, 2017). Land management factors include tillage and crop rotation (Chakraborty et al., 2019), organic matter amendment (Rakshit, Sarkar & Abhilash, 2018), watering regime , and plant cover (Abbas, Zhu & An, 2021). Climate factors include drying-rewetting (Li et al., 2018), freeze-thaw cycles (Xiao et al., 2020b), and temperatures (Guan et al., 2018). Below I describe each of these in detail. A separate section is devoted to the role of microbes.

2.3. Aggregate formation mechanisms

2.3.1. Soil texture and mineralogy (physical)

Soils with different texture behave differently towards aggregate formation and stability. One of the main factors related to soil texture is the clay content and the associated mineral binding through “surface sorption” of organic/inorganic chemicals within aggregates. Therefore, the mineralogy of the soil can define the stability of the aggregates structure against microbial

decomposition according to the type of clay and through various types of bonds (for instance, hydrogen/coordination bonds and Van der Waals force strongly affect attachment of nano-sized particles in the soil (Hu et al., 2015; Li et al., 2022)). However, the degree of organic matter protection within an aggregate also depends on the physical and chemical characteristics of the binding material itself (Baldock & Skjemstad, 2000; Davet, 2004; Sarkar et al., 2018). Effects of plant and microbial residue composition are described below. Another point addressing the importance of soil texture for aggregation is that the same plant residue can decompose differently within varying soil textures. One study found that more clay and silt content resulted in more sequestration of plant residue C in newly formed aggregates (Angst et al., 2021). The same study attributed the smaller ability of sandy soils to sequester plant residue C compared to clay soils to the high rate of decomposition of the plant residue C, due to the lack of organo-mineral associations in the sandy soil. However, the sandy soil in the same study was found to be able to retain a low amount of relatively untransformed plant litter and was suggested to be more stable than the plant litter C sequestered in clayey soil. A recent study by Olagoke et al. (2022) found more extracellular enzymes in soils with higher clay content. They suggested that there is a mechanism of enhancing the microbial production of enzymes in response to the increase in soil clay content. Based on the above results, it was emphasized that the increased extracellular enzyme production with increased clay content is caused either by direct stress induced by the increase in clay minerals on microbes (toxicity) from aluminum complexes in the clay particles, or due to the clay mineral adsorption of microbial extracellular enzymes for resources acquisition, as adsorption will deactivate the enzymes in soil. The exact mechanisms explaining the relations between clay mineralogy and soil microbes remain unclear.

2.3.2 Plant roots and mycorrhiza (biological and physical)

Plant roots along with the growth of hyphae by mycorrhizal fungi can enmesh microaggregates (<0.25 mm in size) into macroaggregates (>0.25 mm in size) and enhance the soil structure (Helfrich et al., 2008; Tisdall & Oades, 1982). The mycorrhizal hyphae co-exist with the plant roots in a symbiotic relationship. The fungi benefit from the plant produced sugars that enter the plant roots and are used by the fungi as a C and energy source, and in return, the fungi provide the roots with water and nutrients. Both roots and fungal hyphae produce organic complexes that can glue the soil microaggregates into stable macroaggregates (Hoorman, Sá & Reeder, 2011). A study by Haynes and Beare (1997) compared six crops for aggregate stability enhancement, where the results showed an increase of up to 100% in aggregate stability due to the existence of live roots compared with root free soil. Nevertheless, a study by Daynes et al. (2013) concluded that plant roots are associated more in mediating the formation of aggregates than in stabilizing them. In the same study, the stabilization of root mediated aggregates was attributed to the fungal hyphae that was coexisting with live roots. Moreover, plant species differ in root architecture/morphology and chemical composition that can influence soil aggregation differently, as discussed below.

Because live roots explore soil for water and nutrients, soil components endure physical stress from the roots growing through the soil causing local compaction of soil particles, which can contribute to the formation of soil microaggregates (Dorioz, Robert & Chenu, 1993; Six et al., 2004). The penetration of growing roots can destroy pores in the aggregates but at the same time they form new ones especially when the roots happen to decay (Angers & Caron, 1998). Moreover, studies showed that the wettability of soil can be influenced by the presence of live roots where it was seen to be able to modify soil moisture through water uptake (Angers & Caron, 1998;

Materechera et al., 1994), where the importance of soil moisture for aggregation will be discussed later in this review.

Several studies found an increase in macroaggregates formation with the presence of live roots compared to soils with no live roots (Poirier et al., 2018; Wang et al., 2020b) and aggregate stability (Xiao et al., 2020a). However, the impact of live roots on aggregation can vary between different species according to their root traits (*e.g.,* root density and length) on aggregate size classes and on MWD. For instance, the density of fine roots had a significant effect on the formation of macroaggregates due to their high production of labile compounds compared to thick roots (Poirier et al., 2018). A more recent study confirmed the earlier addressed positive influence of fibrous-rooted plants on soil macroaggregates and also reported a negative effect on microaggregates (<0.25 mm in size) (Ali et al., 2022). Both studies attributed the positive effect on macroaggregate formation to the low lignin content of the decaying fibrous roots compared to the tap-rooted plants. As lignin is hydrophobic, roots with a lower concentration of lignin may favor binding of soil particles, but may also produce more dissolved organic C during decomposition, which was found strongly linked to the microbial community's abundance and diversity (bacteria/fungi) and thus the decomposition process of plant residues for potential C sequestration (Campbell et al., 2022) in soil aggregates (discussed in detail in later sections in this review).

2.3.3 Plant rhizodeposition and litter production (biological)

Input of organic matter in the soil is mostly produced by plants through photosynthesis. Some of the products produced by plants are secreted by roots to the rhizosphere (or rhizodeposition) that can interact with associated soil microbes (Hütsch, Augustin & Merbach, 2002). These root secretions of mostly labile sugars (like glucose) get decomposed very fast (few minutes to hours)

by soil microbes. Thus, the main benefit of those provided energy sources to aggregate formation is to activate the dormant microbes in the soil (Bartoli et al., 1993).

Plant litter and root residues in the soil also contribute to soil aggregate formation by supplying soil with organic matter. This organic matter can directly increase aggregate formation or indirectly through the formation of microbial products after microbial decomposition. Other sources of organic matter in the soil are provided by all soil microbes and fauna in the soil either through in situ production or by releasing by-products of decomposition of plant residues, soil organisms' residues and dead cells (Gregory, 2008; Gunina & Kuzyakov, 2015; Lee & Foster, 1991; Lynch & Bragg, 1985).

Plant residues vary in decomposability from easy to decompose (labile compounds) to hard-to decompose (recalcitrant compounds). Labile compounds like carbohydrates and amino acids will be decomposed quickly by soil microbes for energy production and biomass synthesis (Gunina & Kuzyakov, 2015). Consequently, the addition of these more labile compounds to soils are not directly involved in the formation of soil aggregates. However, their role in aggregate formation will also depend on how efficiently C from these compounds is used for microbial growth vs how much is lost through respiration, defined as the microbial carbon use efficiency, which is discussed in more detail below. Recalcitrant plant residues are associated with the presence of humus-forming materials, and are often associated with lignin (Yanni et al., 2011) and tannins (Erktan et al., 2017). In contrast to labile compounds, these recalcitrant compounds can play a direct role in aggregate formation, where they form organo-mineral associations in microaggregates (Guhra, Stolze & Totsche, 2022; Six et al., 2004) Nevertheless, the relative role of labile compounds (mostly from rhizodeposition) and recalcitrant compounds (mostly from plant litter) on soil aggregate formation remains unclear.

The increased concerns of soil sustainability therefore has led to hundreds of research works exploring the potential effects of various plant residue soil amendments from different residue origin, pre-treatments, application rate and conditions on aggregation, soil C, and CO_2 soil emission rates (Islam et al., 2021; Rakshit, Sarkar & Abhilash, 2018; Yanni et al., 2011)

2.3.4 Land management

I discussed above how plant litter contributes to soil aggregate formation and how different types of organic matter can have a different influence on aggregate formation and stability. In terms of land management, retaining nutrients in soil is important for agricultural sustainability. Plant residues, animal manure, and sewage sludge are some of the organic wastes added to soil to tackle degraded soils (like nutrient depletion) caused by intensified farming for food security and to mitigate the effects of soil disturbing agricultural practices such as tillage on soil structure (discussed below). Soil organic amendments with plant origin have been used as fresh, composted, or charred amendments (Rakshit, Sarkar & Abhilash, 2018). Halder et al. (2021) studied the effect of 9 plant residue amendments (3 types of vetch: alfalfa, hairy, and Chinese milk and 6 types of straw: rice, maize, wheat, miscanthus, sorghum, and soybean) on aggregation and MWD through a 56-day incubation study. The vetch increased the aggregation more than the straw amendment. The positive influence of vetch on aggregation was related to the high content of soluble sugars and low lignin compared to straw, where lignin was found negatively related to the MWD. The results showed a short-term aggregate stability (up to 28d) followed by a decrease towards the end of incubation due to the decrease in the available soluble sugars for microbes to facilitate aggregation. Consistent with the above, Sarker et al. (2022) emphasized that soil residue amendments rich in carbohydrates (like sugars) are more capable of affecting soil aggregates than aromatic compounds (like lignin). On the other hand, a meta-analysis by Husain and Dijkstra

(2023) found that the effectiveness of the plant residue amendments is generally dependent on the soil initial organic C and pH, where the highest effects were observed in low initial organic C and alkaline soils.

Despite the proven benefits of mulching and composted plant residue soil amendments to the soil moisture and vegetation, there was not observed effect on improving soil aggregation or microbial abundance (Leger et al., 2022).

A review by Siedt et al. (2021a) compared the effects of fresh plant residue (straw), composted biowastes and charred plant residue soil amendments on soil structure. The review noted a positive effect of straw and char soil amendments on aggregation and confirmed the low benefits of composted plant residue for soil aggregation. The increase in aggregation was variable throughout the studies, which was related to the different properties of amendment origins (nutrients content), experimental, climatic, and soil conditions. The same review suggested that soil amendment material needs to be analyzed chemically prior to soil application in order to evaluate each material's suitability for soil amendment, highlighted the importance of continuous research, and reported a lack of meta-analysis for evaluating current research findings regarding various types of soil amendment benefits for soil.

Production of char (or biochar) derived from different plant origins at different processing conditions (through pyrolysis) can result in variations in chemical (C content, pH, aromaticity) and physical properties (particles surface area or porosity) of the biochar, which will impact other soil chemical, physical and biological factors. However, the native soil properties such as texture can also interact with the effect of biochar on soil properties (Gul et al., 2015). A review by Zhang et al. (2019) summarized the methods of plant residue pyrolysis and differences in yield from biochar applications with variable properties. The same review emphasized also that both feed stock source

and pyrolysis method are important factors in biochar properties. The temperature of pyrolysis effect was attributed to the source of feed stock and the lignin content of plant residues was found to be directly correlated to the biochar yield (Liu et al., 2014b). In terms of biochar stability in the soil and decomposability resistance in the long term, the higher the temperature the more stable is the biochar. In general, the effect of biochar application on soil C enhancement was found to be greatly dependent on the initial soil C content or the degree of saturation of soil C (Stewart et al., 2007) to harness the benefits of biochar soil amendments for increasing soil C storage. That means there is no linear relationship between the addition of biochar (C input) and the soil C content. On the other hand, a meta-analysis by Islam et al. (2021) found an overall positive effect of the biochar soil amendment on improving soil aggregation. Moreover, it reported that increasing biochar addition rate to soil will significantly increase the soil aggregate formation. Consistent with the effect of biochar soil amendment on soil C, the meta-analysis also found the effect on aggregation dependent on the pyrolysis temperature and biochar origin, where the highest positive effect was from high pyrolysis temperatures (>600 °C), and biochar made from the wood, and soil factors like pH and texture were also found to be influential in the effectiveness of the biochar application to improve soil aggregation, where biochar application improved soil aggregation in neutral and acidic soil with no change observed in the alkaline soil. In addition, the same meta-analysis reported that biochar amendment improved aggregation in loam-textured soils more than in sandy soils.

2.3.5 Climate factors

Climate factors like precipitation and temperature can subject the soil to drying-rewetting cycles and can have direct and indirect effects on aggregation through induced changes to soil nutrients and microbial activity in the soil (Kaiser, Kleber & Berhe, 2015; Lamparter et al., 2009). For

instance, a study by Guan et al. (2018) found a decrease in MWD, which was related to the significant increase in the silt and clay fraction (<0.053) following a 5 year warming experiment. With respect to soil C pools, the study found no significant effect on the total soil C content or microbial biomass C compared to un-warmed soil (control), an increase in soluble organic C and silt and clay associated C, and a decrease in phenol C, microaggregate associated C, which led to a decrease in aggregate stability. Based on the above results, the authors suggested that in the studied region (alpine meadow) mineral associations are more important than biochemical factors for C stabilization in the soil.

As a result of drought and rain patterns, drying-rewetting cycles indirectly impact aggregation stability by reducing the decomposers (*i.e.*, soil microbes) accessibility to soil organic C (Lamparter et al., 2009). However, the significance of the impact on aggregate associated C may depend on length and intensity of drying-rewetting cycles (Li et al., 2018; Semmel et al., 1990; Zhang, Wang & Li, 2022). In warmer conditions (25°C) drying-rewetting cycles increased macroaggregates and decreased microaggregates compared to cooler temperatures (5°C). The drying-rewetting cycles in warmer conditions also reduced soil respiration more, thereby negatively affecting the priming effect in the soil (Najera et al., 2020). A negative priming effect in the soil may result in a potential C storage in soil aggregates.

On the other hand, assessing soil aggregation in cold regions can be related to the effects of naturally occurring freeze-thaw cycles and soil initial moisture content. While soil moisture content was found to significantly increasing aggregate formation (both macro and micro) and MWD with the increasing in soil moisture content, freeze-thaw cycles were found to affect aggregation negatively (Liu et al., 2023). The freeze-thaw cycles were found to be only beneficial to soil aggregation stability in the presence of vegetation cover (Xiao et al., 2020b). Wang et al.

(2020a) suggested that the mechanisms that govern soil aggregate destruction by freeze-thaw cycles have similarities with the mechanisms affecting soil disintegration (erosion) due to freeze-thaw cycles such as soil moisture content, soil organic matter and clay content. Soil erosion tended to decrease (less erodible) to an extent with the increase in soil moisture (Xia et al., 2018). A recent study by Liu et al. (2023) evaluating the effect of freeze-thaw cycles on macroaggregates and macropores, related the destructive effect of the freeze-thaw cycles on soil macroaggregates to the enlarging effect of freeze-thaw cycles on the macropores. As a result, it causes a weak structure of the soil macroaggregates that eventually lead to a low macroaggregate stability in the long term.

2.4 Aggregate destruction mechanisms

2.4.1. Live roots (biological)

As mentioned above, live plant roots can contribute to the formation of soil aggregates in many ways. On the other hand, plant roots can also destroy the structure of aggregates if root growth happens towards the small spaces (pores) within aggregate structures (Angers & Caron, 1998). In a recent study, Lu et al. (2019) proposed three indirect destruction mechanisms of soil aggregates by live roots. First, through providing energy sources to soil microbes, this can enhance microbial organic matter decomposition, thereby removing the glue that binds soil particles together. Second, roots can compete with microbes for nitrogen (N), forcing them to decompose aggregate associated organic matter to compensate for N deficiency in the surrounding environment. The third mechanism was classified as a physical effect, relating to the destruction of aggregates by (large) roots that can also intensify drying-rewetting cycles (discussed below).

2.4.2 Mechanical forces (physical)

Three types of forces can be listed as aggregate destructive forces, two of which are influenced by nature, and one is an anthropogenic force. The ability of aggregate to hold water in their pores

causes it to swell whereby the water causes tension against the stability of aggregates (Davet, 2004; Tisdall & Oades, 1982). However, the destructive effect of water depends on the intensity of watering events and the degree of soil structure stability (Römkens, Helming & Prasad, 2002). The other natural factor relates to soil fauna movements in the soil such as earthworms and the tunnels they carve in the soil (Lee & Foster, 1991). Anthropogenic destruction of soil aggregates are mostly attributed to farming practices like tillage and the use of farming vehicles that can either destroy the architecture of the aggregates' pore system or force more compaction of the soil (Hoorman, Sá & Reeder, 2011; Lal, Hall & Miller, 1989). Management practices like surface mulching either by using plant residues (de Melo et al., 2022) or even by using recycled tires chips that have large elasticity and can sustain in soil longer than plant residues (Lee & Roh, 2007), can provide a (temporary) protection by cushioning the effects of heavy farming machinery compaction. Another management practice was suggested by Shubha et al. (2020) to mitigate soil compaction by machinery through reducing or minimizing machinery traffic between the farming lanes. There is also an indirect negative impact of tillage on soil aggregation that is related to the destruction of fungal structures, and consequently fungi cannot survive in soils exposed to tillage. The reduced abundance of fungi that contributes to the microaggregate enmeshment would then result in less macroaggregates formation (Hoorman, Sá & Reeder, 2011). Ali et al. (2022) proposed that bio-tillage can be adopted as an alternative to mechanical tillage for preserving soil structure (aggregates) while preparing soil beds for new crops. Bio-tillage can be achieved by crop rotating of fibrous rooted plants with tap-rooted plants especially in high clayey soils. Moreover, the study also found that fibrous rooted crops can enhance the macroaggregate formation. The benefits of growing fibrous rooted crops to aggregation were attributed to: i) the low root lignin/cellulose ratio (when decomposing), ii) the large root length density, and iii) the high percentage of fine roots

penetrating the soil. Despite the proven benefits of bio-tillage, more research is needed to identify the best crop rotating practices for soil structure sustainability.

2.4.3 Soil enzymes (biological)

previously it was discussed that soil enzymes can play an important role in aggregate formation, but they also play an important role in aggregate destruction. The release of extracellular enzymes in the soil by microbes and plants can enhance decomposition of organic matter (Olagoke et al., 2022), and therefore potentially destroy aggregates. The production of extracellular enzymes by microbes often increases upon the availability of organic matter where different types of enzymes are produced involved in the breakdown of specific organic compounds. For instance, there are hydrolytic enzymes responsible for decomposition of more labile organic compounds, oxidative enzymes responsible for more recalcitrant compounds, Enzymes remaining in the soil may get diffused or mobilized upon water availability into aggregate pores exposing sequestered C inside aggregates to decomposition (Angers & Caron, 1998; Gunina & Kuzyakov, 2015). Soil mineralogy (clay content) was found to have an important effect on the microbial production of extracellular enzymes. It was related to the implied stress of clay minerals on microbes such as changing the soil pH (Xu et al., 2022). The released extracellular enzymes by microbes into the soil can also change the clay minerals chemically and physically (Hong et al., 2016). Consistent with the above, an increase in the production of microbial extracellular enzymes was observed by Olagoke, Kalbitz and Vogel (2019) with the increase in clay content of the soil. However, the same study reported that the enzyme activity can be reduced due to the adsorption to clay minerals triggering more microbial production (when energy sources permit), but the exact mechanisms remained unexplained.

2.5 Role of microbes on aggregate formation and destruction

Decomposition of plant residues in the soil is mostly governed by microbes living in the soil habitat apart from earth worms and some insects that digest dead cells and tissues to release organic matter in the soil solution (Adl, 2003). Soil microbes are highly diverse, and most soil microbes are still unexplored. While the importance of their role in the soil ecosystem is well established (Miransari, 2011), the role of specific microbial groups in soil aggregation and structure stability remains less understood. Understanding the dynamics of specific soil microbial groups can help in informing soil management practices to restore or conserve the soil health status (Heneghan et al., 2008; Six et al., 2006). Most of the soil microbes (>90%) consist of bacteria and fungi (Six et al., 2006). Bacteria contributed about (0.03 - 0.3 %) of the soil dry weight and 1% of the soil volume due to their high-water content and relative low density. Bacteria in soil are mostly heterotrophic; they rely on organic compounds continuously added to the soil from plants and animals to grow and produce energy. Some bacteria (autotrophic groups) can perform photosynthesis and fix carbon dioxide (CO_2) directly from the atmosphere and rely on inorganic mineral sources to survive (Clark, 1951). In this work, the focus will be on the heterotrophic bacteria and fungi that decompose plant residues in the soil into simple compounds (like CO_2) as a component of concern in the changing global C-cycle.

Soil fungi are less abundant in number (if counted as individuals) than bacteria, but they often make a larger proportion of the soil biomass than bacteria. They can be either parasitic or symbiotic around plant roots (mycorrhiza). Free-living fungi as well as some mycorrhiza (ectomycorrhiza) decompose plant organic matter in the soil. Fungi tend to have more efficient decomposition enzymes than soil bacteria to decompose complex organic matter (Davet, 2004; Griffin, 1972). Generally, fungi are more dominant in rich soils like forests, while bacteria are more abundant in

agricultural soils where soil is less rich with a lower C:N ratio (Högberg, Högberg & Myrold, 2007). Soil fungi high production of extracellular enzymes will increase their C consumption, especially at times of stress (such as drought) leading to a lower fungal carbon use efficiency (CUE) compared to soil bacteria (Ullah, Carrillo & Dijkstra, 2021b). However, results from other studies had shown variations in fungal and bacterial CUE and showed variations in the effect of fungal and bacterial abundances on the level of CUE due to environmental variations such as type of substrate and climate (temperature) (Qiao et al., 2019) or the availability of inorganic N (*e.g.,* NO_3^-) in the soil (McGee et al., 2019). Silva-Sánchez, Soares and Rousk (2019) observed that while increasing the mineral N stimulated the growth of fungal communities, the growth of bacteria can be stimulated with the increase in the pH. Microbial CUE and its association with soil aggregation and C stabilization is discussed in more detail below.

Previous studies have shown that soil microbes have different influences on soil aggregation dynamics through alterations in the C turnover rates in the soil (Brant, Sulzman & Myrold, 2006; Le Guillou et al., 2012). For instance, bacteria using labile substrates can possibly contribute to soil aggregation through production of necromass. Fungi however produce hyphae that can bind soil particles together, while they also grow slowly on recalcitrant organic matter thereby producing less microbial necromass for soil aggregation. Bacteria have the potential to produce more necromass per unit of plant C input than fungi do due to the higher microbial CUE for bacteria than fungi.

A challenge is that continuous additions of plant residues to the soil will continue to change microbial succession patterns, leading to a continuous change in C flow through the global C-cycle (Bai et al., 2016; Xu et al., 2018). While some microbes like bacteria are corelated with the early stages of the aggregation process, later microbial successions like fungi within the soil matrix can

stabilize aggregates for the long term. Experiments in this field also showed that the role of microbes in aggregation depends on the soil mineral contents (i.e., N availability) and the quality of introduced substrate to the decomposing microbes. However, the exact mechanisms of microbial-aggregation dynamics are not fully understood yet (Bossuyt et al., 2001; Le Guillou et al., 2012).

2.6 Role of soil aggregation for carbon stabilization

As I discussed above, stabilization of organic C from old organic matter (humus), new organic matter (from plant as labile or recalcitrant) or microbes (synthesized or byproducts of decomposition) needs the protection from potential decomposition to sustain in the soil. The formation of microaggregates can stabilize the old organic C in the soil either through adsorption to clay minerals by forming the organo-mineral associations as discussed above, while macroaggregate formation is key to physically protect formed microaggregates through the root or fungal enmeshments (Kumar et al., 2013). Consequently, the stabilization of soil aggregates against soil and plant variables -as contributors of aggregate formation and stability- has become of a great concern to soil scientists in the recent decades. For instance, Xiao et al. (2020a) studied the effects of soil organic C and plant properties (roots) on aggregate stability (MWD value). Results showed that both soil organic C content and root properties are important for aggregate stability. However, there was a stronger positive effect from plant roots than soil organic C on the MWD and the variation in MWD was explained through root properties by 40% more than it was explained by the soil organic C. The higher positive effect of plant roots can be explained by the increase of soil organic C caused from both the growth of plant roots and the root exudates (Materechera et al., 1994). The discussion above is clearly showing how there is a strong

correlation between soil organic C sequestration and soil aggregation stability. All important factors for both processes should be considered for improving soil as a C sink.

2.7 Microbial CUE associated with soil carbon in aggregates

As discussed above, CUE is an important parameter that could potentially influence soil aggregation and C sequestration. Microbial CUE is defined as the net amount of soil C used for growth that is incorporated into the microbial biomass compared to the total C use (growth plus respiration) (Brant, Sulzman & Myrold, 2006). In terms of aggregation, it can be argued that an increase in CUE induces greater aggregate formation and C sequestration in the soil (McGee et al., 2019), while a decrease in CUE leads to increased CO_2 emissions to the atmosphere. Thus, the determination of microbial CUE dynamics is crucial for improving soil management and sustainability (Cotrufo et al., 2013; Kästner & Miltner, 2018).

Studies on substrate decomposition suggested that microbial CUE is highly influenced by soil pH and exchangeable Al^{3+} levels with evidence showing that low soil pH (lower than 5.5) is critically reducing the microbial CUE, because the negative impact of high acidity results in stress mitigation (acidity and cell detoxification) activities that are C inefficient, or to shifts in the microbial communities (Jones et al., 2019; Silva-Sánchez, Soares & Rousk, 2019). However, the results were not interpreted into drawing a direct relationship between microbial CUE and soil pH. On the other hand, a study by Malik et al. (2018) suggested a soil pH threshold value of 6.2, where pH<6.2 will have a great detrimental effect on the microbial CUE.

Nitrogen concentration in the soil can have a positive impact on the microbial CUE as it decreases the N mining by microbes and therefore reduces the mineralization of C stored in the soil, which can C inefficient (Spohn et al., 2016). Climate, substrate chemistry and the diversity of soil

microbial communities have also been considered when studying microbial CUE (Domeignoz-Horta et al., 2020; Luiz et al., 2020). Brant, Sulzman and Myrold (2006) reported that alterations in added substrates to the soil induced a change in the patterns of microbial CUE. The explanation of the substrate impact on C dynamics in the soil was attributed to the substrate direct influence on microbial communities inhabiting the studied soils. Moreover, Wang et al. (2021) linked microbial CUE to the climate induced modifications in microbial biomass, where microbial biomass was also found to be associated with observed changes in the soil organic C.

Microbial CUE interactions with soil aggregates can be conceptualized by incorporating all discussed factors above with a complex interaction between factors (Figure 2.1). Factors that increase microbial CUE also have the potential to increase soil aggregation and C sequestration. A new conceptualized framework is described in the section below.

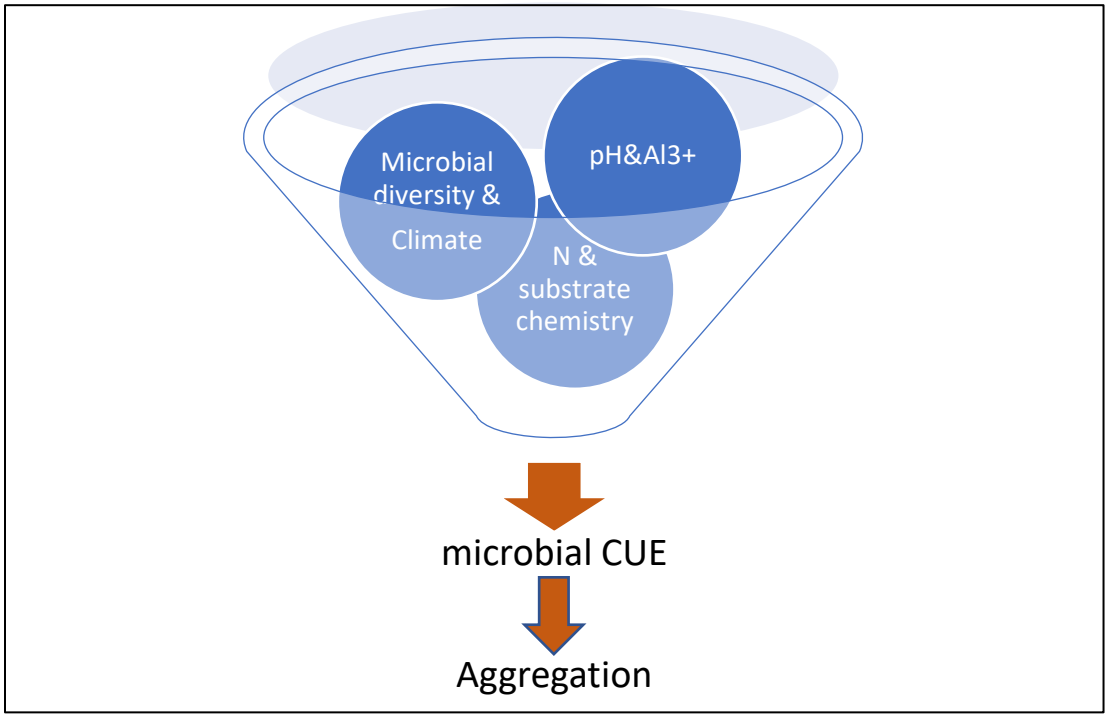

Figure 2.1: Factors influencing microbial CUE and aggregation.

2.8 A conceptual framework

Processes addressed in this conceptual framework are aggregate formation, and aggregate stability. Microbial decomposition of C substrates (primarily from plants) as root exudates (labile organic C) or residues (recalcitrant organic C) is governed by many microbial (bacterial and fungal) and substrate properties. The significance of the role of fungi and bacteria in the aggregate formation depends partly on the fungal and bacterial CUE. CUE is higher for labile than recalcitrant substates during microbial decomposition, while fungi are associated with the decomposition of recalcitrant substrates would have a lower CUE. However, both fungi and bacteria are also associated in the decomposition of labile substrates, which may increase their CUE. The stability of aggregates depends on the resistance of aggregates to aggregate destruction mechanisms such as root activity, soil drying-rewetting, plant/microbe enzymes and land managements (Figure 2.2).

Soil structure/aggregation and texture (*i.e.,* grain size/type) can also determine microbial populations, microbial activity, and C turnover dynamics. For example, aggregate pore systems (like sizes and intensities) within and between soil aggregates can affect the survival of bacteria against predation. Heijnen, Chenu and Robert (1993) reported that pore sizes (< 6 μm) can be a suitable habitat for protecting bacteria from predators. However, a later study of bacterial activity *vs*. pore size, found that there is more bacterial activity within pores of size (6-30 μm) even with increased potential predation on bacteria (Wright et al., 1995). Based on the above, bacteria are better protected from predation than fungi in the soil. However, fungi produce hyphae, bacteria do not, and therefore affect soil aggregation differently. On the other hand, fungi are better adapted to draught than bacteria, and therefore may play a greater role in soil aggregation under draught conditions. Aggregate pore aeration and moisture content can also determine the type of active microbes within soil aggregates depending on the provided conditions by aggregate architecture

against the microbial life preferences (Ebrahimi & Or, 2015). In terms of soil chemistry, fungi dominate more in soil with low pH, leading to a greater role of fungi to improve aggregation in acidic soils.

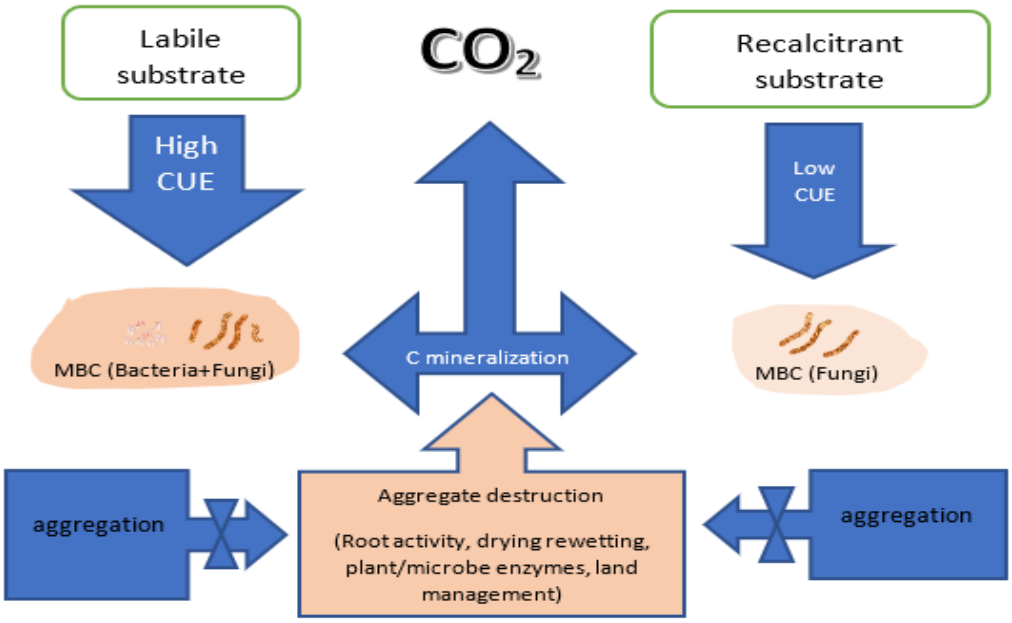

Figure 2.2: Factors influencing soil aggregation, including plant roots, residue type (labile and recalcitrant substrate), soil microbial groups (bacteria and fungi), land management and abiotic factors on soil aggregation and C-substrate turnover. The valve represents aggregate destruction mechanisms (root activity, soil drying rewetting, plant/microbe enzymes and land management).

Since microbial activity in the soil and formation of aggregate stable complexes are both sensitive to soil pH (Xu et al., 2018), the relationship between soil aggregation and microbial activity may be variable in different soils and associated soil microbial communities, that depend itself on the status of soil pH.

This review has confirmed that many factors influence soil aggregation and C sequestration in interactive ways, and that there is a greater need to understand these interactive processes.

2.9 References

Abbas, F, Zhu, Z & An, S 2021, 'Evaluating aggregate stability of soils under different plant species in Ziwuling Mountain area using three renowned methods', *CATENA,* vol. 207, pp. 105616.

Adl, SM 2003, *The ecology of soil decomposition*, CABI, Wallingford, Oxfordshire, UK.

Ali, W, Yang, M, Long, Q, Hussain, S, Chen, J, Clay, D & He, Y 2022, 'Different fall/winter cover crop root patterns induce contrasting red soil (Ultisols) mechanical resistance through aggregate properties', *Plant and Soil,* vol. 477, no. 1, pp. 461-474.

Angers, DA & Caron, J 1998, 'Plant-induced Changes in Soil Structure: Processes and Feedbacks', *Biogeochemistry,* vol. 42, no. 1, pp. 55-72.

Angst, G, Pokorný, J, Mueller, CW, Prater, I, Preusser, S, Kandeler, E, Meador, T, Straková, P, Hájek, T, Van Buiten, G & Angst, Š 2021, 'Soil texture affects the coupling of litter decomposition and soil organic matter formation', *Soil biology & biochemistry,* vol. 159, pp. 108302.

Bai, Z, Liang, C, Bodé, S, Huygens, D & Boeckx, P 2016, 'Phospholipid 13C stable isotopic probing during decomposition of wheat residues', *Applied Soil Ecology,* vol. 98, pp. 65-74.

Baldock, JA & Skjemstad, JO 2000, 'Role of the soil matrix and minerals in protecting natural organic materials against biological attack', *Organic Geochemistry,* vol. 31, no. 7, pp. 697-710.

Bartoli, F, Burtin, G, Philippy, R & Gras, F 1993, 'Influence of fir root zone on soil structure in a 23 m forest transect: the fractal approach', *Soil structure/soil biota interrelationships*, Elsevier.

Beck, T, Joergensen, R, Kandeler, E, Makeschin, F, Nuss, E, Oberholzer, H & Scheu, S 1997, 'An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C', *Soil Biology and Biochemistry,* vol. 29, no. 7, pp. 1023-1032.

Bhattacharya, P, Maity, PP, Ray, M & Mridha, N 2021, 'Prediction of mean weight diameter of soil using machine learning approaches', *Agronomy Journal,* vol. 113, no. 2, pp. 1303-1316.

Blagodatskaya, Đ & 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,* vol. 45, no. 2, pp. 115-131.

Bossuyt, H, Denef, K, Six, J, Frey, SD, Merckx, R & Paustian, K 2001, 'Influence of microbial populations and residue quality on aggregate stability', *Applied Soil Ecology,* vol. 16, no. 3, pp. 195-208.

Bradford, MA, Fierer, N & Reynolds, JF 2008, 'Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils', *Functional Ecology,* vol. 22, no. 6, pp. 964-974.

Brant, JB, Sulzman, EW & Myrold, DD 2006, 'Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation', *Soil Biology and Biochemistry,* vol. 38, no. 8, pp. 2219-2232.

Bucka, FB, Kölbl, A, Uteau, D, Peth, S & Kögel-Knabner, I 2019, 'Organic matter input determines structure development and aggregate formation in artificial soils', *Geoderma,* vol. 354, pp. 113881.

Bureau of Metreology 2020, *State of the climate 2019*, http://www.bom.gov.au/climate/current/annual/nsw/archive/2019.summary.shtml#summary

Campbell, TP, Ulrich, DEM, Toyoda, J, Thompson, J, Munsky, B, Albright, MBN, Bailey, VL, Tfaily, MM & Dunbar, J 2022, 'Microbial Communities Influence Soil Dissolved Organic Carbon Concentration by Altering Metabolite Composition', *Frontiers in Microbiology,* vol. 12.

Castellano, MJ, Mueller, KE, Olk, DC, Sawyer, JE & Six, J 2015, 'Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept', *Global change biology,* vol. 21, no. 9, pp. 3200-3209.

Chakraborty, D, Mondal, S, Das, A, Paul, A, Thomas, P, Aggarwal, P, Behera, U & Sharma, A 2019, 'Tillage and Crop Rotation Effects on Mechanical Properties and Structural Stability of a Sandy Loam Soil

in a Semi-arid Environment', *Journal of the Indian Society of Soil Science,* vol. 67, no. 2, pp. 174-182.

Chao, L, Liu, Y, Freschet, GT, Zhang, W, Yu, X, Zheng, W, Guan, X, Yang, Q, Chen, L, Dijkstra, FA & Wang, S 2019, 'Litter carbon and nutrient chemistry control the magnitude of soil priming effect', *Functional Ecology,* vol. 33, no. 5, pp. 876-888.

Clark, F 1951, 'Bacteria in the soil', *Experientia,* vol. 7, no. 2, pp. 78-80.

Cotrufo, MF, Wallenstein, MD, Boot, CM, Denef, K & Paul, E 2013, 'The M icrobial E fficiency- M atrix S tabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter?', *Global Change Biology,* vol. 19, no. 4, pp. 988-995.

Davet, P 2004, *Microbial ecology of the soil and plant growth*, Science Publishers, Enfield, (NH).

Daynes, CN, Field, DJ, Saleeba, JA, Cole, MA & Mcgee, PA 2013, 'Development and stabilisation of soil structure via interactions between organic matter, arbuscular mycorrhizal fungi and plant roots', *Soil Biology and Biochemistry,* vol. 57, pp. 683-694.

De Deyn, GB, Cornelissen, JH & Bardgett, RD 2008, 'Plant functional traits and soil carbon sequestration in contrasting biomes', *Ecology letters,* vol. 11, no. 5, pp. 516-531.

De Gryze, S, Six, J, Brits, C & Merckx, R 2005, 'A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture', *Soil Biology and Biochemistry,* vol. 37, no. 1, pp. 55-66.

De Melo, RO, Da Fonseca, AA, De Barros, NF, Fernandes, RBA, Teixeira, RDS, Melo, IN & Martins, RP 2022, 'Retention of eucalyptus harvest residues reduces soil compaction caused by deep subsoiling', *Journal of forestry research,* vol. 33, no. 2, pp. 643-651.

Deng, S, Zheng, X, Chen, X, Zheng, S, He, X, Ge, T, Kuzyakov, Y, Wu, J, Su, Y & Hu, Y 2021, 'Divergent mineralization of hydrophilic and hydrophobic organic substrates and their priming effect in

soils depending on their preferential utilization by bacteria and fungi', *Biology and Fertility of Soils,* vol. 57, no. 1, pp. 65-76.

Dijkstra, FA & Cheng, W 2007, 'Moisture modulates rhizosphere effects on C decomposition in two different soil types', *Soil biology & biochemistry,* vol. 39, no. 9, pp. 2264-2274.

Domeignoz-Horta, LA, Pold, G, Liu, X-JA, Frey, SD, Melillo, JM & Deangelis, KM 2020, 'Microbial diversity drives carbon use efficiency in a model soil', *Nature Communications,* vol. 11, no. 1, pp. 3684.

Dorioz, JM, Robert, M & Chenu, C 1993, 'The role of roots, fungi and bacteria on clay particle organization. An experimental approach', *Geoderma,* vol. 56, no. 1, pp. 179-194.

Ebrahimi, A & Or, D 2015, 'Hydration and diffusion processes shape microbial community organization and function in model soil aggregates', *Water Resources Research,* vol. 51, no. 12, pp. 9804-9827.

Elliott, ET 1986, 'Aggregate Structure and Carbon, Nitrogen, and Phosphorus in Native and Cultivated Soils', *Soil Science Society of America journal,* vol. 50, no. 3, pp. 627-633.

Erktan, A, Balmot, J, Merino-Martín, L, Monnier, Y, Pailler, F, Coq, S, Abiven, S, Stokes, A & Le Bissonnais, Y 2017, 'Immediate and long-term effect of tannins on the stabilization of soil aggregates', *Soil Biology and Biochemistry,* vol. 105, pp. 197-205.

Fang, Y, Nazaries, L, Singh, BK & Singh, BP 2018, 'Microbial mechanisms of carbon priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils', *Global Change Biology,* vol. 24, no. 7, pp. 2775-2790.

Felde, VJMNL, Schweizer, SA, Biesgen, D, Ulbrich, A, Uteau, D, Knief, C, Graf-Rosenfellner, M, Kögel-Knabner, I & Peth, S 2021, 'Wet sieving versus dry crushing: Soil microaggregates reveal different physical structure, bacterial diversity and organic matter composition in a clay gradient', *European journal of soil science,* vol. 72, no. 2, pp. 810-828.

Field, DJ, Minasny, B & Gaggin, M 2006, 'Modelling aggregate liberation and dispersion of three soil types exposed to ultrasonic agitation', *Australian Journal of Soil Research,* vol. 44, no. 5, pp. 497.

Fierer, N & Jackson, RB 2006, 'The diversity and biogeography of soil bacterial communities', *Proceedings of the National Academy of Sciences of the United States of America,* vol. 103, no. 3, pp. 626-631.

Fomin, DS, Valdes-Korovkin, IA, Holub, AP & Yudina, AV 2019, 'Dry sieving analysis of soil by vibratory sieve shaker: modification and optimization', *Бюллетень Почвенного института им. В.В. Докучаева*, no. 96, pp. 149-177.

Fontaine, S, Bardoux, G, Abbadie, L & Mariotti, A 2004, 'Carbon input to soil may decrease soil carbon content', *Ecology Letters,* vol. 7, no. 4, pp. 314-320.

Franzluebbers, AJ 2022, 'Soil mean-weight diameter and stability index under contrasting tillage systems for cotton production in North Carolina', *Soil Science Society of America journal,* vol. 86, no. 5, pp. 1327-1337.

Garcia-Pausas, J & Paterson, E 2011, 'Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon', *Soil Biology and Biochemistry,* vol. 43, no. 8, pp. 1705-1713.

Garcia, C, Nannipieri, P & Hernandez, T 2018, 'Chapter 9 - The Future of Soil Carbon', in Garcia, C, Nannipieri, P & Hernandez, T (eds.), *The Future of Soil Carbon*, Academic Press.

Gregory, PJ 2008, *Plant Roots : Growth, Activity and Interactions with the Soil*, 1 edn, Wiley, Hoboken.

Griffin, DM 1972, *Ecology of soil fungi*, London, Chapman & Hall.

Guan, S, An, N, Zong, N, He, Y, Shi, P, Zhang, J & He, N 2018, 'Climate warming impacts on soil organic carbon fractions and aggregate stability in a Tibetan alpine meadow', *Soil biology & biochemistry,* vol. 116, pp. 224-236.

Guhra, T, Stolze, K & Totsche, KU 2022, 'Pathways of biogenically excreted organic matter into soil aggregates', *Soil Biology and Biochemistry,* vol. 164, pp. 108483.

Gul, S & Whalen, JK 2022, 'Chapter Six - Perspectives and strategies to increase the microbial-derived soil organic matter that persists in agroecosystems', in Sparks, DL (ed.), *Advances in Agronomy*, Academic Press.

Gul, S, Whalen, JK, Thomas, BW, Sachdeva, V & Deng, H 2015, 'Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions', *Agriculture, ecosystems & environment,* vol. 206, pp. 46-59.

Gulde, S, Chung, H, Amelung, W, Chang, C & Six, J 2008, 'Soil Carbon Saturation Controls Labile and Stable Carbon Pool Dynamics', *Soil Science Society of America journal,* vol. 72, no. 3, pp. 605-612.

Gunina, A & Kuzyakov, Y 2015, 'Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate', *Soil Biology and Biochemistry,* vol. 90, pp. 87-100.

Guo, LB & Gifford, RM 2002, 'Soil carbon stocks and land use change: a meta analysis', *Global change biology,* vol. 8, no. 4, pp. 345-360.

Halder, M, Liu, S, Zhang, ZB, Guo, ZC & Peng, XH 2021, 'Effects of residue stoichiometric, biochemical and C functional features on soil aggregation during decomposition of eleven organic residues', *Catena (Giessen),* vol. 202, pp. 105288.

Haynes, RJ & Beare, MH 1997, 'Influence of six crop species on aggregate stability and some labile organic matter fractions', *Soil Biology and Biochemistry,* vol. 29, no. 11-12, pp. 1647-1653.

Heijnen, CE, Chenu, C & Robert, M 1993, 'Micro-morphological studies on clay-amended and unamended loamy sand, relating survival of introduced bacteria and soil structure', *Geoderma,* vol. 56, no. 1, pp. 195-207.

Helfrich, M, Ludwig, B, Potthoff, M & Flessa, H 2008, 'Effect of litter quality and soil fungi on macroaggregate dynamics and associated partitioning of litter carbon and nitrogen', *Soil Biology and Biochemistry,* vol. 40, no. 7, pp. 1823-1835.

Heneghan, L, Miller, SP, Baer, S, Callaham Jr., MA, Montgomery, J, Pavao-Zuckerman, M, Rhoades, CC & Richardson, S 2008, 'Integrating Soil Ecological Knowledge into Restoration Management', *Restoration Ecology,* vol. 16, no. 4, pp. 608-617.

Hernandez, T, Hernandez, MC & Garcia, C 2017, 'The effects on soil aggregation and carbon fixation of different organic amendments for restoring degraded soil in semiarid areas: Effect of amendments on soil aggregation and C fixation', *European journal of soil science,* vol. 68, no. 6, pp. 941-950.

Hinsinger, P, Bengough, A, Vetterlein, D & Young, I 2009, 'Rhizosphere: biophysics, biogeochemistry and ecological relevance', *An International Journal on Plant-Soil Relationships*, Springer Netherlands, Dordrecht.

Högberg, MN, Högberg, P & Myrold, DD 2007, 'Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three?', *Oecologia,* vol. 150, no. 4, pp. 590-601.

Hong, H, Fang, Q, Cheng, L, Wang, C & Churchman, GJ 2016, 'Microorganism-induced weathering of clay minerals in a hydromorphic soil', *Geochimica et Cosmochimica Acta,* vol. 184, pp. 272-288.

Hoorman, JJ, Sá, J & Reeder, R 2011, 'The biology of soil compaction', *Soil Tillage Res,* vol. 68, pp. 49-57.

Hu, F, Xu, C, Li, H, Li, S, Yu, Z, Li, Y & He, X 2015, 'Particles interaction forces and their effects on soil aggregates breakdown', *Soil and Tillage Research,* vol. 147, pp. 1-9.

Hu, X, Chen, J & Zhu, L 2020, 'Soil aggregate size distribution and stability of farmland as affected by dry and wet sieving methods', *Žemdirbystė (Akademija),* vol. 107, no. 2, pp. 179-184.

Husain, H & Dijkstra, FA 2023, 'The influence of plant residues on soil aggregation and carbon content: A meta-analysis', *Journal of plant nutrition and soil science*.

Hütsch, BW, Augustin, J & Merbach, W 2002, 'Plant rhizodeposition — an important source for carbon turnover in soils', *Journal of Plant Nutrition and Soil Science,* vol. 165, no. 4, pp. 397-407.

Isbell, R 2002, *The Australian Soil Classification*, 2nd ed. edn, CSIRO PUBLISHING, Melbourne.

Islam, MU, Jiang, F, Guo, Z & Peng, X 2021, 'Does biochar application improve soil aggregation? A meta-analysis', *Soil & tillage research,* vol. 209, pp. 104926.

John, B, Yamashita, T, Ludwig, B & Flessa, H 2005, 'Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use', *Geoderma,* vol. 128, no. 1, pp. 63-79.

Jones, D & Hinsinger, P 2008, 'The rhizosphere: complex by design', *Plant and Soil,* vol. 312, no. 1, pp. 1-6.

Jones, DL, Cooledge, EC, Hoyle, FC, Griffiths, RI & Murphy, DV 2019, 'pH and exchangeable aluminum are major regulators of microbial energy flow and carbon use efficiency in soil microbial communities', *Soil Biology and Biochemistry,* vol. 138.

Juarez, S, Nunan, N, Duday, A-C, Pouteau, V & Chenu, C 2013, 'Soil carbon mineralisation responses to alterations of microbial diversity and soil structure', *Biology and Fertility of Soils,* vol. 49, no. 7, pp. 939-948.

Kaiser, M, Kleber, M & Berhe, AA 2015, 'How air-drying and rewetting modify soil organic matter characteristics: An assessment to improve data interpretation and inference', *Soil Biology and Biochemistry,* vol. 80, pp. 324-340.

Kästner, M & Miltner, A 2018, 'Chapter 5 - SOM and Microbes—What Is Left From Microbial Life', in Garcia, C, Nannipieri, P & Hernandez, T (eds.), *The Future of Soil Carbon*, Academic Press.

Kopittke, PM, Dalal, RC, Finn, D & Menzies, NW 2017, 'Global changes in soil stocks of carbon, nitrogen, phosphorus, and sulphur as influenced by long-term agricultural production', *Global change biology,* vol. 23, no. 6, pp. 2509-2519.

Kumar, R, Kisan Singh, R, Singh, J, Singh, A & Rai, A 2013, 'Soil aggregation dynamics and carbon sequestration', *Journal of Applied and Natural Science,* vol. 5, no. 1, pp. 250-267.

Kuzyakov, Y 2010, 'Priming effects: Interactions between living and dead organic matter', *Soil Biology and Biochemistry,* vol. 42, no. 9, pp. 1363-1371.

Ladan, S & Jacinthe, P-A 2016, 'Evaluation of antibacterial and antifungal compounds for selective inhibition of denitrification in soils', *Environmental Science: Processes & Impacts,* vol. 18, no. 12, pp. 1519-1529.

Lal, R, Hall, G & Miller, F 1989, 'Soil degradation: I. Basic processes', *Land Degradation & Development,* vol. 1, no. 1, pp. 51-69.

Lamparter, A, Bachmann, J, Goebel, MO & Woche, SK 2009, 'Carbon mineralization in soil: Impact of wetting–drying, aggregation and water repellency', *Geoderma,* vol. 150, no. 3, pp. 324-333.

Le Guillou, C, Angers, DA, Maron, PA, Leterme, P & Menasseri-Aubry, S 2012, 'Linking microbial community to soil water-stable aggregation during crop residue decomposition', *Soil Biology and Biochemistry,* vol. 50, pp. 126-133.

Lee, HJ & Roh, HS 2007, 'The use of recycled tire chips to minimize dynamic earth pressure during compaction of backfill', *Construction & building materials,* vol. 21, no. 5, pp. 1016-1026.

Lee, K & Foster, R 1991, 'Soil fauna and soil structure', *Soil Research,* vol. 29, no. 6, pp. 745-775.

Leger, AM, Ball, KR, Rathke, SJ & Blankinship, JC 2022, 'Mulch more so than compost improves soil health to reestablish vegetation in a semiarid rangeland', *Restoration Ecology,* vol. 30, no. 6, pp. e13698.

Lehmann, A, Zheng, W & Rillig, MC 2017, 'Soil biota contributions to soil aggregation', *Nature ecology & evolution,* vol. 1, no. 12, pp. 1828-1835.

Leifeld, J, Bassin, S, Conen, F, Hajdas, I, Egli, M & Fuhrer, J 2013, 'Control of soil pH on turnover of belowground organic matter in subalpine grassland', *Biogeochemistry,* vol. 112, no. 1, pp. 59-69.

Li, J-T, Wang, J-J, Zeng, D-H, Zhao, S-Y, Huang, W-L, Sun, X-K & Hu, Y-L 2018, 'The influence of drought intensity on soil respiration during and after multiple drying-rewetting cycles', *Soil Biology and Biochemistry,* vol. 127, pp. 82-89.

Li, Q, Li, T, Shen, W, Li, H, Li, P, Li, Y & Hou, X 2022, 'Significance of the van der Waals force on the formation of the microstructure of wind-blown loess: An investigation using modified Discontinuous Deformation Analysis', *Computers and Geotechnics,* vol. 148, pp. 104833.

Li, XG, Jia, B, Lv, J, Ma, Q, Kuzyakov, Y & Li, F-M 2017, 'Nitrogen fertilization decreases the decomposition of soil organic matter and plant residues in planted soils', *Soil Biology and Biochemistry,* vol. 112, pp. 47-55.

Liu, B, Fan, H, Jiang, Y & Ma, R 2023, 'Evaluation of soil macro-aggregate characteristics in response to soil macropore characteristics investigated by X-ray computed tomography under freeze-thaw effects', *Soil and Tillage Research,* vol. 225, pp. 105559.

Liu, C, Lu, M, Cui, J, Li, B & Fang, C 2014a, 'Effects of straw carbon input on carbon dynamics in agricultural soils: a meta-analysis', *Global change biology,* vol. 20, no. 5, pp. 1366-1381.

Liu, C, Wang, H, Karim, AM, Sun, J & Wang, Y 2014b, 'Catalytic fast pyrolysis of lignocellulosic biomass', *Chemical Society Reviews,* vol. 43, no. 22, pp. 7594-7623.

Lu, J, Dijkstra, FA, Wang, P & Cheng, W 2019, 'Roots of non-woody perennials accelerated long-term soil organic matter decomposition through biological and physical mechanisms.(Report)', *Soil Biology and Biochemistry,* vol. 134, pp. 42.

Luiz, AD-H, Grace, P, Xiao-Jun Allen, L, Serita, DF, Jerry, MM & Kristen, MD 2020, 'Microbial diversity drives carbon use efficiency in a model soil', *Nature communications,* vol. 11, no. 1, pp. 1-10.

Lynch, JM & Bragg, E 'Microorganisms and Soil Aggregate Stability', New York, NY, Springer New York, pp. 133-171.

Ma, S, Zhu, W, Wang, W, Li, X & Sheng, Z 2023, 'Microbial assemblies with distinct trophic strategies drive changes in soil microbial carbon use efficiency along vegetation primary succession in a glacier retreat area of the southeastern Tibetan Plateau', *Science of The Total Environment,* vol. 867, pp. 161587.

Malik, AA, Puissant, J, Buckeridge, KM, Goodall, T, Jehmlich, N, Chowdhury, S, Gweon, HS, Peyton, JM, Mason, KE & Van Agtmaal, M 2018, 'Land use driven change in soil pH affects microbial carbon cycling processes', *Nature communications,* vol. 9, no. 1, pp. 1-10.

Martin, JP, Martin, WP, Page, JB, Raney, WA & De Ment, JD 1955, 'Soil Aggregation', in Norman, AG (ed.), *Advances in Agronomy*, Academic Press.

Materechera, SA, Kirby, JM, Alston, AM & Dexter, AR 1994, 'Modification of soil aggregation by watering regime and roots growing through beds of large aggregates', *Plant and Soil,* vol. 160, no. 1, pp. 57-66.

Mcgee, K, Eaton, W, Shokralla, S & Hajibabaei, M 2019, 'Determinants of Soil Bacterial and Fungal Community Composition Toward Carbon-Use Efficiency Across Primary and Secondary Forests in a Costa Rican Conservation Area', *Microbial Ecology,* vol. 77, no. 1, pp. 148-167.

Mehnaz, KR, Corneo, PE, Keitel, C & Dijkstra, FA 2019, 'Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen mineralization and nitrous oxide emission from soil', *Soil Biology and Biochemistry,* vol. 134, pp. 175-186.

Mikha, MM, Rice, CW & Milliken, GA 2005, 'Carbon and nitrogen mineralization as affected by drying and wetting cycles', *Soil Biology and Biochemistry,* vol. 37, no. 2, pp. 339-347.

Miransari, M 2011, *Soil microbes and environmental health*, Nova Science Publishers, Inc., Hauppauge, N.Y.

Mo, F, Ren, C, Yu, K, Zhou, Z, Phillips, RP, Luo, Z, Zhang, Y, Dang, Y, Han, J, Ye, J-S, Vinay, N, Liao, Y, Xiong, Y & Wen, X 2022, 'Global pattern of soil priming effect intensity and its environmental drivers', *Ecology,* vol. 103, no. 11, pp. e3790.

Na, M, Hicks, LC, Zhang, Y, Shahbaz, M, Sun, H & Rousk, J 2022, 'Semi-continuous C supply reveals that priming due to N-mining is driven by microbial growth demands in temperate forest plantations', *Soil Biology and Biochemistry,* vol. 173, pp. 108802.

Naga Raju, M, Golla, N & Vengatampalli, R 2017, 'Soil Microbiological Properties', in Maddela, NR, Golla, N & Vengatampalli, R (eds.), *Soil Enzymes: Influence of Sugar Industry Effluents on Soil Enzyme Activities*, Springer International Publishing, Cham.

Najera, F, Dippold, MA, Boy, J, Seguel, O, Koester, M, Stock, S, Merino, C, Kuzyakov, Y & Matus, F 2020, 'Effects of drying/rewetting on soil aggregate dynamics and implications for organic matter turnover', *Biology and Fertility of Soils,* vol. 56, no. 7, pp. 893-905.

Nandan, R, Singh, V, Singh, SS, Kumar, V, Hazra, KK, Nath, CP, Poonia, S, Malik, RK, Bhattacharyya, R & Mcdonald, A 2019, 'Impact of conservation tillage in rice–based cropping systems on soil aggregation, carbon pools and nutrients', *Geoderma,* vol. 340, pp. 104-114.

Oades, J 1984, 'Soil organic matter and structural stability: mechanisms and implications for management', *Plant and Soil,* vol. 76, no. 1, pp. 319-337.

Oades, JM 1993, 'The role of biology in the formation, stabilization and degradation of soil structure', *Geoderma,* vol. 56, no. 1-4, pp. 377-400.

Olagoke, FK, Bettermann, A, Nguyen, PTB, Redmile-Gordon, M, Babin, D, Smalla, K, Nesme, J, Sørensen, SJ, Kalbitz, K & Vogel, C 2022, 'Importance of substrate quality and clay content on microbial extracellular polymeric substances production and aggregate stability in soils', *Biology and Fertility of Soils,* vol. 58, no. 4, pp. 435-457.

Olagoke, FK, Kalbitz, K & Vogel, C 2019, 'Control of Soil Extracellular Enzyme Activities by Clay Minerals—Perspectives on Microbial Responses', *Soil Systems,* vol. 3, no. 4, pp. 64.

Pantigoso, HA, Newberger, D & Vivanco, JM 2022, 'The rhizosphere microbiome: Plant–microbial interactions for resource acquisition', *Journal of Applied Microbiology,* vol. 133, no. 5, pp. 2864-2876.

Poirier, V, Roumet, C, Angers, DA & Munson, AD 2018, 'Species and root traits impact macroaggregation in the rhizospheric soil of a Mediterranean common garden experiment', *Plant and soil,* vol. 424, no. 1/2, pp. 289-302.

Pronk, GJ, Heister, K, Vogel, C, Babin, D, Bachmann, J, Ding, G-C, Ditterich, F, Gerzabek, MH, Giebler, J, Hemkemeyer, M, Kandeler, E, Kunhi Mouvenchery, Y, Miltner, A, Poll, C, Schaumann, GE, Smalla, K, Steinbach, A, Tanuwidjaja, I, Tebbe, CC, Wick, LY, Woche, SK, Totsche, KU, Schloter, M & Kögel-Knabner, I 2017, 'Interaction of minerals, organic matter, and microorganisms during biogeochemical interface formation as shown by a series of artificial soil experiments', *Biology and Fertility of Soils,* vol. 53, no. 1, pp. 9-22.

Qiao, Y, Wang, J, Liang, G, Du, Z, Zhou, J, Zhu, C, Huang, K, Zhou, X, Luo, Y, Yan, L & Xia, J 2019, 'Global variation of soil microbial carbon-use efficiency in relation to growth temperature and substrate supply', *Scientific Reports,* vol. 9, no. 1.

Rabot, E, Wiesmeier, M, Schlüter, S & Vogel, HJ 2018, 'Soil structure as an indicator of soil functions: A review', *Geoderma,* vol. 314, pp. 122-137.

Rahman, MT, Liu, S, Guo, ZC, Zhang, ZB & Peng, XH 2019, 'Impacts of residue quality and N input on aggregate turnover using the combined 13C natural abundance and rare earth oxides as tracers', *Soil & tillage research,* vol. 189, pp. 110-122.

Rakshit, A, Sarkar, B & Abhilash, P 2018, *Soil Amendments for Sustainability : Challenges and Perspectives*, First edition. edn, CRC Press, Boca Raton, FL.

Römkens, MJM, Helming, K & Prasad, SN 2002, 'Soil erosion under different rainfall intensities, surface roughness, and soil water regimes', *CATENA,* vol. 46, no. 2, pp. 103-123.

Rousk, J, Brookes, PC & Bååth, E 2010, 'Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil', *Soil Biology and Biochemistry,* vol. 42, no. 6, pp. 926-934.

Sarkar, B, Singh, M, Mandal, S, Churchman, GJ & Bolan, NS 2018, 'Chapter 3 - Clay Minerals—Organic Matter Interactions in Relation to Carbon Stabilization in Soils', in Garcia, C, Nannipieri, P & Hernandez, T (eds.), *The Future of Soil Carbon*, Academic Press.

Sarker, TC, Zotti, M, Fang, Y, Giannino, F, Mazzoleni, S, Bonanomi, G, Cai, Y & Chang, SX 2022, 'Soil Aggregation in Relation to Organic Amendment: a Synthesis', *Journal of Soil Science and Plant Nutrition,* vol. 22, no. 2, pp. 2481-2502.

Semmel, H, Horn, R, Hell, U, Dexter, AR & Schulze, ED 1990, 'The dynamics of soil aggregate formation and the effect on soil physical properties', *Soil Technology,* vol. 3, no. 2, pp. 113-129.

Shoshany, M, Goldshleger, N & Chudnovsky, A 2013, 'Monitoring of agricultural soil degradation by remote-sensing methods: a review', *International journal of remote sensing,* vol. 34, no. 17, pp. 6152-6181.

Shubha, K, Singh, NR, Mukherjee, A, Maity, A & Dubey, R 2020, 'Controlled Traffic Farming: An Approach to Minimize Soil Compaction and Environmental Impact on Vegetable and Other Crops', *Current science (Bangalore),* vol. 119, no. 11, pp. 1760.

Siedt, M, Schäffer, A, Smith, KEC, Nabel, M, Roß-Nickoll, M & Van Dongen, JT 2021a, 'Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides', *The Science of the total environment,* vol. 751, pp. 141607-141607.

Siedt, M, Schäffer, A, Smith, KEC, Nabel, M, Roß-Nickoll, M & Van Dongen, JT 2021b, 'Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil

structure, nutrient leaching, microbial communities, and the fate of pesticides', *Science of The Total Environment,* vol. 751, pp. 141607.

Silva-Sánchez, A, Soares, M & Rousk, J 2019, 'Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality', *Soil Biology and Biochemistry,* vol. 134, pp. 25-35.

Sinsabaugh, RL, Manzoni, S, Moorhead, DL & Richter, A 2013, 'Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling', *Ecology Letters,* vol. 16, no. 7, pp. 930-939.

Six, J, Bossuyt, H, Degryze, S & Denef, K 2004, 'A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics', *Soil & Tillage Research,* vol. 79, no. 1, pp. 7-31.

Six, J, Conant, RT, Paul, EA & Paustian, K 2002, 'Stabilization mechanisms of soil organic matter: implications for C-saturation of soils', *Plant and soil,* vol. 241, no. 2, pp. 155-176.

Six, J, Elliott, ET & Paustian, K 2000, 'Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture', *Soil Biology and Biochemistry,* vol. 32, no. 14, pp. 2099-2103.

Six, J, Frey, SD, Thiet, RK & Batten, KM 2006, 'Bacterial and fungal contributions to carbon sequestration in agroecosystems.(Author abstract)', *Soil Science Society of America Journal,* vol. 70, no. 2, pp. 555.

Soares, M, Kritzberg, ES & Rousk, J 2017, 'Labile carbon ‘primes’ fungal use of nitrogen from submerged leaf litter', *FEMS Microbiology Ecology,* vol. 93, no. 9, pp. fix110.

Soares, M & Rousk, J 2019, 'Microbial growth and carbon use efficiency in soil: links to fungal-bacterial dominance, SOC-quality and stoichiometry', *Soil Biology and Biochemistry,* vol. 131, pp. 195-205.

Sokol, NW, Sanderman, J & Bradford, MA 2019, 'Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry', *Global change biology,* vol. 25, no. 1, pp. 12-24.

Spohn, M, Pötsch, EM, Eichorst, SA, Woebken, D, Wanek, W & Richter, A 2016, 'Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland', *Soil Biology and Biochemistry,* vol. 97, no. C, pp. 168-175.

Stewart, CE, Paustian, K, Conant, RT, Plante, AF & Six, J 2007, 'Soil carbon saturation: concept, evidence and evaluation', *Biogeochemistry,* vol. 86, no. 1, pp. 19-31.

Stewart, CE, Plante, AF, Paustian, K, Conant, RT & Six, J 2008, 'Soil Carbon Saturation: Linking Concept and Measurable Carbon Pools', *Soil Science Society of America journal,* vol. 72, no. 2, pp. 379-392.

Tang, J, Mo, Y, Zhang, J & Zhang, R 2011, 'Influence of biological aggregating agents associated with microbial population on soil aggregate stability', *Applied Soil Ecology,* vol. 47, no. 3, pp. 153-159.

Tisdall, JM & Oades, JM 1982, 'Organic matter and water-stable aggregates in soils', *Journal of Soil Science,* vol. 33, no. 2, pp. 141-163.

Totsche, KU, Amelung, W, Gerzabek, MH, Guggenberger, G, Klumpp, E, Knief, C, Lehndorff, E, Mikutta, R, Peth, S & Prechtel, A 2018, 'Microaggregates in soils', *Journal of Plant Nutrition and Soil Science,* vol. 181, no. 1, pp. 104-136.

Ullah, MR, Carrillo, Y & Dijkstra, FA 2021a, 'Biocides provide a source of carbon and nitrogen directly to surviving microbes and indirectly through a pulse in microbial necromass', *Applied Soil Ecology,* vol. 160, pp. 103862.

Ullah, MR, Carrillo, Y & Dijkstra, FA 2021b, 'Drought-induced and seasonal variation in carbon use efficiency is associated with fungi:bacteria ratio and enzyme production in a grassland ecosystem', *Soil biology & biochemistry,* vol. 155, pp. 108159.

Ullah, MR, Carrillo, Y & Dijkstra, FA 2023, 'Relative contributions of fungi and bacteria to litter decomposition under low and high soil moisture in an Australian grassland', *Applied Soil Ecology,* vol. 182, pp. 104737.

Védère, C, Lebrun, M, Honvault, N, Aubertin, M-L, Girardin, C, Garnier, P, Dignac, M-F, Houben, D & Rumpel, C 2022, 'How does soil water status influence the fate of soil organic matter? A review of processes across scales', *Earth-Science Reviews,* vol. 234, pp. 104214.

Von Lützow, M, Kögel-Knabner, I, Ekschmitt, K, Flessa, H, Guggenberger, G, Matzner, E & Marschner, B 2007, 'SOM fractionation methods: relevance to functional pools and to stabilization mechanisms', *Soil Biology and Biochemistry,* vol. 39, no. 9, pp. 2183-2207.

Walia, MK & Dick, WA 2018, 'Selected soil physical properties and aggregate-associated carbon and nitrogen as influenced by gypsum, crop residue, and glucose', *Geoderma,* vol. 320, pp. 67-73.

Wang, C, Qu, L, Yang, L, Liu, D, Morrissey, E, Miao, R, Liu, Z, Wang, Q, Fang, Y & Bai, E 2021, 'Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon', *Global change biology,* vol. 27, no. 10, pp. 2039-2048.

Wang, L, Zuo, X, Zheng, F, Wilson, GV, Zhang, XJ, Wang, Y & Fu, H 2020a, 'The effects of freeze-thaw cycles at different initial soil water contents on soil erodibility in Chinese Mollisol region', *Catena (Giessen),* vol. 193, pp. 104615.

Wang, Q, Wang, S, He, T, Liu, L & Wu, J 2014, 'Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils', *Soil Biology and Biochemistry,* vol. 71, pp. 13-20.

Wang, X, Sale, P, Hayden, H, Tang, C, Clark, G & Armstrong, R 2020b, 'Plant roots and deep-banded nutrient-rich amendments influence aggregation and dispersion in a dispersive clay subsoil', *Soil Biology and Biochemistry,* vol. 141, pp. 107664.

Wang, X, Yin, L, Dijkstra, FA, Lu, J, Wang, P & Cheng, W 2020c, 'Rhizosphere priming is tightly associated with root-driven aggregate turnover', *Soil Biology and Biochemistry,* vol. 149, pp. 107964.

Wright, DA, Killham, K, Glover, LA & Prosser, JI 1995, 'Role of Pore Size Location in Determining Bacterial Activity during Predation by Protozoa in Soil', *Applied and Environmental Microbiology,* vol. 61, no. 10, pp. 3537.

Xia, D, Zhao, B, Liu, D, Deng, Y, Cheng, H, Yan, Y, Ding, S & Cai, C 2018, 'Effect of soil moisture on soil disintegration characteristics of different weathering profiles of collapsing gully in the hilly granitic region, South China', *Plos one,* vol. 13, no. 12, pp. e0209427.

Xiao, L, Yao, K, Li, P, Liu, Y, Chang, E, Zhang, Y & Zhu, T 2020a, 'Increased soil aggregate stability is strongly correlated with root and soil properties along a gradient of secondary succession on the Loess Plateau', *Ecological Engineering,* vol. 143, pp. 105671.

Xiao, L, Yao, K, Li, P, Liu, Y & Zhang, Y 2020b, 'Effects of freeze-thaw cycles and initial soil moisture content on soil aggregate stability in natural grassland and Chinese pine forest on the Loess Plateau of China', *Journal of soils and sediments,* vol. 20, no. 3, pp. 1222-1230.

Xu, Y, Liu, K, Yao, S, Zhang, Y, Zhang, X, He, H, Feng, W, Ndzana, GM, Chenu, C, Olk, DC, Mao, J & Zhang, B 2022, 'Formation efficiency of soil organic matter from plant litter is governed by clay mineral type more than plant litter quality', *Geoderma,* vol. 412, pp. 115727.

Xu, Y, Seshadri, B, Sarkar, B, Rumpel, C, Sparks, D & S. Bolan, N 2018, 'Chapter 6 - Microbial Control of Soil Carbon Turnover', in Garcia, C, Nannipieri, P & Hernandez, T (eds.), *The Future of Soil Carbon*, Academic Press.

Yan, S, Yin, L, Dijkstra, FA, Wang, P & Cheng, W 2023, 'Priming effect on soil carbon decomposition by root exudate surrogates: A meta-analysis', *Soil Biology and Biochemistry,* vol. 178.

Yang, C, Li, J & Zhang, Y 2019, 'Soil aggregates indirectly influence litter carbon storage and release through soil pH in the highly alkaline soils of north China', *PeerJ,* vol. 7, pp. e7949-e7949.

Yanni, SF, Whalen, JK, Simpson, MJ & Janzen, HH 2011, 'Plant lignin and nitrogen contents control carbon dioxide production and nitrogen mineralization in soils incubated with Bt and non-Bt corn residues', *Soil biology & biochemistry,* vol. 43, no. 1, pp. 63-69.

Zhang, X, Xin, X, Zhu, A, Yang, W, Zhang, J, Ding, S, Mu, L & Shao, L 2018, 'Linking macroaggregation to soil microbial community and organic carbon accumulation under different tillage and residue managements', *Soil & tillage research,* vol. 178, pp. 99-107.

Zhang, Z, Wang, D & Li, M 2022, 'Soil respiration, aggregate stability and nutrient availability affected by drying duration and drying-rewetting frequency', *Geoderma,* vol. 413, pp. 115743.

Zhang, Z, Zhu, Z, Shen, B & Liu, L 2019, 'Insights into biochar and hydrochar production and applications: A review', *Energy (Oxford),* vol. 171, pp. 581-598.

Chapter 3: The influence of plant residues on soil aggregation and carbon storage potential: a meta-analysis

Abstract

Background: Soil aggregation and organic carbon (OC) content are important indicators of soil quality that can be improved with plant residue amendments. The extent of the effects of plant residue amendments on soil aggregation and OC content across different plant residue and soil types is not fully understood.

Aim: In this meta-analysis, we evaluated the effects of plant residue amendments on soil aggregation and OC content for different plant residues (fresh, charred) and soil types varying in clay content, initial OC content, and pH.

Methods: Our meta-analysis included 50 published studies (total of 299 paired observations). We estimated the response ratios of mean weight diameter (MWD) and separate aggregate size classes, total soil OC (TSC), and aggregates-associated OC. We also considered the effect of experimental factors (study duration, residue type, residue amount, initial soil OC, clay content, and pH).

Results: The benefit of plant residue amendment on soil aggregation was larger in soils with initially low OC content and neutral pH. Initial soil OC content and pH were more important than soil clay content for OC storage in soil aggregates. Both fresh and charred plant residue amendments were effective in forming aggregates, whereas charred residues were more effective in increasing TSC. We found only a weak positive relationship between the response ratio of TSC and MWD indicating that other factors besides soil aggregation contributed to the increase in soil C storage.

Conclusions: While plant residue amendments can enhance soil aggregation and TSC, these effects are likely governed by the type of plant residue and soil properties such as the initial soil pH and OC content.

Key words: Carbon sequestration, Crop residue, Soil amendments, Soil structure.

3.1 Introduction

Soil restoration and sustainability has been studied extensively in the recent decades due to growing concerns about the extent of soil degradation and erosion caused by extensive agriculture practices. Agricultural practices such as physical soil disturbance through tillage and plant biomass removal and associated erosion, have resulted in depletion of soil organic carbon (OC) worldwide (Guo and Gifford (2002); (Kopittke et al., 2017), with much of the C lost to the atmosphere. This has contributed to global warming but has also come at the expense of other soil functions in supporting food production and maintaining water quality and filtration (Heneghan et al., 2008; Rakshit, Sarkar & Abhilash, 2018; Shoshany, Goldshleger & Chudnovsky, 2013). It will therefore be critical to enhance soil C stabilization in agricultural soils worldwide.

An important mechanism to stabilize OC in soil is through soil aggregation (Six et al., 2002; von Lützow et al., 2007). Indeed, aggregate formation requires OC to bind soil particles (such as sand, silt, and clay) (Martin et al., 1955), where the primary source of OC is from plant residues (Angers & Caron, 1998) and rhizodeposition (Hütsch, Augustin & Merbach, 2002). Soil aggregation can be considered a hierarchy of smaller aggregates (or microaggregates) that act as building units for larger aggregates (macroaggregates) (Tisdall & Oades, 1982; Totsche et al., 2018). The microaggregates incorporate OC mostly from microbial products through organo-mineral complexes (Gul & Whalen, 2022), while roots, fungal hyphae, and intact plant residues play an

important role in the formation of macroaggregates (John et al., 2005; Totsche et al., 2018). Soil aggregates can directly protect OC from microbial decomposition, but also indirectly through limiting oxygen diffusion, regulating water flow, and reducing run-off and erosion (Six et al., 2004). However, physical disturbance or biological activity such as from plant root penetration, water, and soil microbiota can also result in C exposure and potential loss through microbial decomposition (Angers & Caron, 1998; Baldock & Skjemstad, 2000; Ebrahimi & Or, 2015; Lynch & Bragg, 1985).

Plant residue amendment to soil is an important way to enhance soil aggregation, either by direct application, composting, or by turning the plant residues into char or biochar before applying to the soil (Rakshit, Sarkar & Abhilash, 2018; Siedt et al., 2021b). Yet, the processes by which different types and amounts of plant residues influence soil aggregation are still not fully understood. Plant residues differ in physical and chemical characteristics that could strongly influence soil aggregation (Blanco-Canqui & Lal, 2004; Samahadthai et al., 2010; Tobiašová 2011). For instance, residues that are relatively poor in quality and have a low decomposability (e.g., high C:N ratio, high lignin content) can persist longer in soil and may therefore have a greater potential to improve soil aggregation (Blanco-Canqui & Lal, 2004). Others have shown that cellulose and soluble sugars in plant residues are important ingredients of soil aggregation (Halder et al., 2021; Tobiašová 2011). Furthermore, plant residue characteristics affect the degree of bacterial versus fungal processing of plant residues, which could further influence soil aggregation (Helfrich et al., 2008). Plant residues can also be converted into biochar before they are added to the soil with potential consequences for soil aggregation. However, the few studies where effects of biochar and fresh plant residues on soil aggregation were compared are inconsistent. For example, Bai et al. (2019) observed that both straw and straw-derived biochar in a rice-wheat

rotation system increased soil aggregation, although biochar amendments had slightly stronger effects deeper in the soil profile. On the other hand, Yoo et al. (2017) observed that the effect of biochar made from corn residues disappeared after 60 days, while Zhang et al. (2017) observed no effect on soil aggregation with addition of either wheat straw or addition of wheat straw-derived biochar to soil. Clearly, a better understanding is needed about plant residue effects on soil aggregation and associated soil OC for different residue types.

The effect of plant residue amendment on soil aggregation and OC sequestration may further depend on soil and environmental factors. An important soil factor affecting aggregate formation and stabilization is the amount of clay in the soil matrix (De Gryze et al., 2005; Olagoke et al., 2022). For instance, the production of extracellular polymeric substances produced by microbes can be influenced by clay content, thereby affecting soil aggregate stability (Olagoke et al., 2022), while the amount and type of clay mineral could further influence protection of OC within aggregates (Angst et al., 2021; Xu et al., 2022). Interestingly, in a previous meta-analysis, Liu et al. (2014a) observed a significant negative relationship between clay content and the increase in soil OC in response to straw return, which they attributed to slower transformation of straw into OC in soils with more clay. Soil pH could indirectly influence soil aggregation by affecting microbial activity and diversity that influence soil OC turnover and microbial C use efficiency, and thus how much OC remains in the soil to form aggregates (Jones et al., 2019). Soil pH could also influence plant growth and litter production (Wang et al., 2021b), and therefore the amount of plant residues added to the soil in subsequent rotations involved in forming soil aggregates. Another important soil factor that could influence the efficiency by which plant residue amendments could affect soil aggregation and OC sequestration is the initial amount of soil OC present. Soils with low soil OC content are often poorly structured and therefore have greater

potential for soil aggregation and OC sequestration, particularly when these soils are still unsaturated with OC (Castellano et al., 2015; Stewart et al., 2008). Besides these soil factors, environmental factors such as temperature and moisture can have strong effects on soil aggregation (Guan et al., 2018; Wang et al., 2012). Nevertheless, understanding of soil aggregate formation and associated OC storage with plant residue amendments for a wide array of soil types and environmental conditions remains limited.

In most studies only specific plant residue types (*e.g.,* fresh residue *vs* charred residue) applied in specific amounts to specific soil types have been investigated, usually for a single climate, with locally available residues and specific duration only. While several reviews and meta-analyses have been conducted assessing the effects of tillage and biochar on soil aggregation and/or soil OC (*e.g.*, Gul et al., 2015; Islam et al 2021; Li et al., 2019; Lorenz & Lal, 2014; Omondi et al 2016), here we conducted a metanalysis, where we aimed to assess the effect of the amount, type, origin, and duration of plant residue amendments on soil aggregation and, soil OC (both the total soil OC (TSC) and OC in individual aggregate size classes), and the role of soil parameters (clay content, initial OC content, pH). Although inorganic fertilizers can also influence decomposition of plant residues (e.g., Li et al. (2017), due to a limited number of observations, here we focus on soil parameters only. We hypothesized that plant residue soil amendment will enhance the formation of macroaggregates and microaggregates and will increase OC storage in the soil (TSC and aggregate-associated OC), particularly for fresh residues, and in soils with high clay content, high soil pH, low initial OC content, and with increased duration.

3.2 Materials and methods

3.2.1 Data collection

Data sets for the effect of plant residue soil amendment on aggregation and soil C were sourced from literature published in 2021 or earlier. The search for literature was conducted by searching the combination of the following terms: “residue”, “aggregate”, “soil”, and “carbon” in the title, abstract or key words using the database Scopus.

The selection of studies was limited to the following criteria: studies on soil only (excluding studies on mining residues), studies with a control without any plant residue soil amendment, studies recording results on aggregation of the following sizes: macroaggregates (>250 μm), microaggregates (250-53 μm), and silt and clay fraction <53 μm and/or studies reporting mean weight diameter (MWD) of aggregates and/or water stable aggregates (WSA) from both laboratory and field studies. Reviews and modeling studies were excluded.

The final data set included 50 research papers with a total of 299 paired observations (Supplementary Information). Studies covered various climate zones from 6 continents and 14 countries (Figure 3.1). Most studies used for our metanalysis were in China and India.

3.2.2 Aggregation and soil carbon data collection and calculations

Data (mean, standard error or standard deviation, and number of replicates for control and plant residue soil amendment treatments) were extracted when presented in the text, in tables or in graphs within the publications or in supplementary documents. For macroaggregates, microaggregates, and the silt and clay fraction, values were expressed as a percentage of the total soil weight. GetData Graph Digitizer 2.26, Germany software was used to extract data from graphs.

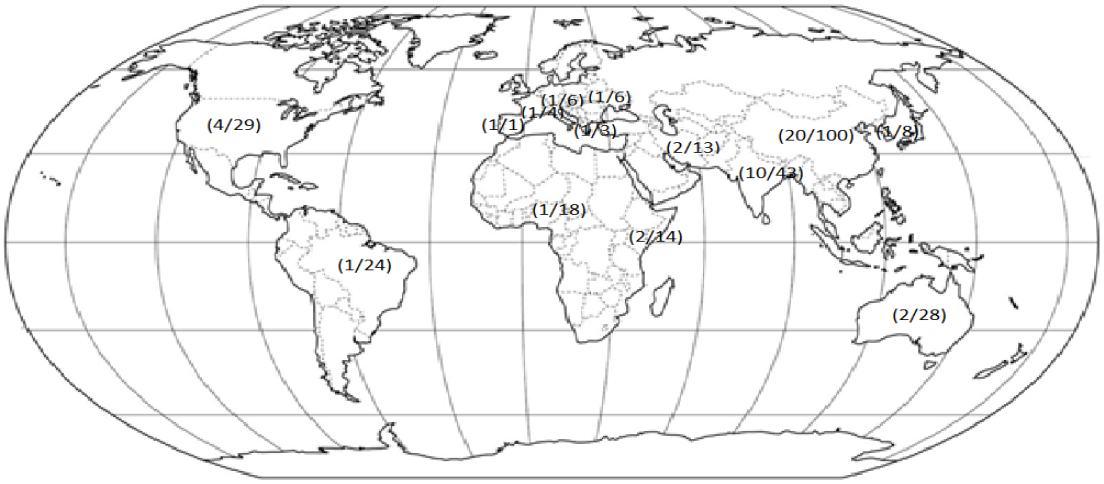

Figure 3.1: Map of global distribution of selected studies and observation (# of studies/# of observations) used in this metanalysis. Map source: https://worldmapblank.com/blank-map-of-world/

We recorded the type (fresh residue *vs* char), origin of fresh residue, amount and duration of plant residues applied. We directly extracted clay % when reported, and we estimated the clay % using the USDA soil textural triangle for studies that only reported soil textural class. We directly extracted TSC when reported, or it was calculated for studies reporting both percentage of aggregates and their associated OC according to equation (1):

$$TSC = (A1\% * A1_C + A2* A2_C + A3\% * A3_C) / (A1 + A2 + A3) \quad (1)$$

where A1%, A2%, A3% are the percentages of each aggregate size, $A1_C$, $A2_C$, $A3_C$ are the concentration of OC in each aggregate size. We further recorded initial soil OC, pH and mean annual temperature (field studies only) when reported. We decided not to record mean annual precipitation, because in most cases we were unclear whether field studies were supplemented with irrigation.

3.2.3 Meta-analysis

A metanalysis was conducted using the software MetaWin (Version 2.1) for 9 parameters: MWD, WSA, macroaggregates, microaggregates, silt and clay fraction, TSC, and OC associated with each of the 3 targeted aggregate sizes. In this metanalysis, 8 experimental factors were considered for each parameter: experimental mode (field, laboratory), study duration (short: ≤ 3 years, long: > 3 years), residue amount (low: ≤ 10 t ha, high: > 10 t ha^{-1}), residue class (char, fresh plant residue), clay % (low: ≤ 20 %, high: > 20 %), initial soil C (low: ≤ 1 %, high: > 1 %), and soil pH (low: ≤ 6,neutral: 6 - 7.5, high: ≥ 7.5). We further subdivided fresh plant residue by origin (sugarcane, rice, maize, wheat, barley, alfalfa, and other (mostly a mixture of different residues, often containing one or more of the 6 identified residues earlier). For MWD, aggregates size classes, and TSC we examined whether effects varied with experimental mode, duration, residue amount, residue class, residue origin (MWD and TSC only), clay content, soil total OC and pH. We were unable to do this for other parameters, because there were not enough observations to provide robust results. In addition to the metanalysis, we investigated the relationships between some parameters and the TSC (MWD vs TSC, macroaggregates vs TSC, microaggregates vs TSC, and silt and clay fraction vs TSC). The natural log of the response ratio (lnRR) of plant residue soil amendment for each of the 9 parameters was calculated according to equation (2):

$$\ln RR = \ln (\mu_T/\mu_C) \quad (2)$$

where μ_T and μ_C are the parameter mean values for treatment and control, respectively. For collected data with no reported standard error or standard deviation (σ) we estimated σ by first calculating the coefficient of variation (CV) for observations where σ was reported (equation (3)):

$$CV = \sigma / \mu \quad (3)$$

We then calculated an average CV across all observations, and multiplied this average CV with the reported mean (μ) to estimate σ for all other observations. To calculate variance of lnRR (Var lnRR) we used equation (4):

$$\text{Var lnRR} = \sigma_C^2 / (n_C \mu_C^2) + \sigma_T^2 / (n_T \mu_T^2) \quad (4)$$

Where σ_C and σ_T are the standard deviations of control and treatment, respectively, n_C and n_T are the number of replicates of the control and treatment, respectively. For studies with more than one observation, we adjusted each Var lnRR by multiplying with the number of observations within each study (van Groenigen et al., 2011).

First, we calculated mean response ratios (lnRR) across all observations and generated 95% bootstrapped confidence intervals (CI) using the random-effects model in MetaWin. For bootstrapping, we used 4999 iterations. We used the inverse of the adjusted variance as the weighting factor (Hedges & Olkin, 1985; van Groenigen et al., 2011). Treatment effects were considered significant if the 95% CI did not overlap with zero. We further examined differences among categories using the $Q_{between}$ statistics (heterogeneity in effect sizes associated to differences between categories). Differences between categories were considered significant at $P_{random} < 0.05$.

To examine relationships of the lnRR of TSC with the lnRR of MWD, and fractions of macroaggregates, microaggregates, and silt and clay, and the relationships of the mean annual temperature with lnRR of MWD, lnRR of fractions of macroaggregates, microaggregates, and silt and clay, and lnRR of TSC, we ran linear regressions using SigmaPlot (version 10.0.1, Systat Software, San Jose, CA).

3.3 Results

Overall, there was a positive response for most of assessed soil parameters (MWD, WSA, macroaggregates, TSC, and OC in the three sizes of aggregates) towards the plant residue amendments (Figure 3.2). The highest observed positive response towards amendments was for WSA (mean lnRR= 0.5), although it showed high Cls due to the relatively small sample size (n=32). Because plant residue amendments increased the fraction of macroaggregates, this resulted in a decrease in the microaggregate and silt and clay fractions. However, the effect of plant residue amendments on OC concentration was generally similar between the different aggregate size classes. The smallest response of OC concentration was for the silt and clay fraction, whereas macro- and microaggregates showed a similar response.

The effect of plant residue amendment on MWD was significantly larger in laboratory studies than in field studies ($p=0.0002$, Figure 3.3). Effects were larger in short-than in long-term studies, although this was not significant ($p=0.23$). Not surprisingly, effects on MWD were more pronounced with higher amounts of plant residue applied ($p=0.0006$), while MWD increased both with applications of fresh plant residue and char (somewhat higher for plant residues, but this was not significant). The effect on MWD was largest with fresh residues of alfalfa and barley, and smallest with wheat residue. Effects of plant residue amendment on MWD were further largest in soils with high clay content ($p=0.0002$), low initial OC ($p=0.0014$) and neutral pH ($p=0.0002$).

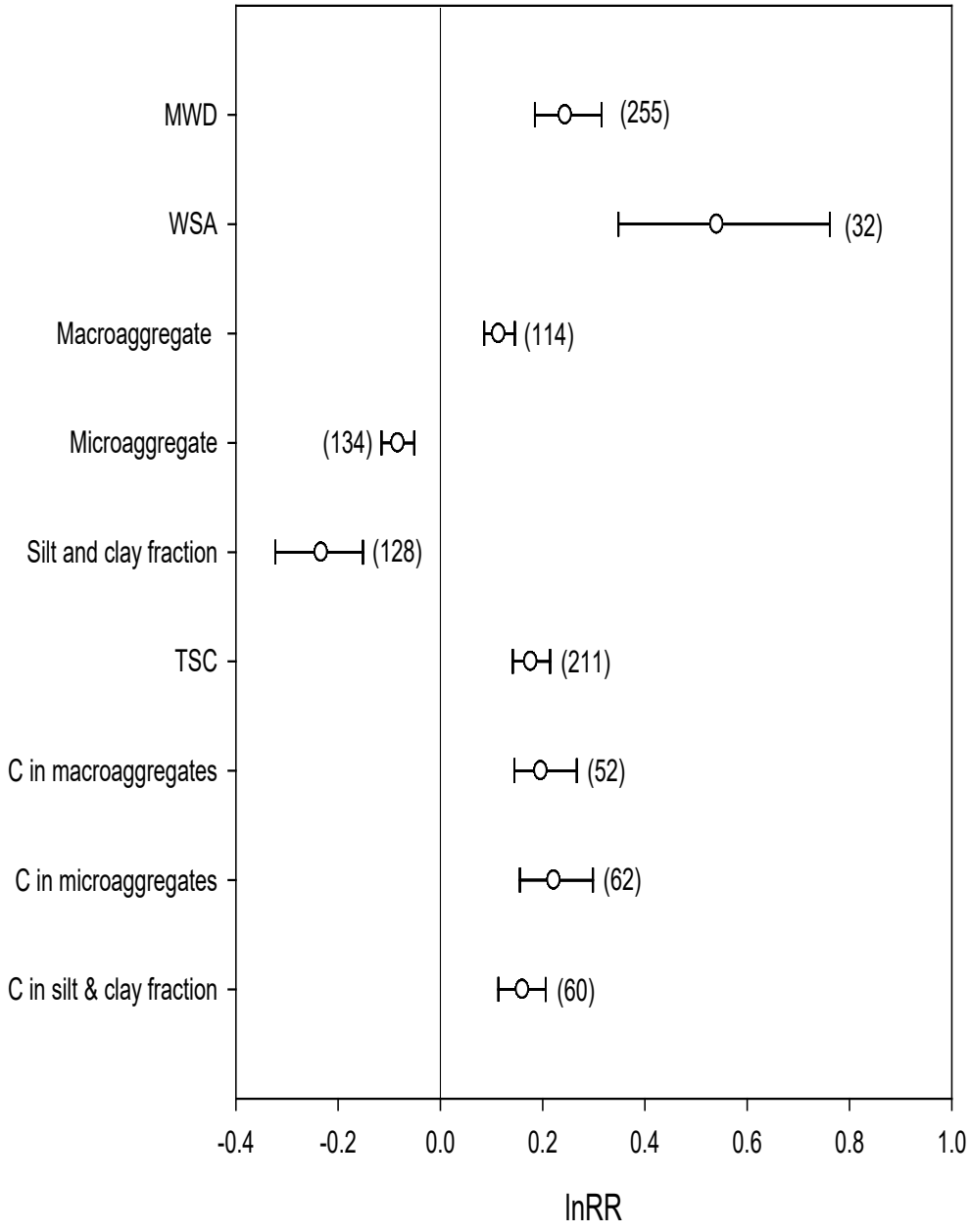

Figure 3.2: Response ratios (lnRR) of mean weight diameter (MWD), water stable aggregates (WSA), macroaggregates, microaggregates, silt and clay fraction, total soil carbon (TSC), C in macroaggregates, C in microaggregates and C in silt and clay fraction, to plant residue amendments. Error bars represent 95 % confidence intervals, (n) is the number of observations.

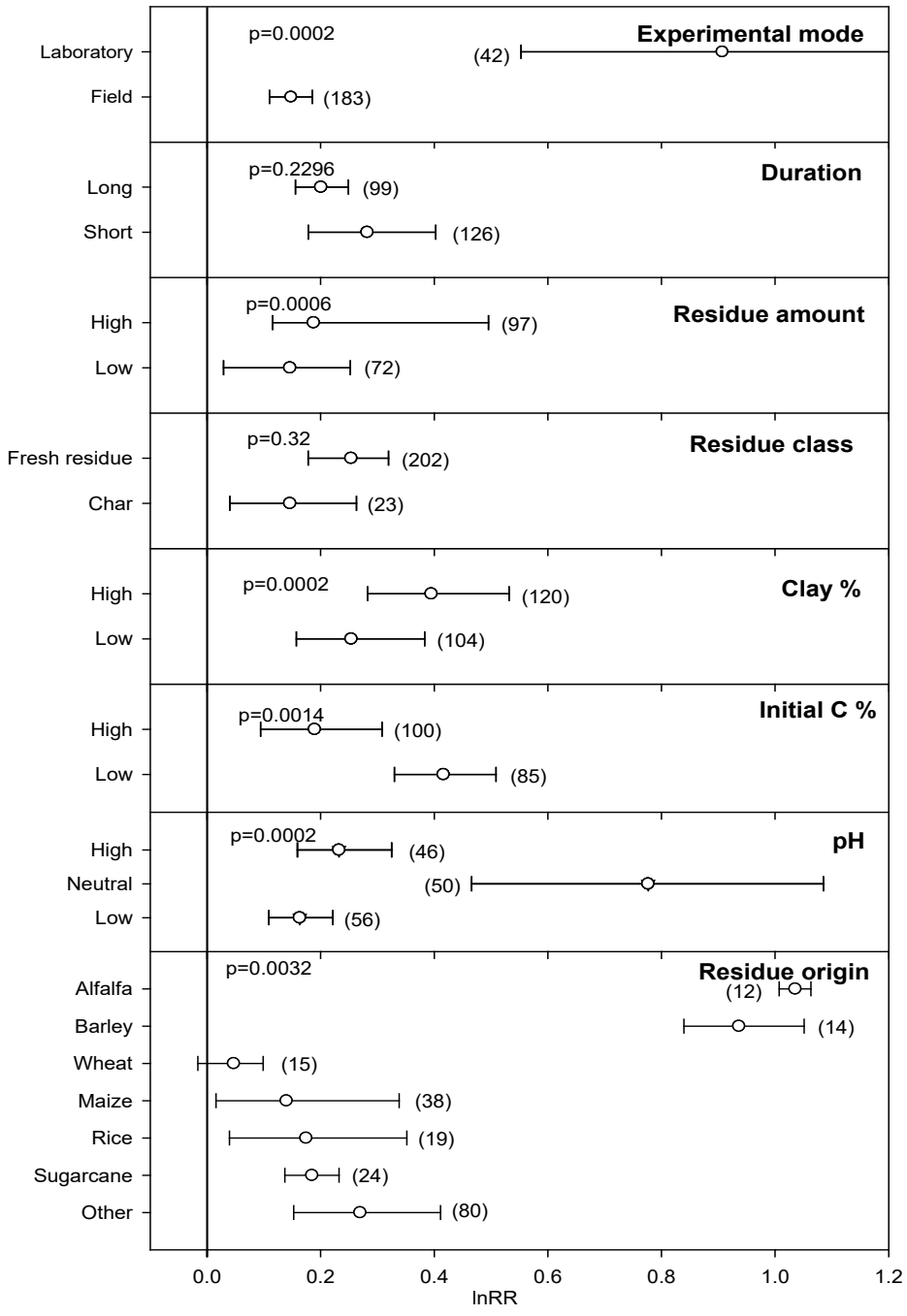

Figure 3.3: The effect of plant residue amendments on mean weigh diameter (MWD) for different experimental conditions and soil factors (experimental mode, duration, residue amount, residue class, clay %, initial C % and pH). Error bars represent 95% confidence intervals.

The plant residue amendment effects on the macroaggregate fraction were positive across all experimental and soil factors (Figure 3.4a), and mostly negative on the microaggregate and silt and clay fraction (Figure 3.4, b, c). However, the effects were mostly not significantly different among categories within each of the experimental and soil factors (Figure 3.4a, b, c). Only initial soil OC content showed a significant effect on soil macroaggregates (increased more with low initial soil OC), and clay content on soil microaggregates (decreased more with high clay content).

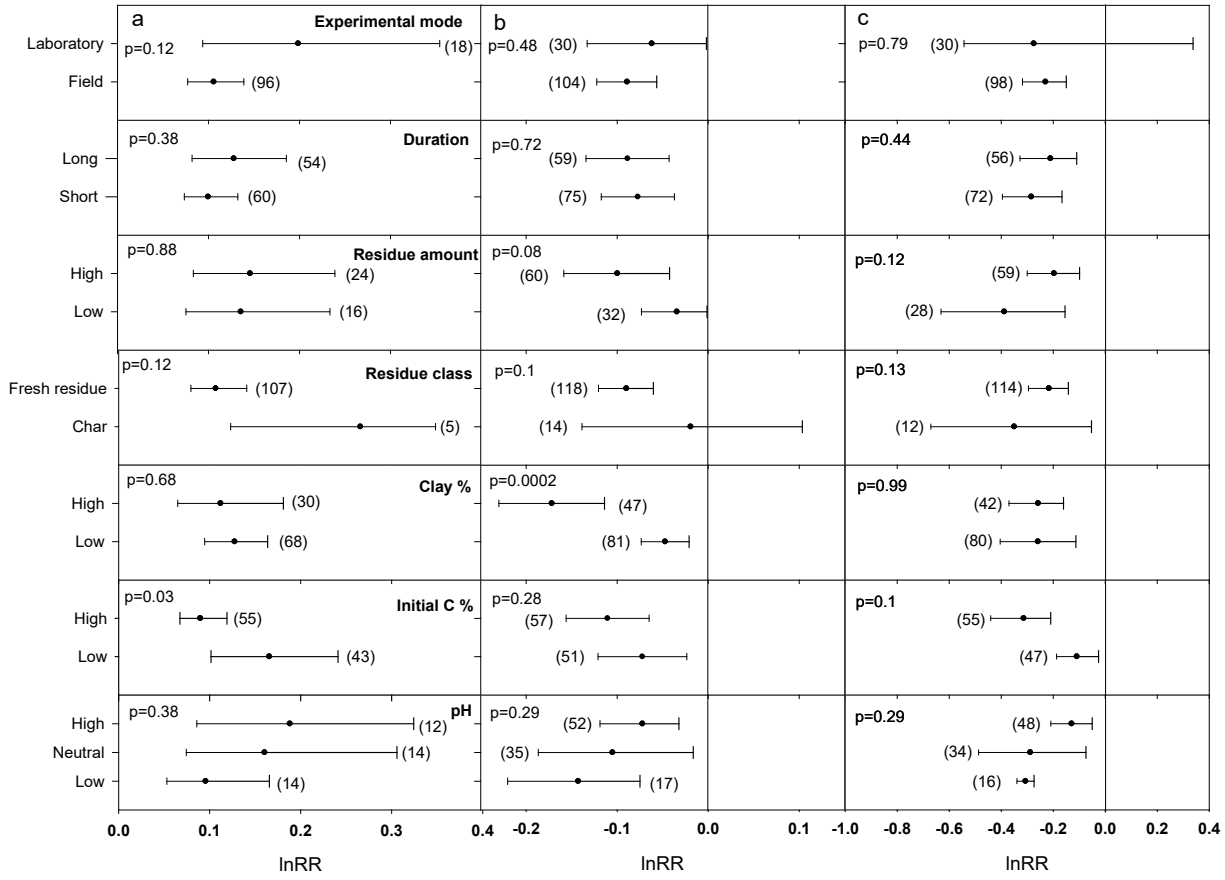

Figure 3.4: The effect of plant residue amendments (lnRR) on the (a) macroaggregate, (b) microaggregate, and (c) silt and clay weight fractions for different experimental conditions and soil factors (experimental mode, duration, residue amount, residue class, clay %, initial C % and pH). Error bars represent 95% confidence intervals.

We observed that there were no significant increases in TSC in response to plant residue amendments in laboratory than in field studies, because of large variation among laboratory studies

(p=0.49, Figure 3.5). Surprisingly, effects on TSC were not influenced by duration and residue amount. However, effects on TSC were larger for char than for fresh plant residues (p=0.0004), and larger when amendments were added to soil that had a low initial OC content (p=0.004) and a-high pH (p=0.001). The effect of the fresh plant residue from different origins on TSC was positive with no significant differences among the different residue origins.

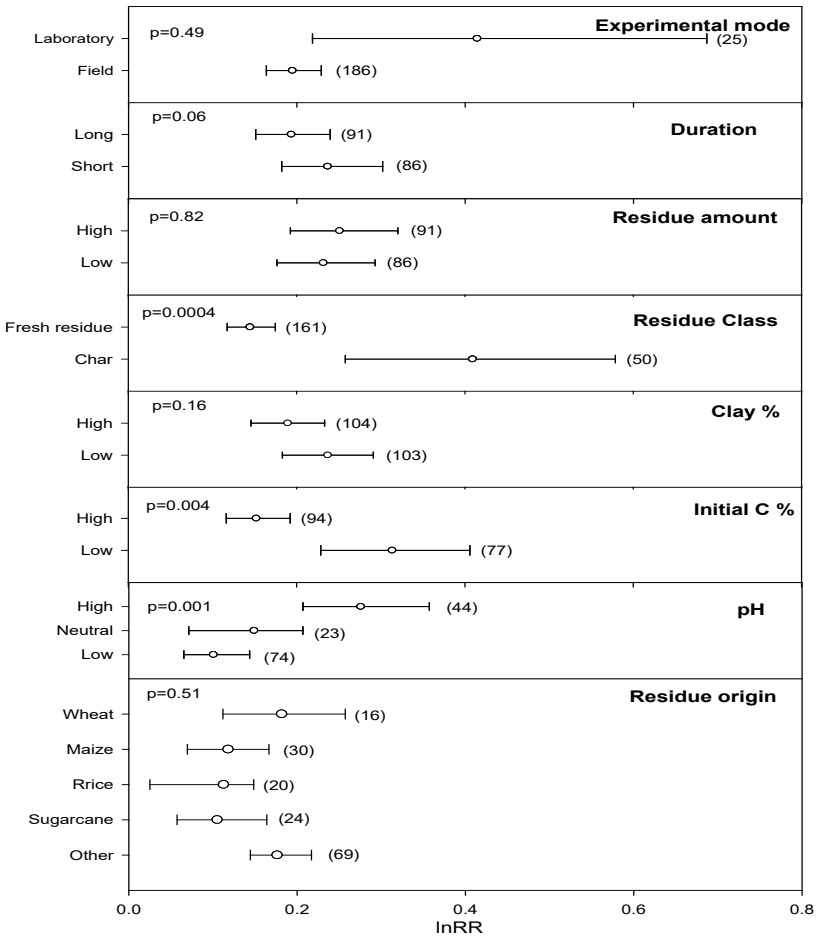

Figure 3.5: The effect of plant residue amendments on total soil carbon (TSC) for different experimental conditions and soil factors (experimental mode, duration, residue amount, residue class, clay %, initial C % and pH). Error bars represent low and high 95% confidence intervals.

We tested whether there were relationships between response ratios of TSC with MWD, macroaggregate, microaggregate, and silt and clay fractions. We only observed significant positive

relationships between response ratio of TSC and MWD and between response ratios of TSC and the macroaggregate fraction (p=0.02 and 0.001, respectively), although the relationships were weak explaining no more than 5% of the variation (Figure 3.6a, b). Relationships were not significant for microaggregate and silt and clay fractions (Figure 3.6c, d).

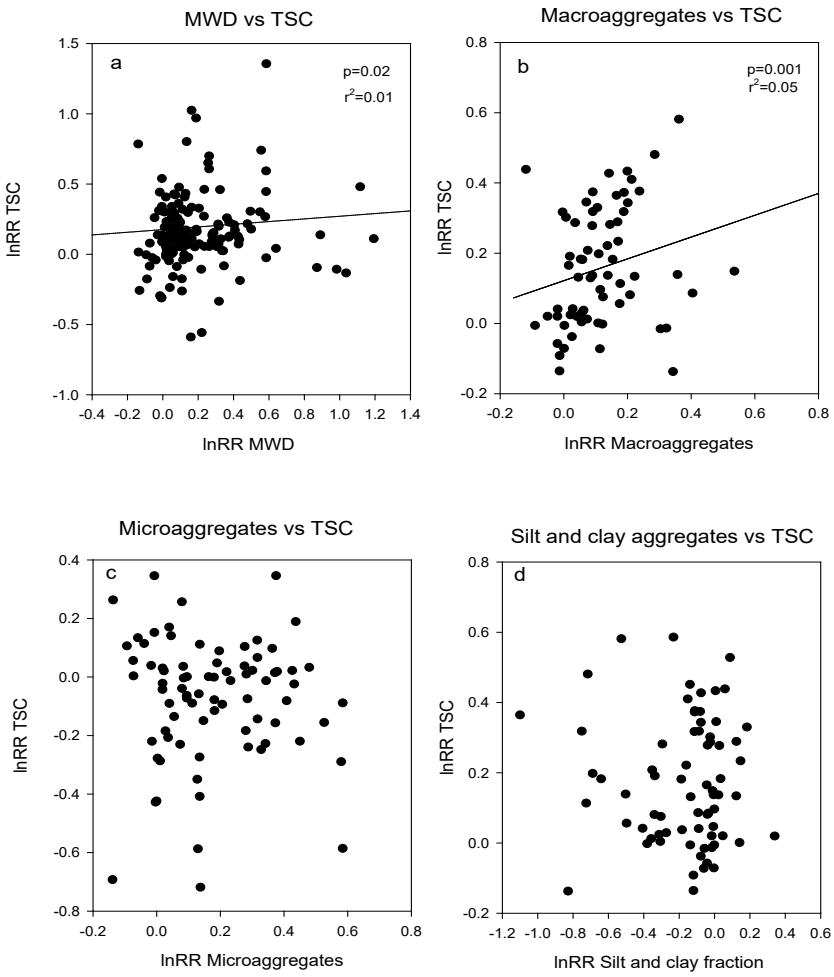

Figure 3.6: Relationships between the response ratio of total soil carbon (lnRR TSC) and (a) mean weight diameter (lnRR MWD), (b) macroaggregate fraction (lnRR Macroaggregates), (c) microaggregate fraction (lnRR Microaggregates), and (d) silt and clay fraction (lnRR Silt and clay fraction).

We further tested whether there were relationships between the mean annual temperature and response ratios of MWD, macroaggregate fraction, microaggregate fraction, silt and clay fraction,

and TSC. We found no direct relationships between the mean annual temperature and any of the tested parameters (data not shown).

3.4 Discussion

Assessed soil parameters showed mostly a positive response towards plant residue soil amendments (apart from microaggregate and silt and clay fractions). The observed positive response for MWD, macroaggregates, TSC, and aggregate-associated OC indicates the importance of this agricultural practice -to an extent- to maintain soil structure and functionality as an environmental resource and as an OC terrestrial reserve.

3.4.1 Plant residue effects on aggregation

The observed negative response to plant residue amendments from both microaggregates and silt and clay fractions (Figure 3.2) can be related to the formation of macroaggregates (given that results were reported as percentages of the total soil weight), since macroaggregates were showing an increase. Furthermore, the increase in MWD with plant residue amendments further indicate that overall larger aggregates were formed. These results confirm the importance of retaining plant residues for improving soil structure through enhancing macroaggregate formation and stabilization. The increase in macroaggregates with plant residue amendments can be related to the activation of soil microbes through supply of substrates from the addition of fresh plant residues, leading to microbial production of byproducts that can increase potential binding of microaggregates into macroaggregates (Six, Elliott & Paustian, 2000), thereby affect OC-mineral interaction (Bucka et al., 2019). Plant residue amendments can also increase fungal activity, which may further have increased the formation and stability of macroaggregates (Tang et al., 2011). However, soil macroaggregates are known to be also sensitive to soil disturbance and type of vegetation that vary root architecture and morphology (Ali et al., 2022; Materechera et al., 1994;

Oades, 1984) which were both not considered in our meta-analysis and may need to be considered in future meta-analysis studies.

The effect of plant residue amendments on MWD was consistent with the positive response for large aggregates (*i.e.,* macroaggregates). The greater responses for MWD in laboratory studies (that were mostly short in length) than field studies can be related to the absence of soil disturbance such as tillage in laboratory studies that may affect the stability of macroaggregates negatively as explained above. Moreover, the natural soil structure in most laboratory studies is destroyed at the start of the experiment due to soil preparation such as sieving and repacking into pots. As such, plant residue amendment effects may be more responsive compared to field studies that already have some soil structure before treatments are applied.

Amendment with fresh plant residue caused a small but non-significant increase in MWD compared to char (Figure 3.3). Although, fresh plant residues have a greater decomposability, so that more microbial products can be produced to form larger aggregates compared to relatively inert charred plant residues, we found no strong evidence for this. On the other hand, it has been suggested that biochar could enhance soil aggregation because if its hydrophobic character, or changes in soil pH and ionic composition of the soil solution (Islam et al., 2021; Wang et al., 2017), but this may also depend on feedstock quality and preparation procedures (Siedt et al., 2021b). Plant residue quality parameters, such as C:N ratio, cellulose and soluble sugars can also have important effects on soil aggregation (Blanco-Canqui & Lal, 2004; Halder et al., 2021; Tobiašová 2011). The large variation in effect sizes on MWD among plant residue origin (Figure 3) also suggests that plant residue quality may be important. Unfortunately, we were unable to extract plant residue quality parameters from most studies, and we were therefore unable to assess effects of plant residue quality directly. We further note that the high effect of alfalfa and barley residues

on MWD came from laboratory studies that in general showed higher effects on MWD (Figure 3.3), and therefore further studies regarding plant residue quality effects on soil aggregation are warranted.

The positive response to plant residue amendments on MWD in high clay soils indicates the importance of clay for enhancing large aggregate formation even in soils with low microbial activity (De Gryze et al., 2005). The average MWD in control plots was on average 0.57 and 1.09 mm for soils with low and high clay content, respectively, possibly because a high clay content resulted in greater soil aggregation. Therefore, the greater relative response in MWD with plant residue amendments in soils with high clay content was even greater in absolute terms. Clay particles have reactive surfaces that can strongly bind to organic compounds such as plant residues and their microbial products, thereby forming aggregates more efficiently (Oades, 1984; Totsche et al., 2018), but we note that formation of aggregates through adsorption of organic compounds to clay particles also strongly depends on the mineral composition of clay (Pronk et al., 2017; Xu et al., 2022). In soils with low initial OC, clay may still be unsaturated with OC (Castellano et al., 2015; Stewart et al., 2008), and may have a higher capacity to sequester more C and therefore a greater effect on aggregation with plant residue amendment, since OC is essential for aggregate formation. Further, low OC soils are more likely to have low aggregation to begin with. As a result, adding plant residues can show a significant effect on aggregation. Plant residue amendment in soils with neutral pH showed the strongest positive effect on MWD, and weakest effect in low and high pH soils. Plant production often increases when increasing the soil pH in acidic soils (Wang et al., 2021b), which could also have stimulated the production of plant residues, although we found no evidence that more plant residues were added in soils with neutral pH. In contrast, others have suggested that adsorption of organic matter to soil minerals, and thus formation of aggregates,

may be higher in acidic soils (Totsche et al., 2018). Another possible explanation for the relatively small effect on aggregation in soils with low pH could be that these soils can have a low microbial C use efficiency and high microbial release of CO_2 (Jones et al., 2019), thereby reducing the potential for soil OC storage and aggregation. Further, high release of CO_2 and low OC storage, and reduced potential for aggregation were observed in high pH soils (Yang, Li & Zhang, 2019) and in low pH soils (Leifeld et al., 2013). Moreover, soil microbial diversity is highly influenced by pH, where neutral soils were found to have the largest microbial diversity (Fierer & Jackson, 2006) for organic matter decomposition. In all, our results suggest that microbial products formed during decomposition of plant residues play an important role in forming macroaggregates in soil.

3.4.2 Plant residue effects on soil C

The OC concentration increased in all aggregate size classes (Figure 3.2), indicating that plant residue amendments affect soil OC in all fractions, despite the fact that plant soil amendments increased the percentage of macroaggregates only, as discussed above. Although the aggregate fractions changed with plant soil amendments, these results indicate that all soil aggregate size classes may be able to sequester OC derived from plant residues, that resulted in an overall increase in TSC. The increase in OC concentration in macroaggregates may have been due to direct inclusion and protection of plant residues, but may also have been caused by incorporation of microaggregates that had higher OC concentrations.

The effect of plant residue amendment on TSC was somewhat larger in laboratory than in field studies (although not significant, Figure 3.5), likely due to similar reasons explained for the effect on MWD above, where soil disturbance may have reduced the impact on TSC in field studies or where laboratory studies may have been more responsive, because they started with soils with little or no soil structure. Surprisingly, the effect on TSC did not change much with the application

duration or amount of residue applied. This could indicate that there is a limit to how much OC can be stored within aggregates (Gulde et al., 2008; Stewart et al., 2008), and that C saturation within aggregates (*i.e.,* in isolated microaggregates or when microaggregates are incorporated into larger aggregates) can be achieved with relatively low amounts of plant residue applied and within a relatively short time. However, our meta-analysis also indicated that this depended on the type of residue. The increase in TSC with the application of char can be related to the low microbial accessibility of OC in char compared to fresh plant residue, resulting in more OC loss from fresh residue than char (Siedt et al., 2021b). Consequently, amendments of plant residues when converted to char may be more efficient in increasing TSC compared to fresh plant residues. In contrast, the increase in MWD varied among fresh plant residues from different origin (Figure 3.3), but the increase in TSC was similar for all fresh plant residues, suggesting that if there were differences in residue quality, this might not be of significance to enhance soil OC.

The increase in TSC following plant residue amendments in soils with low initial OC can be attributed to the fact that low OC soils may be further away from their maximum capacity to store C (or saturation level), therefor allowing for more OC to be sequestered in the soil (Castellano et al., 2015; Stewart et al., 2008). Therefore, soils with low OC saturation may have a greater potential to increase TSC, but we caution that we are referring to a relative increase in OC here, which may not necessarily translate to greater OC sequestration on an absolute basis. However, soils with low OC also happened to be the most responsive in terms of MWD, so the increase in TSC in low initial OC soils could also be influenced by increased aggregation. This was supported by the positive relationship between the response ratio of TSC and MWD (and macroaggregates) that we observed (Figure 3.6), although we would like to point out that this relationship was weak.

Unlike the effect of plant residue amendment on aggregation (MWD) where the effect was greater in soil with more clay (Figure 3.3), the increase in TSC was similar for soils with low and high clay content (Figure 3.5). This suggest that clay plays a more important role in aggregation than in increasing soil OC with plant residue amendments. As discussed above, the initial soil OC content may be more important than clay content for increasing TSC with plant residue amendments. The greatest increase in TSC occurred with high pH (Figure 3.5). We are not clear about potential mechanisms, but possibly other factors, such as availability of nutrients such as phosphorus, may have been lower and reduced microbial decomposition in soils with high pH (Bradford, Fierer & Reynolds, 2008; Fontaine et al., 2004).

The lack of significant positive relationships between the response ratio of MWD and TSC with mean annual temperature is an indication of the complexity of soil and the importance of the soil native physical and chemical properties for the fate of any added OC to the soil. This is consistent with the results of a large-scale study where soil OC content was more important than mean annual temperature in explaining variation in microbial C use efficiency along a 4000 km forest transect in China (Wang et al., 2021), as the microbial C use efficiency of OC will determine the amount of OC remaining in the soil (Sinsabaugh et al., 2013).

We are aware that our meta-analysis has some limitations. They include the lack of adequate number of studies with data on soil parameters such as pH and clay content. This prevented us from conducting a full comparison and analysis of their effects on soil aggregation and associated OC. Because of the relative low number of observations, we were unable to examine whether effects caused by specific parameters were influenced by other parameters. For instance, most of the laboratory studies were short in duration, and therefore we were unable to tease apart experimental mode from duration effects. Studies also use plant residues from different origins,

and their decomposition and subsequent effect on soil aggregation may depend on the interactive effects of residue quality and soil parameters, such as clay content, nutrient availability, and pH (Li et al., 2017; Xu et al., 2022). More research is further needed to evaluate the impacts of soil disturbance (such as tillage), soil pH, and plant residue quality effects on soil structure sustainability and OC sequestration in soils with plant residue amendments.

3.5 Conclusions

From this study, we conclude that plant residue amendments to the soil can improve soil aggregation and OC content, but that these improvements depend on plant residue type (fresh vs charred, and origin of plant residues) and soil characteristics. We found that both fresh and charred plant residue amendments were effective in soil aggregation, but that charred plant materials were more effective in increasing TSC. On the other hand, certain origins of fresh plant residue (from alfalfa and barley) were more effective in soil aggregation than others (wheat), but showed similar effects on TSC. Soil aggregation increased with increased residue amount applied, whereas increases in TSC were similar for low amounts (less than 10 t ha^{-1}) and high amounts (more than 10 t ha^{-1}). As such, preservation of OC in soil aggregates by retention of plant residues significantly depends more on residue type than on quantity.

Importantly, soil properties such as clay content, initial soil OC content and pH were critical factors in influencing aggregate formation and OC storage. Plant residue amendments had much smaller effects on soil aggregation in soils with low clay content, high initial OC content and low or high pH. However, increased soil aggregation did not always result in similar increases in TSC, indicating that other factors besides soil aggregation are important for the increase in TSC with plant residue amendments. We further that we were not able to examine interactive effects of different factors (e.g., residue quality and soil factors). Nevertheless, our results present guidance

on how or when farm managers should apply plant residues. For instance, plant residue amendments in acidic soils to improve soil aggregation and soil OC may be more effective when soil pH is increased at the same time (*e.g.*, with lime). Plant residue amendments may be particularly successful in increasing both soil aggregation and soil OC in soils that have low OC concentrations to begin with. Therefore, plant residue amendments to enhance soil aggregation and soil OC with the ultimate goal of improving soil functions for sustainable agriculture should be adjusted to existing soil conditions.

3.6 Acknowledgement

We acknowledge that this research paper is part of the requirements of a PhD research degree at The University of Sydney.

3.7 References

Ali, W., Yang, M., Long, Q., Hussain, S., Chen, J., Clay, D., & He, Y. (2022). Different fall/winter cover crop root patterns induce contrasting red soil (Ultisols) mechanical resistance through aggregate properties. *Plant and Soil, 477*(1), 461-474. doi:10.1007/s11104-022-05430-4

Angers, D. A., & Caron, J. (1998). Plant-induced Changes in Soil Structure: Processes and Feedbacks. *Biogeochemistry, 42*(1), 55-72. doi:10.1023/a:1005944025343

Baldock, J. A., & Skjemstad, J. O. (2000). Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry, 31*(7), 697-710. doi:https://doi.org/10.1016/S0146-6380(00)00049-8

Bradford, M. A., Fierer, N., & Reynolds, J. F. (2008). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Functional Ecology, 22*(6), 964-974. doi:https://doi.org/10.1111/j.1365-2435.2008.01404.x

Bucka, F. B., Kölbl, A., Uteau, D., Peth, S., & Kögel-Knabner, I. (2019). Organic matter input determines structure development and aggregate formation in artificial soils. *Geoderma, 354*, 113881. doi:https://doi.org/10.1016/j.geoderma.2019.113881

Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E., & Six, J. (2015). Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. *Global Change Biology, 21*(9), 3200-3209.

De Gryze, S., Six, J., Brits, C., & Merckx, R. (2005). A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. *Soil Biology and Biochemistry, 37*(1), 55-66. doi:10.1016/j.soilbio.2004.07.024

Ebrahimi, A., & Or, D. (2015). Hydration and diffusion processes shape microbial community organization and function in model soil aggregates. *Water Resources Research, 51*(12), 9804-9827. doi:10.1002/2015WR017565

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America, 103*(3), 626-631. doi:10.1073/pnas.0507535103

Fontaine, S., Bardoux, G., Abbadie, L., & Mariotti, A. (2004). Carbon input to soil may decrease soil carbon content. *Ecology Letters, 7*(4), 314-320. doi:https://doi.org/10.1111/j.1461-0248.2004.00579.x

Gulde, S., Chung, H., Amelung, W., Chang, C., & Six, J. (2008). Soil Carbon Saturation Controls Labile and Stable Carbon Pool Dynamics. *Soil Science Society of America Journal, 72*(3), 605-612. doi:10.2136/sssaj2007.0251

Guo, L. B., & Gifford, R. M. (2002). Soil carbon stocks and land use change: a meta analysis. *Global Change Biology, 8*(4), 345-360. doi:10.1046/j.1354-1013.2002.00486.x

Heneghan, L., Miller, S. P., Baer, S., Callaham Jr., M. A., Montgomery, J., Pavao-Zuckerman, M., . . . Richardson, S. (2008). Integrating Soil Ecological Knowledge into Restoration Management. *Restoration Ecology, 16*(4), 608-617. doi:10.1111/j.1526-100X.2008.00477.x

Hütsch, B. W., Augustin, J., & Merbach, W. (2002). Plant rhizodeposition — an important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science, 165*(4), 397-407. doi:10.1002/1522-2624(200208)165:4<397::AID-JPLN397>3.0.CO;2-C

Islam, M. U., Jiang, F., Guo, Z., & Peng, X. (2021). Does biochar application improve soil aggregation? A meta-analysis. *Soil & tillage research, 209*, 104926. doi:10.1016/j.still.2020.104926

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*. doi:10.1016/j.soilbio.2019.107584

Kopittke, P. M., Dalal, R. C., Finn, D., & Menzies, N. W. (2017). Global changes in soil stocks of carbon, nitrogen, phosphorus, and sulphur as influenced by long-term agricultural production. *Global Change Biology, 23*(6), 2509-2519.

Leifeld, J., Bassin, S., Conen, F., Hajdas, I., Egli, M., & Fuhrer, J. (2013). Control of soil pH on turnover of belowground organic matter in subalpine grassland. *Biogeochemistry, 112*(1), 59-69.

Liu, C., Lu, M., Cui, J., Li, B., & Fang, C. (2014). Effects of straw carbon input on carbon dynamics in agricultural soils: a meta-analysis. *Global Change Biology, 20*(5), 1366-1381. doi:10.1111/gcb.12517

Lynch, J. M., & Bragg, E. (1985). *Microorganisms and Soil Aggregate Stability*, New York, NY.

Martin, J. P., Martin, W. P., Page, J. B., Raney, W. A., & de Ment, J. D. (1955). Soil Aggregation. In A. G. Norman (Ed.), *Advances in Agronomy* (Vol. 7, pp. 1-37): Academic Press.

Materechera, S. A., Kirby, J. M., Alston, A. M., & Dexter, A. R. (1994). Modification of soil aggregation by watering regime and roots growing through beds of large aggregates. *Plant and Soil, 160*(1), 57-66. doi:10.1007/bf00150346

Oades, J. (1984). Soil organic matter and structural stability: mechanisms and implications for management. *Plant and Soil, 76*(1), 319-337. doi:10.1007/BF02205590

Pronk, G. J., Heister, K., Vogel, C., Babin, D., Bachmann, J., Ding, G.-C., . . . Kögel-Knabner, I. (2017). Interaction of minerals, organic matter, and microorganisms during biogeochemical interface formation as shown by a series of artificial soil experiments. *Biology and Fertility of Soils, 53*(1), 9-22. doi:10.1007/s00374-016-1161-1

Rakshit, A., Sarkar, B., & Abhilash, P. (2018). *Soil Amendments for Sustainability : Challenges and Perspectives* (First edition. ed.). Boca Raton, FL: CRC Press.

Shoshany, M., Goldshleger, N., & Chudnovsky, A. (2013). Monitoring of agricultural soil degradation by remote-sensing methods: a review. *International journal of remote sensing, 34*(17), 6152-6181. doi:10.1080/01431161.2013.793872

Siedt, M., Schäffer, A., Smith, K. E. C., Nabel, M., Roß-Nickoll, M., & van Dongen, J. T. (2021). Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides. *Science of The Total Environment, 751*, 141607. doi:https://doi.org/10.1016/j.scitotenv.2020.141607

Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., & Richter, A. (2013). Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters, 16*(7), 930-939. doi:https://doi.org/10.1111/ele.12113

Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil, 241*(2), 155-176.

Six, J., Elliott, E. T., & Paustian, K. (2000). Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry, 32*(14), 2099-2103. doi:https://doi.org/10.1016/S0038-0717(00)00179-6

Stewart, C. E., Plante, A. F., Paustian, K., Conant, R. T., & Six, J. (2008). Soil Carbon Saturation: Linking Concept and Measurable Carbon Pools. *Soil Science Society of America Journal, 72*(2), 379-392. doi:10.2136/sssaj2007.0104

Tang, J., Mo, Y., Zhang, J., & Zhang, R. (2011). Influence of biological aggregating agents associated with microbial population on soil aggregate stability. *Applied Soil Ecology, 47*(3), 153-159. doi:https://doi.org/10.1016/j.apsoil.2011.01.001

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., & Marschner, B. (2007). SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry, 39*(9), 2183-2207.

Wang, C., Qu, L., Yang, L., Liu, D., Morrissey, E., Miao, R., . . . Bai, E. (2021). Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon. *Global Change Biology, 27*(10), 2039-2048. doi:10.1111/gcb.15550

Yang, C., Li, J., & Zhang, Y. (2019). Soil aggregates indirectly influence litter carbon storage and release through soil pH in the highly alkaline soils of north China. *PeerJ, 7*, e7949-e7949. doi:10.7717/peerj.7949

Supplementary information

References used in this meta-analysis

1. Atere, CT, Gunina, A, Zhu, Z, Xiao, M, Liu, S, Kuzyakov, Y, Chen, L, Deng, Y, Wu, J & Ge, T 2020, 'Organic matter stabilization in aggregates and density fractions in paddy soil depending on long-term fertilization: Tracing of pathways by 13C natural abundance', *Soil biology & biochemistry,* vol. 149, pp. 107931.

2. Bai, N, Zhang, H, Li, S, Zheng, X, Zhang, J, Zhang, H, Zhou, S, Sun, H & Lv, W 2019, 'Long-term effects of straw and straw-derived biochar on soil aggregation and fungal community in a rice-wheat rotation system', *PeerJ (San Francisco, CA),* vol. 6, pp. e6171-e6171.

3. Blanco-Canqui, H, Kaiser, M, Hergert, GW, Creech, CF, Nielsen, R, Maharjan, B, Easterly, AC & Lawrence, NC 2020, 'Can char carbon enhance soil properties and crop yields in low-carbon soils?', *Journal of environmental quality,* vol. 49, no. 5, pp. 1251-1263.

4. Castioni, GaF, Cherubin, MR, Bordonal, RDO, Barbosa, LC, Menandro, LMS & Carvalho, JLN 2019, 'Straw Removal Affects Soil Physical Quality and Sugarcane Yield in Brazil', *Bioenergy research,* vol. 12, no. 4, pp. 789-800.

5. Chakraborty, D, Mondal, S, Das, A, Paul, A, Thomas, P, Aggarwal, P, Behera, U & Sharma, A 2019, 'Tillage and Crop Rotation Effects on Mechanical Properties and Structural Stability of a Sandy Loam Soil in a Semi-arid Environment', *Journal of the Indian Society of Soil Science,* vol. 67, no. 2, pp. 174-182.

6. Chatterjee, S, Bandyopadhyay, K, Pradhan, S, Singh, R & Datta, S 2018, 'Effects of irrigation, crop residue mulch and nitrogen management in maize (Zea mays L.) on soil carbon pools in a sandy loam soil of Indo-gangetic plain region', *Catena,* vol. 165, pp. 207-216.

7. Chen, Z, Ti, J-S & Chen, F 2017, 'Soil aggregates response to tillage and residue management in a double paddy rice soil of the Southern China', *Nutrient cycling in agroecosystems,* vol. 109, no. 2, pp. 103-114.

8. Dai, J, Hu, J, Zhu, A, Lin, X & Nicholson, F 2017, 'No-tillage with half-amount residue retention enhances microbial functional diversity, enzyme activity and glomalin-related soil protein content within soil aggregates', *Soil use and management,* vol. 33, no. 1, pp. 153-162.

9. Das, A, Ghosh, PK, Lal, R, Saha, R & Ngachan, S 2017, 'Soil Quality Effect of Conservation Practices in Maize–Rapeseed Cropping System in Eastern Himalaya', *Land degradation & development,* vol. 28, no. 6, pp. 1862-1874.

10. Dhaliwal, JK, Singh, MJ, Sharma, S, Gupta, N & Kukal, SS 2020, 'Medium-term impact of tillage and residue retention on soil physical and biological properties in dry-seeded rice–wheat system in north-west India', *Soil research (Collingwood, Vic.),* vol. 58, no. 5, pp. 468.

11. Eden, M, Bachmann, J, Cavalaris, C, Kostopoulou, S, Kozaiti, M & Böttcher, J 2020, 'Soil structure of a clay loam as affected by long-term tillage and residue management', *Soil & tillage research,* vol. 204, pp. 104734.

12. Fan, W, Wu, J, Ahmed, S, Hu, J, Chen, X, Li, X, Zhu, W & Opoku-Kwanowaa, Y 2020, 'Short-term effects of different straw returning methods on the soil physicochemical properties and quality index in dryland farming in NE China', *Sustainability (Basel, Switzerland),* vol. 12, no. 7, pp. 2631.

13. Fang, Y, Singh, BP, Cowie, A, Wang, W, Arachchi, MH, Wang, H & Tavakkoli, E 2019, 'Balancing nutrient stoichiometry facilitates the fate of wheat residue-carbon in physically defined soil organic matter fractions', *Geoderma,* vol. 354, pp. 113883.

14. Guo, L-J, Lin, S, Liu, T-Q, Cao, C-G & Li, C-F 2016, 'Effects of Conservation Tillage on Topsoil Microbial Metabolic Characteristics and Organic Carbon within Aggregates under a Rice (Oryza sativa L.)-Wheat (Triticum aestivum L.) Cropping System in Central China', *PloS one,* vol. 11, no. 1, pp. e0146145-e0146145.

15. Guo, Y, Fan, R, Mclaughlin, N, Zhang, Y, Chen, X, Wu, D, Zhang, X & Liang, A 2021, 'Impacts induced by the combination of earthworms, residue and tillage on soil organic carbon dynamics

using 13C labelling technique and X-ray computed tomography', *Soil & tillage research,* vol. 205, pp. 104737.

16. Hernandez, T, Hernandez, MC & Garcia, C 2017, 'The effects on soil aggregation and carbon fixation of different organic amendments for restoring degraded soil in semiarid areas: Effect of amendments on soil aggregation and C fixation', *European journal of soil science,* vol. 68, no. 6, pp. 941-950.
17. Jiang, M, Wang, X, Liusui, Y, Han, C, Zhao, C & Liu, H 2017, 'Variation of soil aggregation and intra-aggregate carbon by long-term fertilization with aggregate formation in a grey desert soil', *Catena (Giessen),* vol. 149, pp. 437-445.
18. Kerré, B, Hernandez-Soriano, MC & Smolders, E 2016, 'Partitioning of carbon sources among functional pools to investigate short-term priming effects of biochar in soil: A 13C study', *The Science of the total environment,* vol. 547, pp. 30-38.
19. Kiboi, MN, Ngetich, FK, Muriuki, A, Adamtey, N & Mugendi, D 2020, 'The response of soil physicochemical properties to tillage and soil fertility resources in Central Highlands of Kenya', *Italian journal of agronomy,* vol. 15, no. 2, pp. 71.
20. Kumar, S, Garg, AK & Aulakh, MS 2016, 'Effect of Conservation Agriculture Practices on Physical, Chemical and Biological Attributes of Soil Health Under Soybean–Rapeseed Rotation', *Agricultural research (India : Online),* vol. 5, no. 2, pp. 145-161.
21. Li, Z, Rui, Z, Zhang, D, Feng, X, Lu, H, Shen, S, Zheng, J, Li, L, Song, Z & Pan, G 2019, 'Macroaggregates as biochemically functional hotspots in soil matrix: Evidence from a rice paddy under long-term fertilization treatments in the Taihu Lake Plain, eastern China', *Applied soil ecology : a section of Agriculture, ecosystems & environment,* vol. 138, pp. 262-273.
22. Liu, Z 2018, 'Effects of long-term various organic materials incorporation on characteristics of soil quality and crop yield', *IOP Conf. Ser.: Earth Environ. Sci*, IOP Publishing.
23. Lu, H, Bian, R, Xia, X, Cheng, K, Liu, X, Liu, Y, Wang, P, Li, Z, Zheng, J, Zhang, X, Li, L, Joseph, S, Drosos, M & Pan, G 2020, 'Legacy of soil health improvement with carbon increase following

one time amendment of biochar in a paddy soil – A rice farm trial', *Geoderma,* vol. 376, pp. 114567.

24. Ma, S, Kan, Z, Qi, J & Zhang, H 2020, 'Effects of straw return mode on soil aggregates and associated carbon in the North China Plain', *Agronomy (Basel),* vol. 10, no. 1, pp. 61.

25. Margenot, AJ, Paul, BK, Sommer, RR, Pulleman, MM, Parikh, SJ, Jackson, LE & Fonte, SJ 2017, 'Can conservation agriculture improve phosphorus (P) availability in weathered soils? Effects of tillage and residue management on soil P status after 9 years in a Kenyan Oxisol', *Soil & tillage research,* vol. 166, pp. 157-166.

26. Mashyekhi, R, Emami, H & Asl, FN 2020, 'The influence of pistachio shell biochar and barley residues on soil properties', *Polish Journal of Soil Science,* vol. 53, no. 1, pp. 21.

27. Mi, W, Wu, Y, Zhao, H, Wu, L & Liu, Y 2018, 'Effects of combined organic manure and mineral fertilization on soil aggregation and aggregate-associated organic carbon in two agricultural soils', *Journal of plant nutrition,* vol. 41, no. 17, pp. 2256-2265.

28. Modak, K, Ghosh, A, Bhattacharyya, R, Biswas, DR, Das, TK, Das, S & Singh, G 2019, 'Response of oxidative stability of aggregate-associated soil organic carbon and deep soil carbon sequestration to zero-tillage in subtropical India', *Soil & tillage research,* vol. 195, pp. 104370.

29. Nandan, R, Singh, V, Singh, SS, Kumar, V, Hazra, KK, Nath, CP, Poonia, S, Malik, RK, Bhattacharyya, R & Mcdonald, A 2019, 'Impact of conservation tillage in rice–based cropping systems on soil aggregation, carbon pools and nutrients', *Geoderma,* vol. 340, pp. 104-114.

30. Nath, CP, Das, TK, Bhattacharyya, R, Pathak, H, Paul, S, Chakraborty, D & Hazra, KK 2019, 'Nitrogen Effects on Productivity and Soil Properties in Conventional and Zero Tilled Wheat with Different Residue Management', *Proceedings of the National Academy of Sciences, India. Section B: Biological sciences,* vol. 89, no. 1, pp. 123-135.

31. Oladele, SO 2019, 'Changes in physicochemical properties and quality index of an Alfisol after three years of rice husk biochar amendment in rainfed rice – Maize cropping sequence', *Geoderma,* vol. 353, pp. 359-371.

32. Paradelo, R, Lerch, TZ, Houot, S & Dignac, M-F 2019, 'Composting modifies the patterns of incorporation of OC and N from plant residues into soil aggregates', *Geoderma,* vol. 353, pp. 415-422.

33. Pramanik, P & Phukan, M 2020, 'Enhanced microbial respiration due to carbon sequestration in pruning litter incorporated soil reduced the net carbon dioxide flux from atmosphere to tea ecosystem', *Journal of the science of food and agriculture,* vol. 100, no. 1, pp. 295-300.

34. Rahman, MT, Liu, S, Guo, ZC, Zhang, ZB & Peng, XH 2019, 'Impacts of residue quality and N input on aggregate turnover using the combined 13C natural abundance and rare earth oxides as tracers', *Soil & tillage research,* vol. 189, pp. 110-122.

35. Ruis, SJ, Blanco-Canqui, H, Jasa, PJ, Ferguson, RB & Slater, G 2017, 'Can Cover Crop Use Allow Increased Levels of Corn Residue Removal for Biofuel in Irrigated and Rainfed Systems?', *Bioenergy research,* vol. 10, no. 4, pp. 992-1004.

36. Saffari, N, Hajabbasi, MA, Shirani, H, Mosaddeghi, MR & Mamedov, AI 2020, 'Biochar type and pyrolysis temperature effects on soil quality indicators and structural stability', *Journal of environmental management,* vol. 261, pp. 110190-110190.

37. Shahbaz, M, Kuzyakov, Y & Heitkamp, F 2017, 'Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls', *Geoderma,* vol. 304, pp. 76-82.

38. Sindelar, M, Blanco-Canqui, H, Jin, VL & Ferguson, R 2019, 'Cover Crops and Corn Residue Removal: Impacts on Soil Hydraulic Properties and Their Relationships with Carbon', *Soil Science Society of America journal,* vol. 83, no. 1, pp. 221-231.

39. Singh, G, Bhattacharyya, R, Das, TK, Sharma, AR, Ghosh, A, Das, S & Jha, P 2018, 'Crop rotation and residue management effects on soil enzyme activities, glomalin and aggregate stability under zero tillage in the Indo-Gangetic Plains', *Soil & tillage research,* vol. 184, pp. 291-300.

40. Singh, VK, Yadvinder, S, Dwivedi, BS, Singh, SK, Majumdar, K, Jat, ML, Mishra, RP & Rani, M 2016, 'Soil physical properties, yield trends and economics after five years of conservation

agriculture based rice-maize system in north-western India', *Soil & tillage research,* vol. 155, pp. 133-148.

41. Sun, Z, Zhang, Z, Zhu, K, Wang, Z, Zhao, X, Lin, Q & Li, G 2020, 'Biochar altered native soil organic carbon by changing soil aggregate size distribution and native SOC in aggregates based on an 8-year field experiment', *The Science of the total environment,* vol. 708, pp. 134829-134829.

42. Walia, MK & Dick, WA 2018, 'Selected soil physical properties and aggregate-associated carbon and nitrogen as influenced by gypsum, crop residue, and glucose', *Geoderma,* vol. 320, pp. 67-73.

43. Wang, X, Qi, J-Y, Zhang, X-Z, Li, S-S, Latif Virk, A, Zhao, X, Xiao, X-P & Zhang, H-L 2019, 'Effects of tillage and residue management on soil aggregates and associated carbon storage in a double paddy cropping system', *Soil & tillage research,* vol. 194, pp. 104339.

44. Ye, G, Lin, Y, Kuzyakov, Y, Liu, D, Luo, J, Lindsey, S, Wang, W, Fan, J & Ding, W 2019, 'Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: Results of a 27-year field experiment', *Soil biology & biochemistry,* vol. 134, pp. 15-24.

45. Yoo, G, Kim, H & Choi, JY 2017, 'Soil Aggregate Dynamics Influenced by Biochar Addition using the 13C Natural Abundance Method', *Soil Science Society of America journal,* vol. 81, no. 3, pp. 612-621.

46. Zhang, M, Cheng, G, Feng, H, Sun, B, Zhao, Y, Chen, H, Chen, J, Dyck, M, Wang, X, Zhang, J & Zhang, A 2017a, 'Effects of straw and biochar amendments on aggregate stability, soil organic carbon, and enzyme activities in the Loess Plateau, China', *Environmental science and pollution research international,* vol. 24, no. 11, pp. 10108-10120.

47. Zhang, W, Li, S, Xu, Y, Wang, Y, Liu, X, Peng, C & Wang, J 2020, 'Residue incorporation enhances the effect of subsoiling on soil structure and increases SOC accumulation', *Journal of soils and sediments,* vol. 20, no. 10, pp. 3537-3547.

48. Zhang, X, Xin, X, Zhu, A, Yang, W, Zhang, J, Ding, S, Mu, L & Shao, L 2018, 'Linking macroaggregation to soil microbial community and organic carbon accumulation under different tillage and residue managements', *Soil & tillage research,* vol. 178, pp. 99-107.

49. Zhang, X, Xin, X, Zhu, A, Zhang, J & Yang, W 2017b, 'Effects of tillage and residue managements on organic C accumulation and soil aggregation in a sandy loam soil of the North China Plain', *Catena (Giessen),* vol. 156, pp. 176-183.

50. Zhu, Z, Angers, DA, Field, DJ & Minasny, B 2017, 'Using ultrasonic energy to elucidate the effects of decomposing plant residues on soil aggregation', *Soil & tillage research,* vol. 167, pp. 1-8.

Chapter 4: Fungi are more important than bacteria for soil C loss through priming effects and C protection through aggregation

Abstract

Plant inputs from root exudates and litter can improve soil aggregation and enhance soil organic carbon (SOC) content, but they can also increase SOC decomposition referred to as the priming effect. While bacteria and fungi are considered as key microbes for organic matter decomposition, their role in decomposing plant inputs and SOC, and their contribution to forming aggregates and SOC protection is still not fully understood. We conducted a 16-day incubation study to investigate the role of bacteria and fungi on decomposition and protection of C derived from glucose (labile C as a surrogate for root exudates) and wheat root biomass added to soil. Bronopol and captan were used to suppress bacterial and fungal activity, respectively, in the soil. Because glucose and wheat root biomass were enriched in ^{13}C, we were able to estimate their decomposition and priming effect, microbial C use efficiency (CUE), and recovery in different aggregate size classes. As expected, the decomposition of both substrates was reduced significantly when biocides were added, and the decomposition rate of glucose was significantly higher than of wheat root biomass. Fungi were more important than bacteria in causing a priming effect, as indicated by the reduced SOC decomposition when captan was added. The mean weight diameter of aggregates at the end of the incubation was significantly reduced when captan was added, either alone or in combination with bronopol, independent of substrate type, suggesting that fungi also played an important role in soil aggregation. However, recovery of C derived from wheat root biomass in macroaggregates was highest when both biocides were added, suggesting that decomposition by both bacteria and fungi may have outweighed the protection of wheat C in new macroaggregates. While we observed variation in the microbial CUE among substrate and biocide treatments, we found no relationship

with soil aggregation. We conclude that both bacteria and fungi were important for decomposition of added substrates to the soil, but that fungi can play a larger role in both soil C loss through priming effects and C protection through aggregation.

Key words: soil carbon decomposition, soil aggregation, bacteria, fungi, microbial carbon use efficiency

4.1 Introduction

Soil aggregates are the unit blocks for soil structure and their stability has important consequences for the quality and function of soil (Rabot et al., 2018). Soil aggregates vary in size and shape affecting water filtration and gas exchange with the atmosphere. The pore spaces between and within soil aggregates provide soil fauna, microbes and plants with resources and a habitat. Moreover, the formation of stable soil aggregates is of great significance for carbon (C) sequestration in the soil, where C inside aggregates can be protected from microbial decomposition and release of atmospheric CO_2 (Six et al., 2002), thereby mitigating climate change.

For aggregates to form, organic matter is essential to bind soil particles (like sand, clay, and silt) into stable clusters (*i.e.*, aggregates, Tisdall and Oades, 1982). Organic matter involved in the formation of aggregates is provided primarily by plants. Plant organic matter input comes in different forms, where it can be labile such as sugars and organic acids produced through rhizodeposition, or it can be more complex like plant litter (Hütsch, Augustin & Merbach, 2002). Much of this plant organic matter input is consumed by microbes and transformed into microbial by-products that also can be used as binding materials for aggregation (Six et al., 2006). Therefore,

soil aggregation may strongly depend on the type of plant inputs and how it is processed by microbes.

Soil aggregates are usually classified into macroaggregates (>250 μm), microaggregates (250-53 μm) and the clay and silt fraction (<53 μm). Sequestration of C in aggregates is facilitated by 1) the adsorption of organic C onto clay minerals in microaggregates, 2) binding materials such as polysaccharides produced by bacteria, plant roots and fungal hyphae to combine clay and silt particles together into larger aggregates, and 3) encapsulation of smaller aggregates with plant roots and hyphal enmeshment into larger aggregates (Six et al., 2002). Fungi have shown to be important for the formation of macroaggregates, while bacteria are important for the formation of both micro- and macroaggregates (Lehmann, Zheng & Rillig, 2017).

Plant organic matter input can also stimulate the microbial decomposition of soil native organic C, referred to as the priming effect (Kuzyakov, 2010). The magnitude of the priming effect depends on microbial composition (Blagodatskaya & Kuzyakov, 2008; Garcia-Pausas & Paterson, 2011; Wang et al., 2014).I In some studies, bacteria have shown greater priming effects than fungi (Deng et al., 2021; Mo et al., 2022), whereas in other studies fungi were more important (Xiao et al., 2022; Zhang et al., 2023), although this may also depend on the type of plant organic matter inputs (Chao et al., 2019; Na et al., 2022). For instance, labile compounds have more energy and may therefore cause a greater priming effect by fast-growing bacteria compared to more complex compounds that could favor fungi (Deng et al., 2021). The enhanced decomposition of soil organic matter caused by the priming effect could potentially destabilize aggregates (Wang et al., 2020c), but it is unclear to what degree this depends on substrate type and microbial composition.

Plant C inputs will not only affect the decomposition rate of organic matter, but also how much of the organic matter remains in the soil through the formation of microbial products (Rahman et al., 2019; Sokol, Sanderman & Bradford, 2019). Important parameters in that regards are the microbial growth and carbon use efficiency (CUE) of the substrate (Sinsabaugh et al., 2013). The CUE of a substrate – the proportion of the total available C consumed for growth of microbes – will not only determine the amount of C stored in soil aggregates (Luiz et al., 2020), but may also affect soil aggregation itself. The microbial growth and CUE may differ between fungi and bacteria (Ma et al., 2023; Soares & Rousk, 2019). For instance, greater investment of C in the production of enzymes by fungi can lead to a reduction in the microbial CUE in the soil (Ullah, Carrillo & Dijkstra, 2021b). Furthermore, previous research has shown the importance of different C sources and types on microbial growth and CUE (Qiao et al., 2019). However, how the CUE will affect soil aggregation and their incorporation of C, and to what degree this is influenced by plant organic matter type and microbial composition, remain unclear.

Fungicides and bactericides have been frequently used to manipulate fungal and bacterial activity in soil (Ladan & Jacinthe, 2016; Rousk, Brookes & Bååth, 2010). However, use of these biocides could potentially complicate interpretation of their effects on soil organic matter decomposition. For instance, most fungicides and bactericides are organic and could potentially provide a source of C to the surviving microbial community (Muñoz-Leoz et al., 2013; Ullah, Carrillo and Dijkstra (2021a). Application of these biocides also frequently results in a pulse in microbial death that could temporarily be used as a C substrate for the remaining microbes (Badalucco et al., 1994; Muñoz-Leoz et al., 2011). Furthermore, the effect of biocide additions is often short-lived because

of their decay and adsorption onto soil particles, which can result in regrowth of target microbes within weeks (Badalucco et al., 1994; Chen et al., 2001). Because of these complications, it was suggested to add biocides at repeated intervals to suppress regrowth of target microbes, and use isotopically labeled substrates to differentiate their decomposition from decomposition of other sources (*i.e.*, soil organic matter, dead microbes and biocides themselves, Ullah et al., 2021a).

The aim of this study was to examine the effect of complex and labile plant substrates and the role of fungi and bacteria on the priming effect, microbial CUE, soil aggregation and C storage in a 16-day laboratory incubation study. We assessed two types of plant substrates: 1) a complex substrate (wheat roots) to represent root litter and, 2) a labile substrate (glucose) to represent rhizodeposition. Both substrates were labeled with ^{13}C, which allowed us to examine the substrate-derived C in respiration, microbial growth on the substrate, and the microbial CUE of each of the substrates, as well as the priming effect. We further assessed substrate-derived C in three different aggregate size classes (macroaggregates, microaggregates, and silt and clay fraction). We used the fungicide captan and the bactericide bronopol to reduce fungal and bacterial activity, respectively. We hypothesized that: 1) by removing the activity of soil bacteria using bronopol, the decomposition and CUE of glucose and formation of macroaggregates (or mean weight diameter -MWD- of the aggregates) will be reduced more than with wheat root biomass, 2) by removing the activity of fungi with the use of captan, the decomposition and CUE of wheat root biomass and formation of macroaggregates (or MWD) will be reduced more than with glucose, 3) microbial growth and CUE are positively correlated to the formation of macroaggregates (or MWD) and substrate-derived C remaining in the soil, and 4) the addition of glucose will increase the

mineralization of soil C (priming effect) more than the addition of wheat root biomass, particularly in the presence of bacteria (*i.e.*, greatest decrease in the priming effect with addition of bronopol).

4.2 Methods and materials

4.2.1 Soil sampling and treatments

The soil used for this laboratory experiment was sampled in November 2019 from the top 0-15 cm at John Bruce Pye Farm on the Camden campus of The University of Sydney, NSW, Australia (33°55′51″S, 150°39 ′ 38″E). The site is at 81 m above sea level. The mean temperature in July is 4.1 ℃, and the mean temperature in January is 29.8 ℃ (Bureau of Metreology). The soil is a clay loam and classified as a red chromosol according to the Australian Soil Classification (Isbell, 2002). The soil had 35% clay, 34% sand, 31% silt, 3.2% organic C, 0.23% N, 0.015% P, and a pH of 5.7 (H_2O).

Soil was air dried at 40 ℃. Stones and visible roots were removed from the soil by sieving through a 2 mm sieve. Then soil was passed through a 0.250 mm sieve to destroy all macroaggregates. For the 16-day incubation, a sample of 150 g of dried soil was used for each treatment. Each sample was divided into two portions (100 g and 50 g), each placed in a 100 ml polyethylene cup. The 100 g portion was used for respiration and aggregate measurements while the 50 g portion was used for microbial biomass measurements. The bactericide bronopol (2-bromo-2-nitropropane-1,3-diol) to reduce the activity of bacteria (B) and the fungicide captan (N-trichlorcmethylthio-4-cyclohexene-l ,2- dicarboximide) to reduce the activity of fungi (F) were added at the rate of 0.5 g kg^1 in solution at day 0, 7 and 15. The selection of the biocides was based on the results of previous studies of the efficiency of several biocides (Ullah, Carrillo & Dijkstra, 2023). Repeated additions

were used to suppress bacterial and fungal activity throughout the incubation period. The two substrates that were used in this study were glucose (99 atom% ^{13}C) and roots from wheat (*Triticum aestivum*, 99 atom% ^{13}C) (purchased from IsoLife, Wageningen, the Netherlands). Both substrates were diluted with non-labeled glucose and wheat root biomass, respectively, to achieve 10 atom% ^{13}C for both substrates. For glucose, 0.33 g kg^{-1} (0.13 g C kg^{-1}) in solution was added to the soil samples together with the biocides on day 0, 7 and 15 of the incubation (total addition of 1 g kg^{-1} of glucose) while 1 g kg^{-1} (0.46 g C kg^{-1}) of finely ground wheat roots was added as one application at the start of the incubation by thoroughly mixing the ground material with the soil. The biocide (no biocides, bronopol only, captan only, and bronopol + captan) and substrate treatments (no substrate, glucose, and wheat root biomass) were done in a full factorial design (12 treatment combinations) with three replicates for each treatment combination (total of 36 samples). All soil samples were watered up to 25 % gravimetric soil moisture content (or approximately 75 % of the water holding capacity). The incubation was run for 16 days at 25 °C to allow enough time for the substrates to be decomposed and for the microbes to form aggregates (Helfrich et al., 2008; Zhang et al., 2021).

4.2.2 Measurements

4.2.2.1 Soil respiration

Soil respiration and the ^{13}C in soil respiration were measured at day 0, 2, 4, 8 and 16 of the incubation. The 100 g samples were placed in 500 mL glass jars. A gas sample was taken from the jars and then jars were immediately closed with a lid fitted with a sampling port. After 2 h another gas sample was taken. For each gas sampling, 30 mL of gas was taken with a syringe and injected into a pre-evacuated 12 mL exetainer with an exhaust needle in the septum to keep the exetainer

at atmospheric pressure (Labco Limited, Lampeter, UK). The gas was then analyzed for CO_2 and ^{13}C-CO_2 on an isotope ratio mass spectrometer (IRMS Delta V Advantage with a Conflo IV and GasBench interface, Thermo Fisher Scientific, Bremen, Germany). The CO_2 concentrations in μL L^{-1} were converted to mg C L^{-1} using the ideal gas law. Total soil respiration ($CO_{2\text{-total}}$ in mg C kg^{-1} d^{-1}) was calculated using the following equation:

$$CO_{2\text{-total}} = (CO_{2\text{-t1}} - CO_{2\text{-t0}}) / t) \times V_{headspace} / m_{soil} \quad (1)$$

where $CO_{2\text{-t1}}$ and $CO_{2\text{-t0}}$ are the initial and final measurement of CO_2 (in mg C L^{-1}), t is the time between initial and final measurements (in d), $V_{headspace}$ is the volume (in L) of the jar used for the incubation, m_{soil} is the mass of dry soil (in kg). We then calculated the substrate-derived respiration ($CO_{2\text{-substrate}}$ in mg C kg^{-1} d^{-1}) (Dijkstra & Cheng, 2007) using the following equation:

$$CO_{2\text{-substrate}} = (^{13}C\text{-}_{resp+} - {}^{13}C_{resp-}) / (^{13}C_{substrate} - {}^{13}C_{resp-}) \times CO_{2\text{-total}} \quad (2)$$

where ^{13}C-$_{resp+}$, $^{13}C_{resp-}$, and $^{13}C_{substrate}$ are the ^{13}C atom % in respiration of soil with substrate (glucose or wheat root biomass), respiration of soil without substrate, and substrate, respectively. We further calculated cumulative total soil respiration and cumulative substrate-derived respiration over the 16-day period of the incubation by summing the average daily soil respiration and substrate-derived CO_2 rate between two measuring dates multiplied with the time interval between these two measuring dates.

Using the calculated cumulative total soil respiration and cumulative substrate-derived respiration over the 16-day period of the incubation, we calculated the primed soil C (mg C kg^{-1}) using the following equation:

$$C_{primed} = (CO_{2\text{-cum}} - CO_{2\text{-cum.substrate}}) - CO_{2\text{-control}} \quad (3)$$

where $CO_{2\text{-cum}}$ and $CO_{2\text{-cum.substrate}}$ are the cumulative total soil respiration and the cumulative substrate-derived respiration over the 16-day period of the incubation, respectively, $CO_{2\text{-control}}$ is the cumulative respiration in the control (without substrate).

4.2.2.2 Microbial biomass and CUE

Microbial biomass C (MBC) was measured on day 2 and 16, while the ^{13}C isotope signature of MBC was also measured on day 2 of the incubation. On each day, two subsamples (12 g each) of the 50 g portion were collected for each sample. One set was extracted by adding 40 mL of 0.05 M K_2SO_4 and filtered after shaking for an hour on an end-to-end shaker and centrifuging for 15 min at 4000 rpm. The other set was fumigated with chloroform for 48 h in a vacuum desiccator, and then extracted following the same procedures above for the non-fumigated samples. The filtered solutions were acidified with 150 µl 1 M H_3PO_4 and then analyzed for total C on a TOC analyzer (Shimadzu TOC-V csh, TNM-1, Kyoto, Japan). Aliquots of the fumigated and non-fumigated extracts collected on day 2 were dried in a ventilated oven at 65 °C, and the salts were analyzed for ^{13}C on the IRMS. The MBC (in mg kg^{-1} soil) was calculated as the difference in total C in fumigated and non-fumigated samples divided by 0.45 (Beck et al., 1997). The ^{13}C atom % in microbial biomass ($^{13}C_{microbial}$) was determined using equation (4) (Mehnaz et al., 2019):

$$^{13}C_{microbial} = (C_{fum} \times {}^{13}C_{fum} - C_{non\text{-}fum} \times {}^{13}C_{non\text{-}fum}) / (C_{fum} - C_{non\text{-}fum}) \quad (4)$$

where C_{fum} and $^{13}C_{fum}$ are the total C and ^{13}C atom % in fumigated extracts respectively, $C_{non\text{-}fum}$ and $^{13}C_{non\text{-}fum}$ are the total C and ^{13}C atom % in non-fumigated extracts, respectively. To calculate the substrate derived C in the microbial biomass ($MBC_{substrate}$ in mg kg^{-1} soil), we used (Mehnaz et al., 2019):

$$MBC_{substrate} = MBC \times ({}^{13}C_{mic+} - {}^{13}C_{mic-}) / ({}^{13}C_{substrate} - {}^{13}C_{mic-}) \quad (5)$$

where MBC is the microbial biomass C in soil with added substrate, $^{13}C_{mic+}$ and $^{13}C_{mic-}$ are the microbial ^{13}C atom% of the soils with and without substrate addition, respectively, and $^{13}C_{substrate}$ is the ^{13}C atom % of the added C substrate. Assuming that during the first 2 days of the incubation there was no microbial death of substrate derived C, the $MBC_{substrate}$ can be considered as the microbial growth on the substrate. We further calculated specific microbial growth on the substrate (d^{-1}) by dividing the $MBC_{substrate}$ by the MBC measured on day 2.

The microbial carbon use efficiency (CUE) during the first 2 days was calculated for each substrate as:

$$\text{Microbial CUE} = MBC_{substrate}/(MBC_{substrate} + CO_{2\text{-}substrate}) \quad (6)$$

where we used substrate-derived respiration during the first 2 days of the incubation for $CO_{2\text{-substrate}}$.

4.2.2.3 Aggregate separation

After 16 days of incubation, the 100 g soil samples used for gas sampling were analyzed for aggregates using a wet sieving method (Elliott, 1986). For aggregate separation, the soil was first gently crumbled in deionized water after slaking for one night at 5 ℃ to pass through a 2 mm sieve. Soils were then placed on a 0.25 mm sieve stacked on a 0.053 mm sieve that were moved up and down 50 times during a 2 min period. The soil on the 0.25 mm sieve (macro-aggregate fraction), 0.053 mm sieve (micro-aggregate fraction) and soil passed through the 0.053 mm sieve (silt and clay fraction) were dried in an oven at 105 ℃ for one day and weighed. The fraction of each aggregate size class was expressed as a % of the total soil weight. The dried aggregate fractions were then ground and analyzed for total C and ^{13}C on the IRMS. The % of aggregate fractions was calculated using the equation:

$$\text{aggregate \%} = (W_a / W_{Ts}) \times 100 \quad (6)$$

where W_a represents the aggregate fraction weight, W_{Ts} is the total weight of soil used for aggregate separation. We used the aggregate fractions to calculate the mean weight diameter (MWD) using the equation:

$$\text{MWD} = \sum_{i=1}^{n} \bar{x}_i \, w_i \quad (7)$$

where $\bar{x}_i$ is the mean diameter of size fraction i, w_i is the fraction of that aggregate size class, n is the number of size fractions (Hernandez, Hernandez & Garcia, 2017). Substrate-derived C in each aggregate (in mg kg^{-1} soil) was estimated using the equation:

$$C_{substrate} = C_{agg} \left(\left({}^{13}C_{agg.+} - {}^{13}C_{agg.-}\right) / \left({}^{13}C_{substrate} - {}^{13}C_{agg.-}\right)\right) \times w_i \quad (8)$$

where C_{agg} is C concentration in the aggregate size class (in mg kg^{-1} aggregate), ${}^{13}C_{agg.+}$ is the ${}^{13}C$ atom% measured in aggregates from glucose or wheat soil treatments, ${}^{13}C_{agg.-}$ is the ${}^{13}C$ atom% measured in aggregates from the no-substrate control, and w_i is the fraction of that aggregate size class.

4.2.3 Statistics

The statistical analysis for experimental data was conducted using JMP Pro 14 software. We used two-way analysis of variance (ANOVA) to examine the main and interactive effects of substrate addition (soil only, glucose, and wheat) and biocide treatments (none, bronopol, captan, and bronopol+ captan) on cumulative total soil respiration, MBC measured on day 2 and day 16, aggregate size fractions and MWD. We also used two-way ANOVA to examine main and interactive effects of substrate type (glucose and wheat) and biocide treatments on cumulative substrate-derived respiration, microbial growth on the substrate and substrate respiration during the first 2 days of the incubation, specific microbial growth on the substrate, microbial CUE, and substrate-derived C in total soil and in each of the aggregate size classes. Post-hoc tests (Tukey test) were performed to compare the means of each treatment combination. When necessary, data

were log-transformed to improve normal distribution and to reduce heteroscedasticity. All mean values of the results are presented with standard error.

4.3 Results

4.3.1 Total soil and substrate-derived respiration

The total cumulative soil respiration over the 16-d period of the incubation decreased with the addition of bronopol and captan individually, and where respiration decreased the most in soil samples receiving both biocides (bronopol+captan) (Figure 4.1a, Table 4.1). The cumulative substrate-derived respiration after 16 days was also lowest in soil samples receiving both biocides together. However, addition of bronopol only had no effect on cumulative respiration of glucose relative to the control (*i.e.,* treatment without substrate addition), while addition of captan increased cumulative respiration of glucose relative to the control (Figure 4.1b). Not surprisingly, cumulative respiration of glucose was in general higher than respiration of wheat root biomass.

4.3.2 Microbial parameters

After 2 days, addition of bronopol only decreased MBC in the wheat root biomass treatment, while addition of captan decreased MBC in the control and wheat root biomass treatments (Figure 4.2a, Table 4.1). When both biocides were added, MBC decreased in the control and glucose treatments. Addition of both biocides decreased MBC in the control and glucose treatments, but not in the wheat root biomass treatment. At the end of the incubation period, there was an overall decrease in the MBC across all treatments (Figure 4.2b). The MBC was generally lower with addition of bronopol alone or in combination with captan across all substrate treatments.

Figure 4.1: Cumulative total soil respiration (a) and cumulative substrate-derived respiration (b) during the 16-day incubation period with respect to substrate treatment (control, glucose, wheat) and biocide treatment (none, bronopol, captan, bronopol+ captan). Error bars represent standard error. P values for ANOVA are reported when significant ($p < 0.05$). bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

Microbial growth on substrate after two days of incubation was generally higher from glucose than from wheat root biomass (Figure 4.3a, Table 4.1). Microbial growth on glucose was not affected by the addition of bronopol but decreased when captan or both biocides were added to the soil. Microbial growth on wheat root biomass strongly decreased when bronopol or captan were added to the soil, while there was also a non-significant reduction by more than 50% in the microbial growth on wheat root biomass when both biocides were added.

Table 4.4: ANOVA p values for the soil biocide and substrate treatments on soil total 16-day cumulative respiration, cumulative 16-day substrate-derived respiration, substrate-derived respiration during the first 2 days, primed C, microbial biomass C (MBC) on day 2 and day 16, substrate derived MBC, specific microbial growth on substrate, C use efficiency (CUE), macroaggregates, microaggregates, silt and clay fraction, mean weight diameter (MWD), and substrate-derived C in macroaggregates, microaggregates, and silt and clay fraction, and total soil. P < 0.05, significant, p< 0.1, marginally significant, ns, not significant.

Parameter	ANOVA P values		
	Biocide	Substrate	Biocide × substrate
Cum total respiration	<0.0001	0.0009	ns
Cum substrate-derived respiration	<0.0001	<0.0001	<0.0001
Substrate-derived resp during first 2 days	<0.0001	<0.0001	0.02
Primed carbon	0.001	ns	ns
Microbial biomass carbon day 2	0.0001	ns	<0.0001
Microbial biomass carbon day 16	<0.0001	ns	ns
Microbial growth on substrate	0.03	0.0002	0.09
Specific microbial growth on substrate	0.03	<0.0001	0.007
Microbial CUE of substrate	0.05	ns	0.005
Macroaggregates (%)	<0.0001	ns	0.03
Microaggregates (%)	<0.0001	ns	0.06
Silt and clay fraction (%)	0.01	ns	ns
Mean weight diameter aggregates	0.0006	ns	ns
Sub-der C macroaggr (mg C kg^{-1} aggregate)	<0.0001	<0.0001	<0.0001
Sub-der C microaggr (mg C kg^{-1} aggregate)	ns	ns	ns
Sub-der C silt and clay (mg C kg^{-1} aggregate)	ns	ns	ns
Sub-der C macroaggr (mg C kg^{-1} soil)	<0.0001	<0.0001	<0.0001
Sub-der C microaggr (mg C kg^{-1} soil)	ns	ns	ns
Sub-der C silt and clay (mg C kg^{-1} soil)	ns	ns	ns
Sub-der C total soil (mg C kg^{-1} soil)	<0.0001	<0.0001	<0.0001

Respiration of substrates during the first two days of the incubation showed similar results, where respiration rates were generally higher for glucose than for wheat root biomass, and where respiration rates decreased the most when both bronopol and captan were added (Figure 4.3b, Table 4.1). However, addition of captan alone had only small effects on glucose respiration, but stronger effects on wheat root biomass respiration.

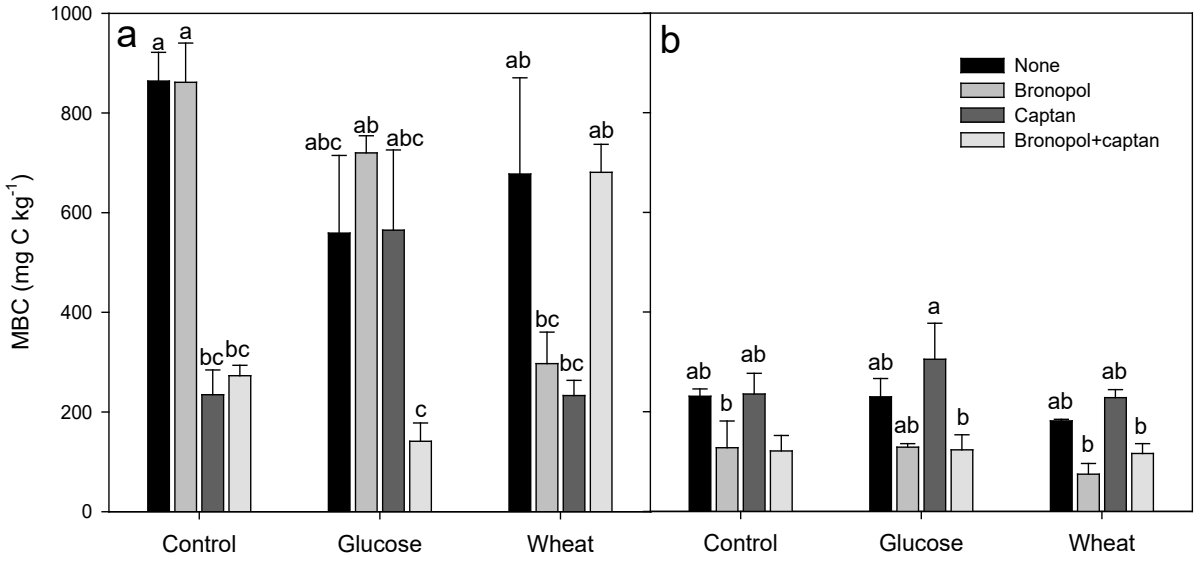

Figure 4.2: Total microbial biomass carbon (MBC, mg kg^{-1} soil) on day 2(a) and day 16 (b) of the incubation with respect to substrate treatment (control, glucose, wheat) and biocide treatment (none, bronopol, captan, and bronopol+captan). Presented values are means ± standard error. Columns with different letters for each day are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

In general, specific microbial growth on substrates did not differ between the two substrates and was not affected by biocide addition (Figure 4.3c, Table 4.1). The exception was that specific microbial growth on glucose significantly increased when both biocides were added.

Microbial carbon use efficiency (CUE) did not differ between the two substrates (across biocide treatments), but showed different biocide effects for each substrate (Figure 4.3d, Table 4.1).

Biocide addition had little effect on microbial CUE of glucose, but had strong effects on microbial CUE of wheat root biomass, where the lowest CUE was observed in soils amended with bronopol.

Primed C (*i.e.*, C loss) from the soil was the highest when bronopol was added (*i.e.*, with bacterial suppression) with no significant difference between glucose and wheat root biomass (Figure 4.4). However, suppressing fungi (using captan only) and both microbes in the soil (using bronopol and captan) led to a negative priming effect, with no significant difference between glucose and wheat treatments. Only the effect of biocide treatment was found to be significant ($p = 0.001$) on the primed C.

4.3.3 Aggregate size classes and MWD

We observed a significant biocide effect on the percentage of macroaggregates (Table 4.1) where macroaggregates decreased with the addition of captan or both biocides, especially in soils with glucose (Table 4.2, although the Tukey's HSD post-hoc test revealed no significant differences among treatments). The addition of bronopol decreased the fraction of microaggregates in both soil substrate treatments, where the lowest fraction was observed in soils treated with glucose and bronopol. The silt and clay fraction was significantly affected by biocide treatment (Table 4.1) with the highest fraction observed when both biocides were applied (Table 4.2).

Table 4.2 Mean ± standard error of soil aggregate fractions (macroaggregates: >250 µm, microaggregates: 53-250 µm, silt and clay fraction: <53 µm) as a percentage, and the substrate-derived carbon (C in macroaggregates, microaggregates, and silt and clay fraction (in mg kg^{-1}aggregate) affected by substrate treatments (control, glucose, and wheat) and biocide treatments (none, bronopol, captan, and bronopol+captan). Values with different letters are significantly different according to Tukey's HSD post-hoc test (only shown for parameters with significant differences, $p < 0.05$).

Treatments		Aggregate fractions (%)			Substrate- derived C in soil aggregate fractions (mg kg^{-1} aggregate)		
Substrate	Biocide	>250 µm	53-250 µm	< 53 µm	> 250 µm	53-250 µm	<53 µm
control	none	51.8 ± 12.4	31.6 ± 9.8 ab	16.6 ± 2.6	-------	-------	-------
glucose	none	53.6 ± 3.6	27.0 ± 1.6 ab	19.4 ± 5.2	61.9 ± 18.0 b	67.7 ± 18.4	43.4 ± 25.0
wheat	none	54.1 ± 11.0	27.9 ± 9.5 ab	18.0 ± 2.1	167 ± 78.8 b	72.0 ± 33.4	36.3 ± 21.0
control	bronopol	55.7 ± 5.5	22.9 ± 2.6 ab	21.4 ± 2.9	-------	-------	-------
glucose	bronopol	73.6 ± 4.9	11.4 ± 3.7 b	15.0 ± 1.1	50.2 ± 25.6 b	25.1 ± 14.1	20.6 ± 11.9
wheat	bronopol	37.3 ± 4.7	36.3 ± 5.0 ab	26.4 ± 2.4	54.4 ± 3.7 b	63.9 ± 33.2	40.7 ± 23.5
control	captan	30.1 ± 4.4	42.6 ± 1.0 ab	27.3 ± 7.8	-------	-------	-------
glucose	captan	20.2 ± 3.3	49.8 ± 4.3 a	30.0 ± 4.0	31.8 ± 3.2 b	23.3 ± 13.5	23.5 ± 13.6
wheat	captan	29.2 ± 0.6	30.6 ± 5.4 ab	32.8 ± 6.5	27.0 ± 2.7 b	28.7 ± 16.6	30.5 ± 17.6
control	bronopol+captan	25.3 ± 5.9	48.9 ± 6.5 a	25.8 ± 0.7	--------	-------	-------
glucose	bronopol+captan	22.3 ± 3.9	41.2 ± 7.9 ab	41.1 ± 10.0	58.7 ± 6.0 b	36.1 ± 20.9	31.3 ± 18.1
wheat	bronopol+captan	29.1 ± 4.7	45.6 ± 5.7 a	25.3 ± 1.0	2534 ± 70.3 a	73.0 ± 48.3	27.1 ± 15.6

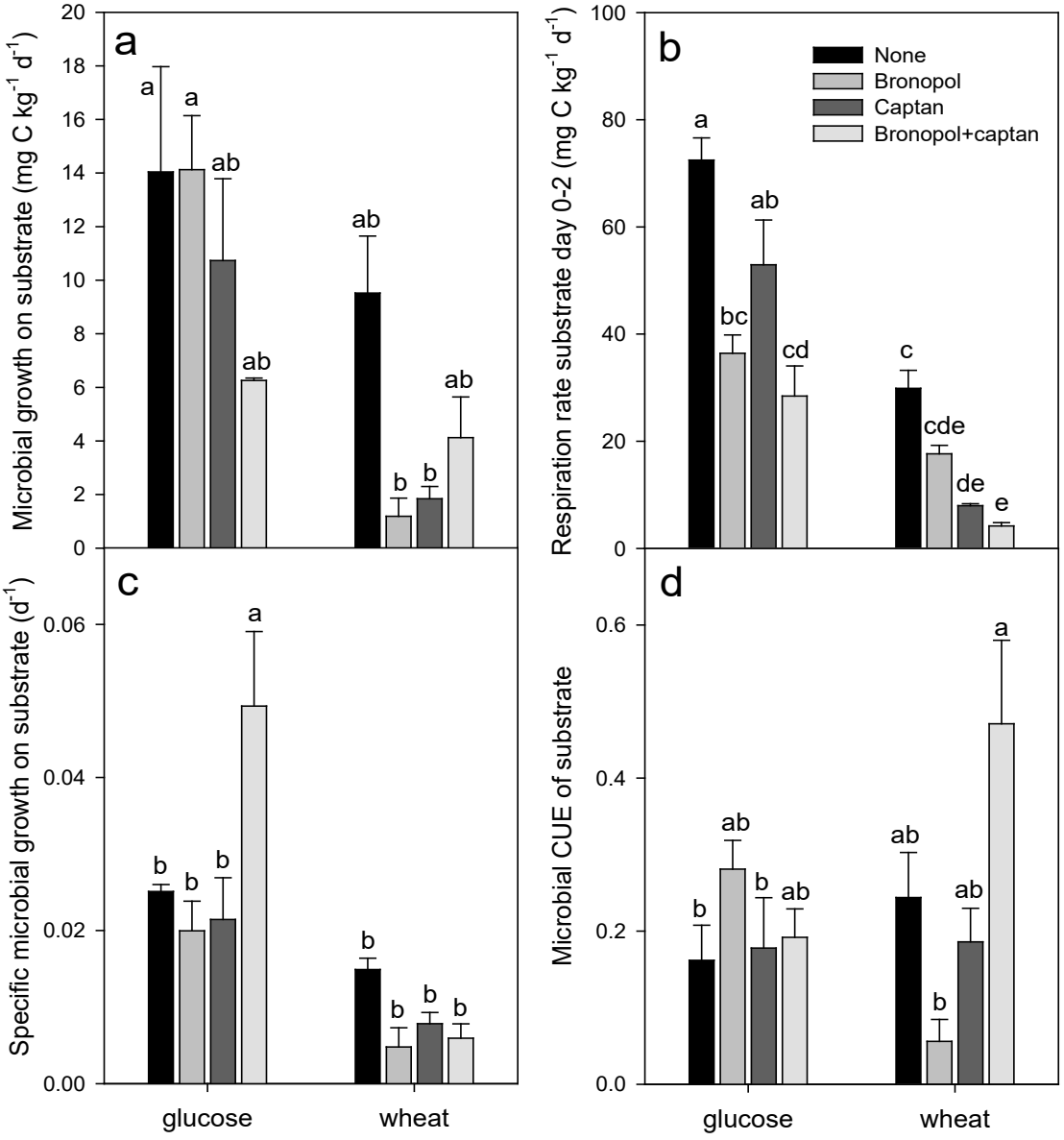

Figure 4.3: Microbial growth (a), respiration (b), specific microbial growth (c), and microbial carbon use efficiency (CUE, d) of glucose and wheat during the first 2 days of the incubation for different biocide treatments (none, bronopol, captan, bronopol+captan). Error bars represent standard error. Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

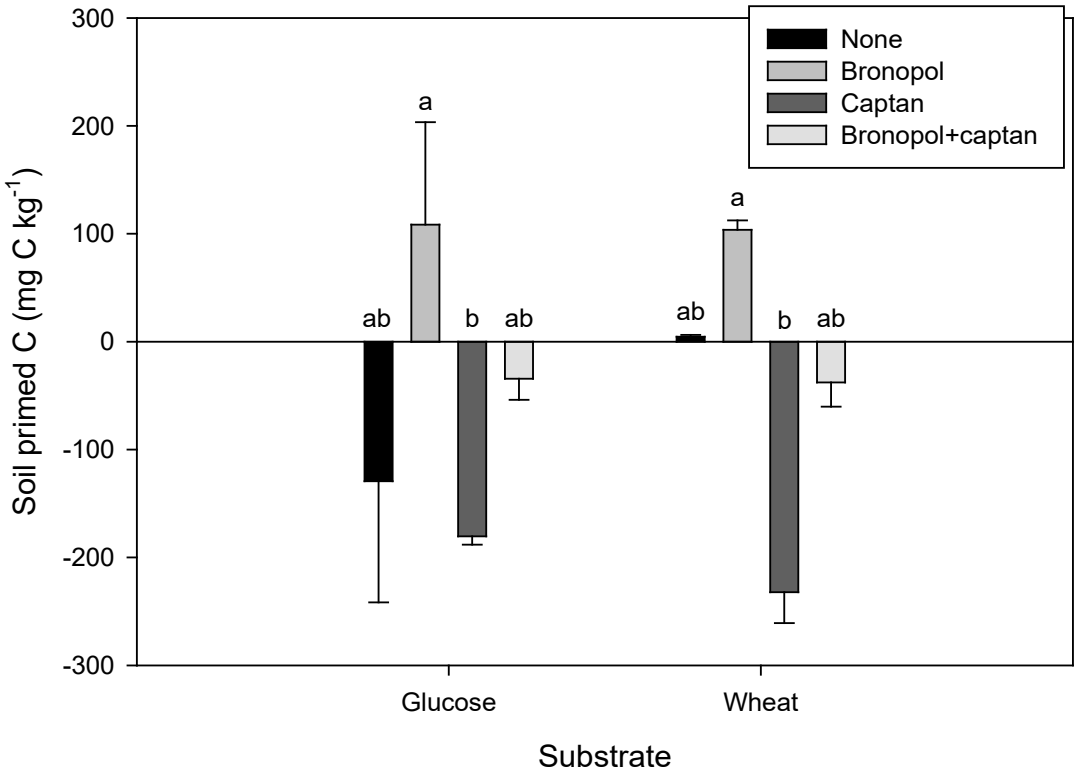

Figure 4.4: The soil primed C in substrate (glucose and wheat) and biocide (none, bronopol, captan, and bronopol+captan) soil treatments. Error bars represent standard error. Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

Across biocide treatments, the MWD after 16 days of incubation did not vary with substrate treatments, but it was significantly affected by the biocide treatment (Table 4.1). In general, the MWD decreased with captan addition, either added alone or in combination with bronopol, although we could not discern significant differences among treatment combinations using the Tukey's HSD post-hoc test (Figure 4.5). The MWD was not related to microbial growth or CUE of the substrates.

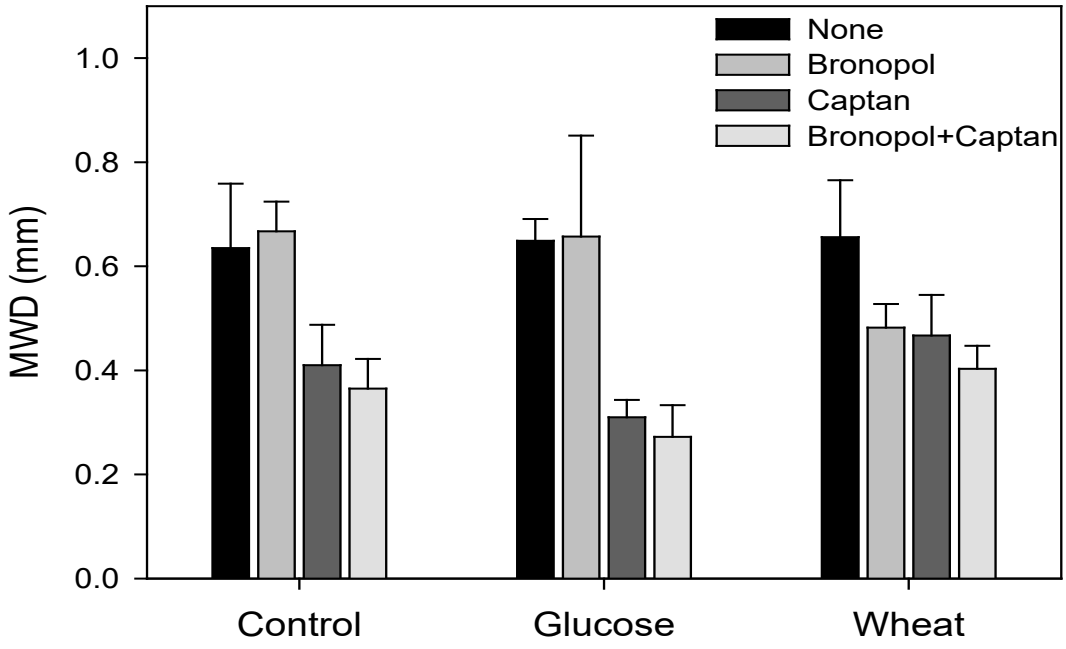

Figure 4.5: Mean weight diameter (MWD) after 16 days of the incubation with respect to substrate treatment (control, glucose, wheat) and biocide treatment (non, bronopol, captan, bronopol+captan). Error bars represent standard errors.

Both biocide and substrate treatments significantly affected the substrate-derived C concentration (mg C kg^{-1} aggregate) in macroaggregates (Table 4.1), where they were highest for wheat root biomass and highest when both biocides were added (Table 4.2). Consequently, the highest substrate-derived C concentration was observed for wheat root biomass when both biocides were added. Treatments had no significant effects on substrate-derived C concentration in the microaggregates and silt and clay fraction (Tables 1, 2).

4.3.4 Recovered substrate C in soil

After accounting for aggregate fraction size, there was generally more of the wheat-derived C than the glucose-derived C in macroaggregates (in mg C kg^{-1} soil) across all biocide treatments (Figure 4.6a, Table 4.1). The addition of either biocide had little effect on the substrate-derived C in macroaggregates for both substrates, but increased wheat-derived C significantly when both biocides were added. The substrate-derived C in microaggregates and in the silt and clay fraction varied across all biocide treatments, but showed no significant differences among treatments (Figure 4.6b, c). The substrate-derived C in total soil presented a similar pattern as the substrate C in macroaggregates (Figure 4.6d).

We observed no relationships between microbial growth on the substrate and the substrate-derived C recovered in the different aggregate size fractions or the total soil. There were also no relationships between microbial CUE and the recovered substrate-derived C in the different aggregate size classes.

4.4 Discussion

In this experiment, we selected two types of organic materials to be used by soil microbes as substrates: a labile organic compound (glucose) and a complex organic compound (wheat root biomass) to differentiate between the impact of each on the soil structure in the form of soil aggregates. We used glucose as a substrate in this experiment to represent root exudates from live plant roots, while we used wheat root biomass to represent plant litter input.

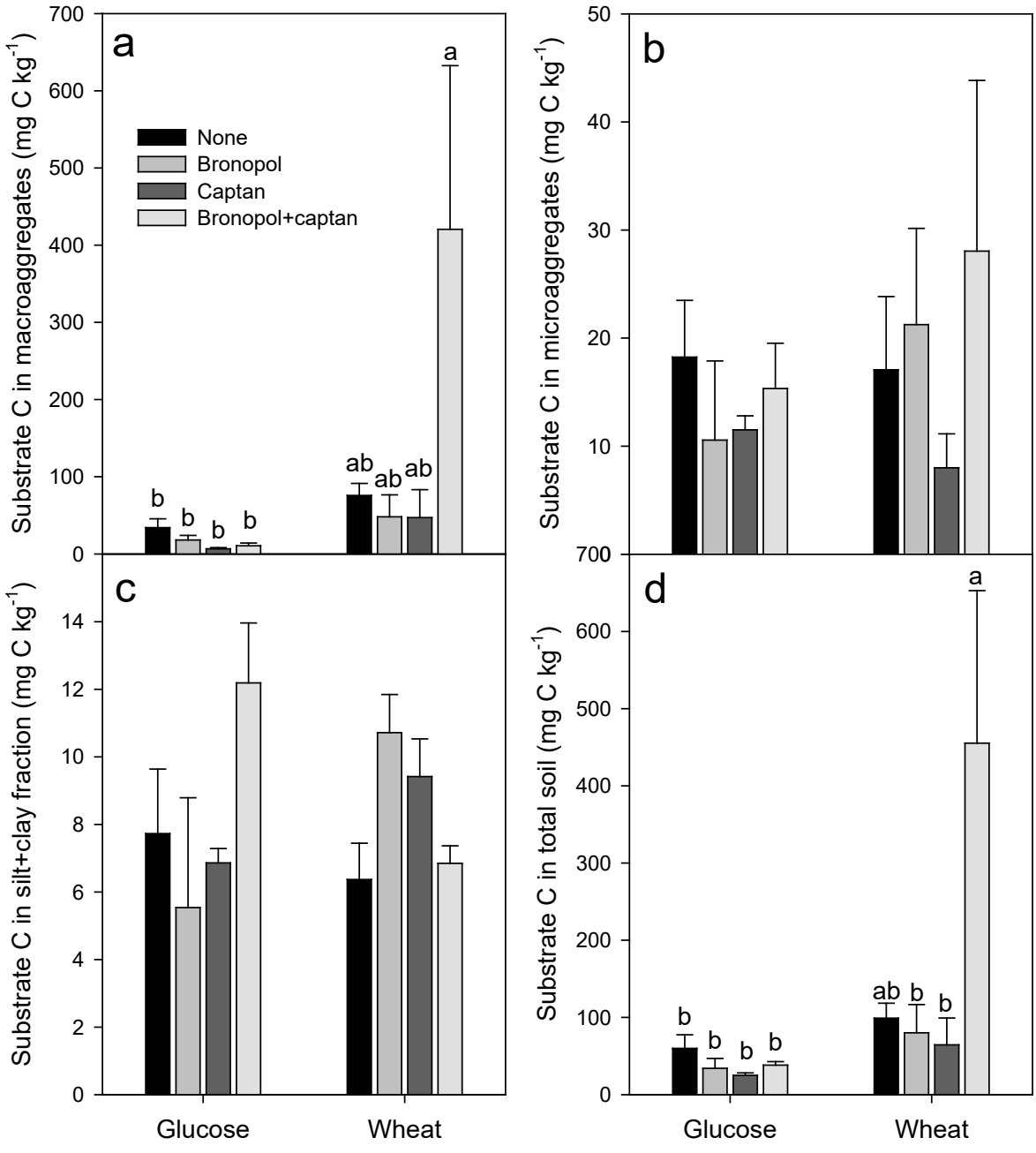

Figure 4.6: The total substrate-derived C recovered in the soil (mg C kg^{-1} soil) at the end of 16 d of incubation, error bars represent standard errors. P values for ANOVA are reported when significant ($p < 0.05$). Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

4.4.1 Bacterial *versus* fungal decomposition of glucose and wheat root biomass in the soil

The total soil respiration during the 16 d of the incubation showed an overall decrease with the addition of biocides due to the reduction in soil microbial activity rate. The significantly lower respiration rates from samples treated with biocides indicated that using bronopol and captan decreased the decomposition of soil organic matter, highlighting the significance of the role of bacteria and fungi for organic matter decomposition compared to other soil biota. As expected and consistent with previous research findings, microbial respiration from the labile C substrate was higher than microbial respiration from the complex C substrate (Olagoke et al., 2022). We note that we added glucose at different times throughout the incubation period to simulate root exudation. Therefore, glucose that was added later-on in the incubation had less time to be decomposed, but more of this could potentially be decomposed compared to wheat root biomass if we continued with the incubation.

The suppression of bacterial activity in the soil (using bronopol) reduced the decomposition of glucose during the first two days more than when fungal activity was suppressed (using captan, Figure 4.3b), indicating that soil bacteria were more important in the initial stages of decomposition of labile C substrates (like glucose) than soil fungi, consistent with the recent findings by Ullah, Carrillo and Dijkstra (2023). However, both soil microbes were important for the decomposition of complex C (wheat root biomass) during the first two days (Figure 4.3b) and during the whole incubation experiment (Figure 4.1b). Based on the above, the first part of our first hypothesis is accepted (removing bacteria using bronopol will reduce the decomposition of glucose in the soil) during the first 2 days, but not over the whole incubation period. Given that both bacteria and fungi are capable of using various C sources (Martinović et al., 2022), it is

possible that with time the decomposition of glucose was replaced by other microbes such as fungi. In support of the second hypothesis, removing the fungi using captan reduced the decomposition of the more complex substrate (wheat root biomass), but over the whole incubation experiment, it also increased the decomposition of the labile substrate (glucose) (Figure 4.1b). As explained for bronopol above, with time other microbes than fungi may have taken over in the decomposition of wheat root biomass.

The total soil microbial biomass carbon (MBC) at the beginning of the incubation (day 2) was generally higher than at the end of incubation (day 16), likely due to the high availability of fresh organic matter addition (glucose or wheat root biomass) and labile C from the soil organic matter in the control treatment providing resources for microbial growth, whereas at the end of the incubation those resources may have depleted reducing their growth (*e.g.*, Zornoza et al. 2016). After the second day of incubation, the observed higher microbial growth on glucose (Figure 4.3a) indicates that glucose was more readily incorporated in microbial biomass compared to wheat root biomass. Microbial growth on glucose reduced the most when both biocides were added, but because MBC also strongly declined (Figure 4.2a), specific microbial growth of the microbial community that remained active was the highest for this treatment (Figure 4.3c). In contrast, microbial growth on wheat was reduced the most when either bronopol or captan was added (Figure 4.3a), with no impact on specific growth rates (Figure 4.3c). We are unclear what the surviving microbial community was, but the specific growth rate of this community on glucose was even higher than the whole microbial community in the treatment without biocides. These results suggest that by suppressing bacteria and/or fungi with biocides may have stimulated growth of the remaining microbial community on wheat root biomass. In comparison with previous

research, suppressing fungi in the soil using captan reduced microbial growth on maize roots and also reduced the root derived microbial growth compared to the soil without captan (Helfrich et al., 2008). Another study found that the microbial growth on a relatively labile C substrate (starch) was also higher than microbial growth on more recalcitrant C (cellulose) (Olagoke et al., 2022). However, the latter study reported that the effect of the substrate on the bacterial communities also depended on the taxonomy of the soil bacteria, where some taxa were found to be more dependent on complex substrates like cellulose.

4.4.2 CUE and priming effects of glucose and wheat root biomass in the soil

The removal of bacteria from the soil using bronopol resulted in a non-significant increase in the CUE for glucose, but significantly decreased the CUE for wheat root biomass, which led us to reject the first hypothesis (we expected a greater reduction in CUE of glucose with bronopol). Our results suggest that bacteria and fungi did not differ in their CUE when decomposing glucose, but that the fungi remaining after bronopol addition had a lower CUE when decomposing wheat root biomass compared to bacteria. Several other studies also reported that fungi had a lower CUE than bacteria (Ullah, Carrillo & Dijkstra, 2021b), although they did not make a distinction between substrate type. Here, we found that fungi only had a lower CUE when decomposing the more complex wheat root biomass. The reduced CUE on wheat root biomass in response to bronopol addition further suggests that bacteria played a significant role in decomposition of wheat root biomass. This is also supported by the reduction in wheat derived respiration with addition of bronopol (Figures 4.1b, 4.3b), and that by removing bacteria with relatively high CUE would then reduce CUE of the remaining microbial community.

The addition of captan alone had no effect on the CUE for glucose and wheat root biomass, and these results do not support our second hypothesis regarding CUE (we expected a reduction in CUE of wheat root biomass with captan). The absence of an effect with captan addition alone on the CUE for glucose may be a result of fungi having played a minor role in glucose decomposition (as suggested by Figures 4.1b, 4.3b), but it is unclear why it did not affect the CUE for wheat root biomass. If fungi decomposing wheat root biomass had a lower CUE than bacteria, and bacteria played an important role in decomposing wheat root biomass, as argued above, then the CUE should have increased after captan addition. Others observed that oligotrophic microbial communities (including fungi) had higher CUEs decomposing more recalcitrant C than copiotrophic communities in soils from the Tibetan Plateau (Ma et al., 2023), suggesting that fungi specialized in decomposing recalcitrant C have relatively high CUEs. The microbial CUE can vary strongly among different microbial groups (Manzoni et al., 2012) and other microbial groups may have been responsible for wheat biomass decomposition, so that removal of fungi had no effect on the CUE. Interestingly, the highest CUE was observed in the wheat root biomass treatment where both bronopol and captan were added (Figure 4.3d). Possibly, the addition of both biocides can provide an abundance of C and nitrogen (N) resources for surviving soil microbes as suggested by Ullah, Carrillo and Dijkstra (2021a), where these resources can mediate an increase in the microbial growth and thus resulting in a higher CUE.

There was no significant difference in the effect of the addition of different substrates on soil primed C (Figure 4.4), which led us to reject the fourth hypothesis (we expected a higher positive effect from glucose addition than wheat root biomass on the soil priming effect). Furthermore, we expected that bacteria would be mostly responsible for the priming effect caused by glucose, but

instead we observed that removal of bacteria with bronopol addition resulted in the largest priming effect, while removal of fungi with captan addition resulted in negative priming effect, both for glucose and wheat root biomass. Our results therefore indicate that fungi, not bacteria, played an important role in the priming effect. Some have argued that fungi are more efficient than bacteria in using added substrates to mine for nutrients that are locked in soil organic matter (De Deyn, Cornelissen & Bardgett, 2008; Fang et al., 2018; Soares, Kritzberg & Rousk, 2017). Given that we did not add any inorganic nutrients to our soils, it is therefore possible that addition of substrate, either as glucose or as wheat root biomass, stimulated fungi more than bacteria to mine for nutrients in soil organic matter. The fact that there was no difference in the substrate type on the priming effect (Table 4.1), further indicates that the substrate complexity or addition of nutrients with wheat root biomass played no important role on the priming effect in our study.

4.4.3 The role of bacteria and fungi on soil structure and carbon sequestration in the soil with glucose and wheat root biomass addition

In general, effects of substrate and biocide addition on soil structure (aggregate formation and MWD) were small compared to their effects on microbial parameters mentioned above. Likely, the 16-day incubation was not long enough to cause large effects on soil structure. However, across all substrate treatments the MWD was significantly affected by the biocide treatment ($p = 0.0006$, Table 4.1), where the MWD was reduced when captan was added by itself or combined with bronopol (Figure 4.5). This effect on MWD was largely due to biocide effects on the percentage of macroaggregates (Table 4.2). This suggests that fungi were more important for soil aggregation than bacteria, regardless of the type and presence of substrate added. Previous research by Zhang et al. (2018) found that the addition of wheat straw increased the fungal abundance more than

bacterial abundance, and significantly increased macroaggregate formation, after 9 years of continuous straw return in a field study under conservation tillage. They attributed the increase in fungal community abundance to the improvement of soil moisture and aeration. In a meta-analysis, Lehmann, Zheng and Rillig (2017) highlighted that while both bacteria and fungi were found to be important influencers of the formation of macro and microaggregates, fungi were found more influential in the macroaggregate formation, likely due to their formation of hyphae binding microaggregates together into macroaggregates.

There was no significant relationship found between CUE of added substrates and soil aggregate formation. Consequently, the third hypothesis is rejected. Again, one of the reasons of not observing relationships between CUE of added substrate and aggregate formation could be that the study was not long enough to detect relationships. We further note that we only measured CUE of the added substrate but that both fungi and bacteria also use organic matter already present in the soil, and we did not measure CUE on soil organic matter. Therefore, production of microbial necromass from soil organic matter may have contributed to aggregate formation, but unfortunately, that is something we did not measure, and this may have obscured any relationship between aggregate formation and microbial CUE on added substrates in our study.

The addition of bronopol and captan together significantly increased wheat-derived C in macroaggregates, both expressed per unit of aggregate and per unit of soil (Table 2, Figure 6). This suggests that although fungi may be more important for forming soil aggregates, decomposition of wheat root biomass by both bacteria and fungi may have outweighed the protection of wheat root C in new macroaggregates. However, we note that there was a large variation in wheat-derived

C in macroaggregates among the three replicates treated with both biocides (bronopol+captan). The results of the wheat-derived C in macroaggregates may indicate that when both biocides were added together, very little of the wheat root biomass was decomposed, as was also shown in Figure 4.1b. Although the wheat root biomass was finely ground, it may have remained on the top sieve with the macroaggregate size fraction when aggregate size classes were separated, and therefore not directly incorporated in the macroaggregates. Regardless, our results indicate that suppression of bacteria and fungi strongly reduced decomposition of wheat root biomass.

In comparison with our results, previous research has indicated a positive effect of both labile (glucose) and complex plant organic matter (maize crop residue) amendments on aggregate formation and aggregate C content when each type of substrate was applied alone, but where the addition of both substrates together increased soil aggregation and associated C content in aggregates the most after a year-long laboratory study (Walia & Dick, 2018). In our study we were mostly interested in differentiating between the decomposition and effects of labile (glucose) and complex (wheat root biomass) substrates on soil aggregation and soil C when added to soil alone, and therefore the addition of both substrates was not considered. Future research is therefore needed to investigate if the addition of both substrates used in this work together will be more beneficial to soil aggregation and soil C sequestration in soil aggregates.

There are several assumptions we made in our study. First of all, we assumed that the biocides we used would be target-specific, *i.e.*, that the bactericide bronopol would reduce the activity of bacteria and the fungicide captan would reduce the activity of fungi. While this was observed in other studies (*e.g.*, Pan et al., 2019; Ullah et al., 2021a), there is also evidence that these biocides

can affect non-target organisms in soil (Milenkovski et al., 2010; Piotrowska-Seget et al., 2008). Since we did not measure the microbial community composition in our study, it is therefore possible that we also reduced activity of non-targeted microorganisms. Second, we assumed that addition of these biocides would not directly affect soil aggregation. These biocides could potentially have acted as binding agents, although this is unlikely given that this would have resulted in an increase in aggregation with biocide addition, while we found that the biocides reduced soil aggregation (Table 4.1, Figure 4.5).

4.5 Conclusions

We conclude that the soil bacterial communities were more important than soil fungi for the decomposition of the labile substrate glucose, but only in the initial stages of the decomposition, whereas both microbes were responsible for the decomposition of the more complex substrate wheat root biomass over the entire 16-day incubation period. In contrast to our hypothesis, we observed no relationship between the microbial CUE of added substrates and the formation of aggregates. Furthermore, there was no strong difference between the microbial CUE of glucose and wheat root biomass when added to the soil. Soil primed C was strongly linked to fungal activity rather than to bacterial activity, while the priming effect was less influenced by the type of substrate added to the soil. Soil fungi were also more important than soil bacteria for soil aggregate formation, while the substrate type was less important for soil aggregation. These results therefore highlight the importance of fungi for soil priming (C loss pathway) and soil aggregation (C protection pathway) in the soil that we studied. The loss of C due to soil priming was often higher than the substrate-C that remained in the soil after 16 days, suggesting that these soils on balance lost C with substrate addition, even after suppressing bacterial and/or fungal activity. The

exception was when we suppressed both bacterial and fungal activity with biocides and observed a strong increase in wheat-derived C in macroaggregates, indicating that decomposition by both bacteria and fungi can strongly reduce the potential for wheat root biomass C to be incorporated in soil macroaggregates. We recommend the use of specific biomarkers (*e.g.*, amino sugars and phospholipid fatty acids) in future research for a better understanding of how bacteria and fungi and their necromass affect decomposition and aggregation for different substrates.

4.6 Acknowledgements

I thank Milad Bagheri Shirvan for running samples on the isotope ratio mass spectrometer and TOC analyzer.

4.7 References

Badalucco, L., Pomare, F., Grego, S., Landi, L., Nannipieri, P., 1994. Activity and degradation of streptomycin and cycloheximide in soil. Biol. Fertil. Soils 18, 334-340.

Beck, T., Joergensen, R., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29, 1023-1032.

Blagodatskaya, D., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45, 115-131.

Chao, L., Liu, Y., Freschet, G.T., Zhang, W., Yu, X., Zheng, W., Guan, X., Yang, Q., Chen, L., Dijkstra, F.A., Wang, S., 2019. Litter carbon and nutrient chemistry control the magnitude of soil priming effect. Funct. Ecol. 33, 876-888.

Chen, S.-K., Edwards, C.A., Subler, S., 2001. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biol. Biochem. 33, 1971-1980.

De Deyn, G.B., Cornelissen, J.H., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecol. Lett. 11, 516-531.

Deng, S., Zheng, X., Chen, X., Zheng, S., He, X., Ge, T., Kuzyakov, Y., Wu, J., Su, Y., Hu, Y., 2021. Divergent mineralization of hydrophilic and hydrophobic organic substrates and their priming effect in soils depending on their preferential utilization by bacteria and fungi. Biol. Fertil. Soils 57, 65-76.

Dijkstra, F.A., Cheng, W., 2007. Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biol. Biochem. 39, 2264-2274.

Domeignoz-Horta, L.A., Pold, G., Liu, X.A., Frey, S.D., Melillo, J.M., DeAngelis, K.M., 2020. Microbial diversity drives carbon use efficiency in a model soil. Nat. Comm. 11, 3684.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627-633.

Fang, Y., Nazaries, L., Singh, B.K., Singh, B.P., 2018. Microbial mechanisms of carbon priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils. Glob. Change Biol. 24, 2775-2790.

Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biol. Biochem. 43, 1705-1713.

Helfrich, M., Ludwig, B., Potthoff, M., Flessa, H., 2008. Effect of litter quality and soil fungi on macroaggregate dynamics and associated partitioning of litter carbon and nitrogen. Soil Biol. Biochem. 40, 1823-1835.

Hernandez, T., Hernandez, M.C., Garcia, C., 2017. The effects on soil aggregation and carbon fixation of different organic amendments for restoring degraded soil in semiarid areas: Effect of amendments on soil aggregation and C fixation. Eur. J. Soil Sci. 68, 941-950.

Hütsch, B.W., Augustin, J., Merbach, W., 2002. Plant rhizodeposition — an important source for carbon turnover in soils. J. Plant Nutr. Soil Sci. 165, 397-407.

Isbell, R., 2002, The Australian Soil Classification, 2nd edn, CSIRO Publishing, Collingwood, Victoria, Australia.

Kooijman, A.M., Bloem, J., van Dalen, B.R., Kalbitz, K., 2016. Differences in activity and N demand between bacteria and fungi in a microcosm incubation experiment with selective inhibition. Appl. Soil Ecol. 99, 29–39.

Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biol. Biochem. 42, 1363-1371.

Ladan, S., Jacinthe, P.A., 2016. Evaluation of antibacterial and antifungal compounds for selective inhibition of denitrification in soils. Environ Sci Process. Impacts 18, 1519-1529.

Lehmann, A., Zheng, W., Rillig, M.C., 2017. Soil biota contributions to soil aggregation. Nat. Ecol. Evol. 1, 1828-1835.

Ma, S., Zhu, W., Wang, W., Li, X., Sheng, Z., 2023. Microbial assemblies with distinct trophic strategies drive changes in soil microbial carbon use efficiency along vegetation primary

succession in a glacier retreat area of the southeastern Tibetan Plateau. Sci. Tot. Environ. 867, 161587.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79-91.

Martinović, T., Mašínová, T., López-Mondéjar, R., Jansa, J., Šturs ová, M., Starke, R., Baldrian P., 2022. Microbial utilization of simple and complex carbon compounds in a temperate forest soil. Soil Biol. Biochem. 173, 108786.

Mehnaz, K.R., Corneo, P.E., Keitel, C., Dijkstra, F.A., 2019. Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen mineralization and nitrous oxide emission from soil. Soil Biol. Biochem. 134, 175-186.

Milenkovski, S., Bååth, E., Lindgren, P.-E., Berglund, O., 2010. Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. Ecotoxicol. 19, 285–294.

Mo, F., Ren, C., Yu, K., Zhou, Z., Phillips, R.P., Luo, Z., Zhang, Y., Dang, Y., Han, J., Ye, J.S., Vinay, N., Liao, Y., Xiong, Y., Wen, X., 2022. Global pattern of soil priming effect intensity and its environmental drivers. Ecol. 103, e3790.

Müller, K., Marhan, S., Kandeler, E., Poll, C., 2017. Carbon flow from litter through soil microorganisms: From incorporation rates to mean residence times in bacteria and fungi. Soil Biol. Biochem. 115, 187-196.

Muñoz-Leoz, B., Garbisu, C., Charcosset, J.Y., Sánchez-Pérez, J.M., Antigüedad, I., Ruiz-Romera, E., 2013. Non-target effects of three formulated pesticides on microbially mediated processes in a clay-loam soil. Sci. Total Environ. 449, 345-354.

Muñoz-Leoz, B., Ruiz-Romera, E., Antigüedad, I., Garbisu, C., 2011. Tebuconazole application decreases soil microbial biomass and activity. Soil Biol. Biochem. 43, 2176-2183.

Na, M., Hicks, L.C., Zhang, Y., Shahbaz, M., Sun, H., Rousk, J., 2022. Semi-continuous C supply reveals that priming due to N-mining is driven by microbial growth demands in temperate forest plantations. Soil Biol. Biochem. 173, 108802.

Olagoke, F.K., Bettermann, A., Nguyen, P.T.B., Redmile-Gordon, M., Babin, D., Smalla, K., Nesme, J., Sørensen, S.J., Kalbitz, K., Vogel, C., 2022. Importance of substrate quality and clay content on microbial extracellular polymeric substances production and aggregate stability in soils. Biol. Fertil. Soils 58, 435-457.

Pan, Y., Wu, Y., Li, X., Zeng, J., Lin, X., 2019. Continuing impacts of selective inhibition on bacterial and fungal communities in an agricultural soil. Microbiol. Ecol. 78, 927–935.

Piotrowska-Seget, Z., Engel, R., Nowak, E., Kozdr´oj, J., 2008. Successive soil treatment with captan or oxytetracycline affects non-target microorganisms. World J. Microbiol. Biotechnol. 24, 2843–2848.

Qiao, Y., Wang, J., Liang, G., Du, Z., Zhou, J., Zhu, C., Huang, K., Zhou, X., Luo, Y., Yan, L., Xia, J., 2019. Global variation of soil microbial carbon-use efficiency in relation to growth temperature and substrate supply. Sci. Rep. 9, 5621.

Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.J., 2018. Soil structure as an indicator of soil functions: A review. Geoderma 314, 122-137.

Rahman, M.T., Liu, S., Guo, Z.C., Zhang, Z.B., Peng, X.H. 2019. Impacts of residue quality and N input on aggregate turnover using the combined ^{13}C natural abundance and rare earth oxides as tracers. Soil Tillage Res. 189, 110-122.

Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biol. Biochem. 42, 926-934.

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecol. Lett. 16, 930-939.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241, 155-176.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555-569.

Soares, M., Kritzberg, E.S., Rousk, J., 2017. Labile carbon 'primes' fungal use of nitrogen from submerged leaf litter. FEMS Microbiol. Ecol. 93, fix110.

Soares, M., Rousk, J., 2019. Microbial growth and carbon use efficiency in soil: links to fungal-bacterial dominance, SOC-quality and stoichiometry. Soil Biol. Biochem. 131, 195-205.

Sokol, N.W., Sanderman, J., Bradford, M.A., 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Glob. Change Biol. 25, 12-24.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163.

Ullah, M.R., Carrillo, Y., Dijkstra, F.A., 2021a. Biocides provide a source of carbon and nitrogen directly to surviving microbes and indirectly through a pulse in microbial necromass. Appl. Soil Ecol. 160, 103862.

Ullah, M.R., Carrillo, Y., Dijkstra, F.A. 2021b. Drought-induced and seasonal variation in carbon use efficiency is associated with fungi:bacteria ratio and enzyme production in a grassland ecosystem. Soil Biol. Biochem. 155, 108159.

Ullah, M.R., Carrillo, Y., Dijkstra, F.A., 2023. Relative contributions of fungi and bacteria to litter decomposition under low and high soil moisture in an Australian grassland. Appl. Soil Ecol. 182, 104737.

Walia, M.K., Dick, W.A., 2018. Selected soil physical properties and aggregate-associated carbon and nitrogen as influenced by gypsum, crop residue, and glucose. Geoderma 320, 67-73.

Wang, Q., Wang, S., He, T., Liu, L., Wu, J., 2014. Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils. Soil Biol. Biochem. 71, 13-20.

Wang, X., Yin, L., Dijkstra, F.A., Lu, J., Wang, P., Cheng, W., 2020. Rhizosphere priming is tightly associated with root-driven aggregate turnover. Soil Biol. Biochem. 149, 107964.

Xiao, D., He, X., Wang, G., Xu, X., Hu, Y., Chen, X., Zhang, W., Su, Y., Wang, K., Soromotin, A.V., Alharbi, H.A., Kuzyakov, Y., 2022. Network analysis reveals bacterial and fungal keystone taxa involved in straw and soil organic matter mineralization. Appl. Soil Ecol. 173, 104395.

Zhang, L., Wang, Y., Chen, J., Zhang, C., Cao, Y., Cai, G., Yu., L., 2023. Exogenous carbon addition reduces soil organic carbon: the effects of fungi on soil carbon priming exceed those of bacteria on soil carbon sequestration. Forests 14, 1268.

Zhang, X., Xin, X., Zhu, A., Yang, W., Zhang, J., Ding, S., Mu, L., Shao, L., 2018. Linking macroaggregation to soil microbial community and organic carbon accumulation under different tillage and residue managements. Soil Tillage Res. 178, 99-107.

Zhang, Y., Liu, Q., Zhang, W., Wang, X., Mao, R., Tigabu, M., Ma, X., 2021. Linkage of aggregate formation, aggregate-associated C distribution, and microorganisms in two different-textured ultisols: A short-term incubation experiment. Geoderma 394, 114979.

Zornoza, R., Acosta, J.A., Faz, A., Bååth, E., 2016. Microbial growth and community structure in acid mine soils after addition of different amendments for soil reclamation. Geoderma 272, 64-72.

Chapter 5: Plant-microbial interactive effects on plant residue decomposition, soil aggregation and soil organic carbon content

Abstract

The plant photosynthesized carbon (C) enters the soil through rhizodeposits. The root secretions (or rhizodeposition) attract soil microbes to reside around the root creating a rhizosphere zone, where rhizodeposits induce the microbial decomposition of soil C (rhizosphere priming effect or RPE). These rhizodeposits, together with plant litter, may also play an important role in soil aggregation and soil C stabilization processes that are still not fully understood. In this study, we conducted a glasshouse pot experiment to investigate the effect of the rye grass (*Lolium perenne*) rhizosphere on soil aggregation, aggregate associated C, and soil C decomposition. We applied a planting treatment (planted with rye grass and unplanted) and a rye grass leaf litter addition treatment (none, low with 1 mg kg^{-1}, and high with 10 mg kg^{-1}). After 30 days, the rye grass shoots were trimmed, and a ^{13}C-CO_2 pulse labeling was conducted. Three days after the ^{13}C-CO_2 pulse labeling we measured total soil respiration, which was separated into root respiration, decomposition of rhizodeposits, and decomposition of soil organic C (including the added litter). This allowed us to calculate the RPE. We further measured microbial biomass C (MBC), soil aggregate fractions (macroaggregates, microaggregates, and silt and clay fraction), and their total C content, as well as ^{13}C from the pulse label (via rhizodeposition) recovered in MBC and aggregates. The presence of live roots increased the total respiration and the MBC, where MBC was highest in treatments with high litter addition. We observed a positive RPE in the treatment with the highest litter treatment (1.27 mg pot^{-1} d^{-1}), but no RPE was discerned in the treatments without litter addition. Both planting and litter addition treatments had little effects on soil aggregation. The ^{13}C from the pulse label was observed in all aggregates, suggesting that

rhizodeposition contributed to formation of all three aggregate size classes. However, we observed no significant effect of litter addition. In general, the results showed that the effects of plant and litter treatments were more evident on the MBC causing a positive RPE when combined with high litter addition, but had little effect on the soil aggregation and C content in soil aggregates.

5.1 Introduction

Soil carbon (C) is of great importance for soil structure (Guhra, Stolze & Totsche, 2022). Soil structure is defined by the formation of stable soil aggregates. While stable soil aggregates can serve as a terrestrial C sink for long periods of time, it can also support the ecosystem such as hosting soil microfauna and plant roots. Good soil aggregate structure is also important for gas exchange in the soil, soil water holding capacity, and water infiltration (Bucka et al., 2019; Sindelar et al., 2019). Agricultural practices -such as tillage and organic amendments- can impact soil C (Chakraborty et al., 2019; Guo & Gifford, 2002) and may impact C stabilization in soil aggregates.

Secretions from plant roots in the surrounding microenvironment known as rhizosphere provides organic C including easy to decompose low molecular compounds. The rhizodeposition of low molecular compounds attracts soil microbes and their predators such as nematodes and protozoa to reside around the plant roots that can form a symbiotic relationship with plants, where microbes provide nutrients for plant growth in return for energy-rich C compounds. The abundance of energy-rich C sources provided by root secretions can increase the microbial growth rate and activity in the rhizosphere (Marschner, 2023). In general, the type of root deposits in the rhizosphere depends on the plant type, nutrient availability, soil type and chemistry, land management and type of stress imposed on the plant. In addition, plant root growth can physically modify soil structure, and interact with microbes that produce organic compounds (like microbial

necromass) in the rhizosphere zone that are used to form soil aggregates (Neumann & Ludewig, 2023).

The plant induced changes in soil also include drying-rewetting cycles caused by root water uptake that can affect the formation of macropores (Lu et al., 2019). The plant effect on microbial activity in the soil can indirectly impact the C turnover in the soil, affecting soil aggregate formation and stability in planted soils (Angers & Caron, 1998). The plant effect on soil C decomposition can be on both the soil native organic C (triggering a so called rhizosphere priming effect, Kuzyakov (2002)) and on the dynamics of microbial decomposition of added plant litter to the soil. However, it is still unclear how plant root exudates regulate C decomposition and subsequent aggregate formation in the soil (Yan et al., 2023).

The addition of organic matter such as fresh plant residue, either composted or charred, has been an ongoing agricultural practice to retain some of the soil depleting nutrients and to restore the soil deteriorated structure due to many negative environmental and land use impacts (Rakshit, Sarkar & Abhilash, 2018). However, results from previous research were not consistently confirming a positive effect from plant residue soil amendments on soil aggregation or soil C content (Husain & Dijkstra, 2023). Furthermore, the rate and type of plant residue additions can also have an impact on the degree of soil aggregation and C content (Sarker et al., 2022).

The soil C content is related to the microbial biomass C (MBC) and the microbial C use efficiency (CUE) of the C resources in the soil (Campbell et al., 2022; Canarini et al., 2016; Soares & Rousk, 2019). In general, the organic C in the soil consists of 1-4 % MBC (Sparling, 1992). In addition, the microbial CUE, which is the C used for growth as a ratio of total C use (growth+respiration) tends to be positively related to the C sequestration in the soil (Geyer et al., 2020; McGee et al.,

2019). However, the role of added plant C resources, either as rhizodeposition or ass plant litter, affecting MBC and microbial CUE are still not fully understood (Wang et al., 2021a).

While soil aggregation needs organic C to glue soil particles (such as sand, silt, and clay), the properties of formed aggregates depend on the type of added organic C to soil (Bucka et al., 2019; Sarker et al., 2022). To form stable aggregates, minerals in these aggregates can protect the soil occluded C from the enzymatic activities of decomposers (soil microbes) through many mechanisms such as clay adsorption of organic matter and organo-mineral associations (Baldock & Skjemstad, 2000). The aggregate particles get enmeshed by plant roots, fungal hyphae, and microbial and plant residues into bigger aggregates. Soil aggregates can be classified according to size fraction as: macroaggregates (>250 μm), microaggregates (53-250 μm), and silt and clay fraction (<53 μm) (Six et al., 2000; Tisdall & Oades, 1982). A mean weight diameter (MWD) value can be calculated from the separation of different soil aggregate size fractions (either by dry or wet sieving), which can be used to represent soil aggregation as a whole (Franzluebbers, 2022).

The aim of this study was to examine: 1. the role of rye grass (*Lolium perenne*) on soil organic C decomposition, MBC, and rhizosphere priming effect (RPE), in the presence of low and high addition rates of rye grass leaf litter (1 and 10 g kg^{-1}, respectively), and 2. the contribution of roots and rhizodeposition from rye grass on soil aggregation and soil organic C in the presence of low and high addition rates of rye grass residue. We used a novel ^{13}C pulse labelling method that allowed us to separate soil respiration into root respiration, rhizodeposition decomposition, and soil organic C decomposition (Wang et al., 2021b). We further assessed the ^{13}C label recovered in MBC, and in different soil aggregates.

We hypothesized that: 1. The presence of live roots (rhizosphere) in the soil will increase the MBC and the decomposition of soil C (including added leaf litter) more than in bare soils (i.e., cause a

positive RPE), particularly with high leaf litter addition, 2. the presence of rhizosphere will induce the formation of macroaggregates or a higher MWD than in bare soils, particularly with high leaf litter addition, and 3. the high application of plant litter will decrease the ^{13}C recovered in MBC and aggregates, because leaf litter would promote a microbial community with a lower microbial CUE.

5.2 Methods and materials

5.2.1 Soil sampling and treatments

The soil used for this experiment was sampled in October 2021 from 0-15 cm depth at John Pye farm, near Camden, NSW, Australia (33°55′51″S, 150°39 ′ 38″E), at 81 m above sea level. The soil is a clay loam and classified as a red Chromosol according to the Australian Soil Classification (Isbell, 2002). The soil had 35% clay, 34% sand, 31% silt, 3.2% organic C, 0.23% N, 0.015% P, and a pH of 5.7 (H_2O). The $\delta^{13}C$ of the soil was -21.1‰.

The soil was air dried and sieved through a 2 mm sieve to remove stones and visible roots, then passed through a 0.250 mm sieve to destroy all soil macroaggregates. The sieved soil was then mixed with 50% fine sand (v/v), to reduce soil compaction and for better root growth. The pots were made from PVC pipes (6 cm in diameter) that were cut into rings of 7 cm in height and cut vertically again in two halves (to minimize destruction of soil cores when sampling later on, see below). To assemble the pots, the halves were tightly joined with mask tape, while the bottom of the pipes were covered with PVC caps. All pots were filled with 200 g of the soil mix.

Two sets of 15 pots each (with and without plants) were prepared with the following rye grass (*Lolium perenne*) leaf litter treatments: no litter, low litter (0.2 g pot^{-1} or 1 g kg^{-1} soil), high litter (2 g pot^{-1} or 10 g kg^{-1} soil). The litter addition rates were based on annual production of rye grass which can be as high as 15 t/ha in Australia (Cayley et al., 1998). The litter contained 43.8% C

and 1.95% N. The $\delta^{13}C$ of the litter was -26.3‰. In total there were 30 pots with 5 replicates for each treatment. All pots received fertilizer solution (for each pot 177 mg N,80 mg P, 277 mg K, 103 mg S, 36 mg Ca, 35 mg Mg, 0.2 mg Zn, 0.1 mg Cu. 0.9 mg Fe, and 1.5 mg Mn) and pots with plants were sown with rye grass seeds. Pots were kept in a glass house with a daily 12 h light (using red and blue LED lights) and 12 h dark cycle at 25 °C. The pots were watered daily (for 30 days) to 70% of the field water holding capacity by weighing pots then water each pot to the targeted water level.

5.2.2 Pulse labeling

After 30 days, a $^{13}CO_2$ pulse labeling was conducted for planted pots adapted from Wang et al. (2021b). The aim of the pulse labeling was to separate total soil respiration into rhizodeposition decomposition, root respiration, and litter and soil C decomposition, and to assess the ^{13}C label recovered in MBC and soil aggregates.

The pulse $^{13}CO_2$ labeling was conducted by placing the pots in a glass chamber placed in the glass house and injecting $^{13}CO_2$ (99 atom% ^{13}C, pressurized gas from Cambridge Isotope Laboratories Inc., Andover, MA, USA) to the chamber through a port at the top of the chamber, so that the CO_2 concentration inside the chamber reached 1000 ppm. The chamber had a fan fitted at the top and ice packs were placed inside the chamber to control the temperature, and a CO_2 sensor (MG811 CO_2 gas module, DFRobot, Shanghai, China) connected to Arduino (Shanghai, China) Nano board with a TFT screen to monitor and the concentration of CO_2. Planted pots were kept in the chamber for 2 hours. To account for any possible $^{13}CO_2$ diffusion to the soil that can cause an over estimation of the respiration rate measurements (Burton et al., 2002), we assessed back diffusion by exposing 4 planted pots to the same pulse labelling treatment in dark chamber (covered with 100% blackout material) to prevent photosynthesis.

5.2.3 Measurements and calculations

Three days after $^{13}CO_2$ pulse labelling, the rye grass shoots were trimmed to 10 mm above soil surface, dried in an oven (65 °C) and ground into a fine powder for ^{13}C analysis. Then the mask tape and bottom cap were removed from the planted pots, and pots were cut into two halves. One halve containing intact soil and roots was placed in a Mason jar covered with lids with sampling ports to collect gas samples. The other halve of the soil was sieved (2 mm) to separate roots from soil. Roots were further rinsed to remove all soil. Rinsed roots and 50 g of sieved soil was also placed in Mason jars with sampling ports. The remaining sieved soil was used for microbial biomass and aggregate measurements (see below). Finally, pots without plants and the 4 extra pots used to assess back diffusion were also placed in Masson jars with sampling ports. Gas samples were taken from each Mason jar at time 0, 1h, and 2h with a 30 ml syringe. The collected gas was then taken transferred into pre-vacuumed 12 ml exetainers (Labco Limited, Lampeter, UK), where excess gas was used to flush the exetainer. The gas was then analyzed for CO_2 and ^{13}C -CO_2 on an isotope ratio mass spectrometer (IRMS delta V advantage with a Conflo IV and Flash HT interface, Thermo Fisher Scientific, Bremen, Germany). After gas sampling, the roots were dried in an oven (65 °C), ground into a fine powder, and then together with the shoot biomass analyzed for total C and ^{13}C on the IRMS. The total soil respiration rate in the planted pots (in mg C pot^{-1} d^{-1}) was calculated based on the change in CO_2 concentration from Mason jars containing half of the intact soil and roots using linear regression, the ideal gas law, and the head space of the Mason jar. Soil respiration in the pots without plants was calculated in the same way. For details, see Wang et al. (2021b).

The $\delta^{13}C$ of CO_2 for total soil respiration (from Mason jars containing half of the intact soil and roots, and from Mason jars containing unplanted pots), root respiration (from Mason jars

containing roots only), and soil + rhizosphere decomposition (from Mason jars containing sieved soil) was calculated using a keeling plot (Hasibeder et al., 2015). We were unable to detect the ^{13}C label in total respiration from the 4 extra pots used for back diffusion, indicating that back diffusion did not play a significant role, and we therefore did not need to correct soil respiration measurements in planted pots for back diffusion effects. We separated total soil respiration in planted pots onto three components. First, root respiration was separated from the total soil respiration (1):

$$Resp_{root} = Resp_{total} (\delta^{13}C\ Resp_{total} - \delta^{13}C\ Resp_{soil+rhizo})/ (\delta^{13}C\ Resp_{root} - \delta^{13}C\ Resp_{soil+rhizo}) \quad (1)$$

where $Resp_{root}$ is root respiration, $Resp_{total}$ is the total soil respiration in planted pots (in mg $C\text{-}CO_2$ pot^{-1} d^{-1}), and $\delta^{13}C\ Resp_{root}$, $\delta^{13}C\ Resp_{total}$, and $\delta^{13}C\ Resp_{soil+rhizo}$ are the ^{13}C values (in ‰) of root respiration, total soil respiration, and soil + rhizodeposition decomposition, respectively. We then calculated the soil + rhizodeposition decomposition ($Resp_{soil+rhizo}$) as:

$$Resp_{soil+rhizo} = Resp_{total} - Resp_{root} \quad (2)$$

We then separated rhizodeposition decomposition ($Resp_{rhizo}$) from ($Resp_{soil+rhizo}$) with:

$$Resp_{rhizo} = Resp_{soil+rhizo} (\delta^{13}C\ Resp_{soil+rhizo} - \delta^{13}C\ Resp_{unpl})/ (\delta^{13}C\ Resp_{root} - \delta^{13}C\ Resp_{unpl}) \quad (3)$$

where $\delta^{13}C\ Resp_{unpl}$ is the $\delta^{13}C$ value of soil respiration in unplanted pots. Here, for simplicity we will associate $Resp_{rhizo}$ to rhizodeposition rates, given that much of rhizodeposition is decomposed quickly, although we note that we only measured rhizodeposition decomposition rates.

Litter and soil C decomposition (here after used as soil C decomposition) in planted pots ($Resp_{soil, pl}$) was then calculated as:

$$Resp_{soil, pl} = Resp_{soil+rhizo} - Resp_{rhizo} \quad (4)$$

Finally, we calculated RPE as the difference litter and soil C decomposition between planted and unplanted pots ($Resp_{soil, unpl}$):

$$RPE = Resp_{soil, pl} - Resp_{soil, unpl} \quad (5)$$

For details and assumptions using these equations we refer to Wang et al. (2021b).

For microbial biomass measurements, two sets of soil samples (7 g each) from planted and unplanted pots were used. One set was fumigated by placing glass beakers of soil samples with a beaker of chloroform in a desiccator for 48 h, and one set remained unfumigated. Both fumigated and unfumigated soil samples were extracted using 40 ml of 0.05 M K_2SO_4. The extracts were then filtered after shaking for an hour on an end-to-end shaker and centrifuging for 15 min at 4000 rpm. To measure the total organic C for soil extracts, they were acidified with 150 µl 1 M H_3PO_4 and then analyzed on a TOC analyzer (Shimadzo TOC-V csh, TNM-1, Kyoto, Japan). Aliquots of the fumigated and non-fumigated extracts were dried in a ventilated oven at 65 °C, and the salts were analyzed for ^{13}C on the IRMS. The MBC (in mg kg^{-1} soil) was calculated as the difference in total C in fumigated and non-fumigated samples divided by 0.45 (Beck et al., 1997). The ^{13}C atom % in microbial biomass ($^{13}C_{mic}$) was determined using equation (6) (Mehnaz et al., 2019):

$$^{13}C_{mic} = (C_{fum} \times {}^{13}C_{fum} - C_{non\text{-}fum} \times {}^{13}C_{non\text{-}fum}) / (C_{fum} - C_{non\text{-}fum}) \quad (6)$$

where C_{fum} and $^{13}C_{fum}$ are the total C and ^{13}C atom % in fumigated extracts respectively, $C_{non\text{-}fum}$ and $^{13}C_{non\text{-}fum}$ are the total C and ^{13}C atom % in non-fumigated extracts, respectively. The ^{13}C label recovered in microbial biomass (MBC_{pl}) was then calculated with:

$$MBC_{pl} = MBC \times {}^{13}C_{mic, excess} \quad (7)$$

where $^{13}C_{mic,\ axcess}$ is the difference in ^{13}C atom % in microbial biomass between planted and unplanted pots.

For aggregate, we separated 3 aggregates size classes (macroaggregates (>250 μm), microaggregates (53-250 μm), and silt and clay fraction (<53 μm)) using a wet sieving method after slaking the soil samples (100 g each) overnight in the cool room (4 °C). The wet sieving was done by moving three sieves (2mm, 250 μm, 53 μm), stacked on top of each other, vertically for 2 min in deionized water, where the soil was placed on the 2mm sieve. The separated soil aggregates were dried in an oven at 105 °C then weighed. The percentage of each aggregate size fraction was calculated using equation (8):

$$\text{aggregate \%} = (W_a / W_{Ts}) * 100 \quad (8)$$

where W_a represents the aggregate fraction weight, W_{Ts} is the total weight of soil used for aggregate separation. Mean weight diameter (MWD) was calculated from the separated aggregate size fractions using equation (9):

$$\text{MWD} = \sum_{i=1}^{n} \bar{x}_i\, w_i \quad (9)$$

where $\bar{x}_i$ is the mean diameter of size fraction i, w_i is the fraction of that aggregate size class, n is the number of size fractions (Hernandez, Hernandez & Garcia, 2017).

Samples from each separated aggregate fraction were analyzed for the ^{13}C using the IRMS. We then calculated the ^{13}C (from rhizodeposition) recovered in each aggregate size fraction, similar to ^{13}C recovered in microbial biomass (equation 7). We also calculated the ^{13}C label recovered in root, shoot, total soil (sum of three aggregate size classes) and total ^{13}C recovered in the plant-soil system (in mg ^{13}C pot_{-1}) in the same way.

5.2.4 Statistical analysis

The statistical analysis of experimental data was conducted using JMP Pro 16 software. A two-way analysis of variance ANOVA was used to examine the main and interactive effects of plant (planted and unplanted) and litter (none, low, and high) treatments on the total respiration rate, MBC, soil aggregate fractions, MWD, and total C in each aggregate fraction. We used one-way ANOVA to examine litter treatment effects on ^{13}C label recovered in soil (sum of three aggregate size classes), roots, shoots, and the whole plant soil system, root respiration, rhizodeposition, soil C decomposition, RPE, and ^{13}C recovered in MBC and aggregate. We used Tukey's HSD post-hoc test to assess differences among treatment combinations.

5.3 Results

5.3.1 The ^{13}C label recovered in soil, plant roots and shoots

Overall, the shoot biomass had more ^{13}C from the pulse labeling than the root biomass (Figure 5.1). The recovered ^{13}C in both roots and shoots increased with the low litter soil treatment then decreased with the increase in litter addition. However, the litter treatment effect on the recovered ^{13}C in the pot components was only significant in shoots and in the total pot ($p=0.03$ and 0.02, respectively).

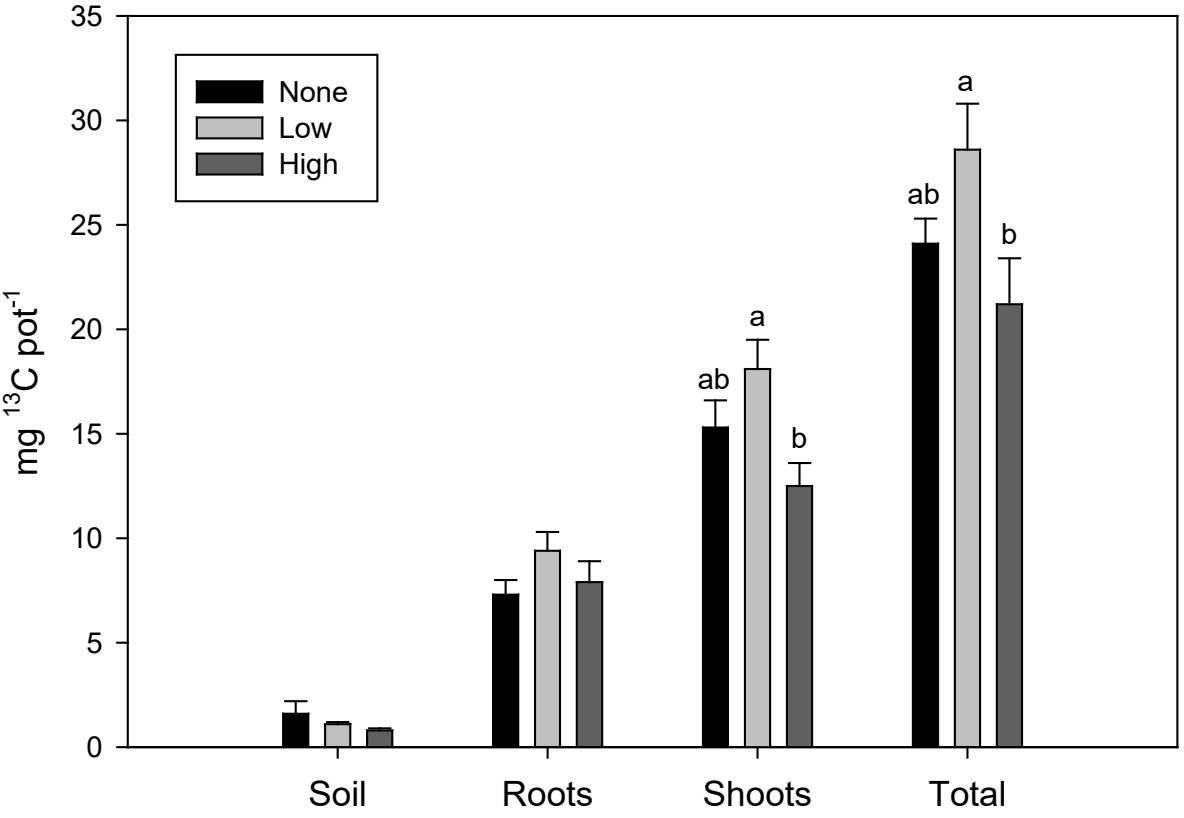

Figure 5.1. The recovered ^{13}C in soil, roots, shoots, and total (soil + plant). Error bars represent standard error, (n=5). Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

5.3.2 Soil respiration

The measured δ^{13}C signatures in respiration from sieved soil (δ^{13}C $Resp_{soil+rhizo}$), plant roots (δ^{13}C $Resp_{root}$), and from intact soil with roots (δ^{13}C $Resp_{total}$) indicated that the method of pulse labeling in this experiment succeeded in labelling the plant C with ^{13}C and that the ^{13}C got transferred to the soil through the rhizodeposition from the labeled plants (Table 5.1).

Table 5.5: The $\delta^{13}C$ values (‰) in respiration from sieved soil ($\delta^{13}C\ Resp_{soil+rhizo}$), plant roots ($\delta^{13}C\ Resp_{root}$), and from intact soil with roots ($\delta^{13}C\ Resp_{total}$). Values are means ± standard error, n=5.

Parameter	Litter treatment		
	None	Low	High
$\delta^{13}C\ Resp_{soil+rhizo}$	3084.4 ± 508.5	3293.6 ± 303.6	2393.4 ± 259.5
$\delta^{13}C\ Resp_{root}$	6538.5 ± 479.3	8420.0 ± 1007.9	7064.5 ± 491.1
$\delta^{13}C\ Resp_{total}$	5811.7 ± 485.1	6867 ± 610.1	4851 ± 449.8

The total respiration rate was significantly affected by the plant treatment across all litter treatments ($p < 0.0001$). However, the effect of litter addition was not significant on the total respiration rate, although litter treatments showed different patterns of total respiration from planted compared to unplanted soils (Figure 5.2a). Consistent with the total respiration rate from planted soils, there was no significant difference between litter treatments on root respiration, rhizodeposition, and soil C decomposition (Figure 5.2b). While root respiration decreased with the increased litter addition, the soil C decomposition in planted soils increased with the increase in litter addition, although litter effects were not significant ($p > 0.05$).

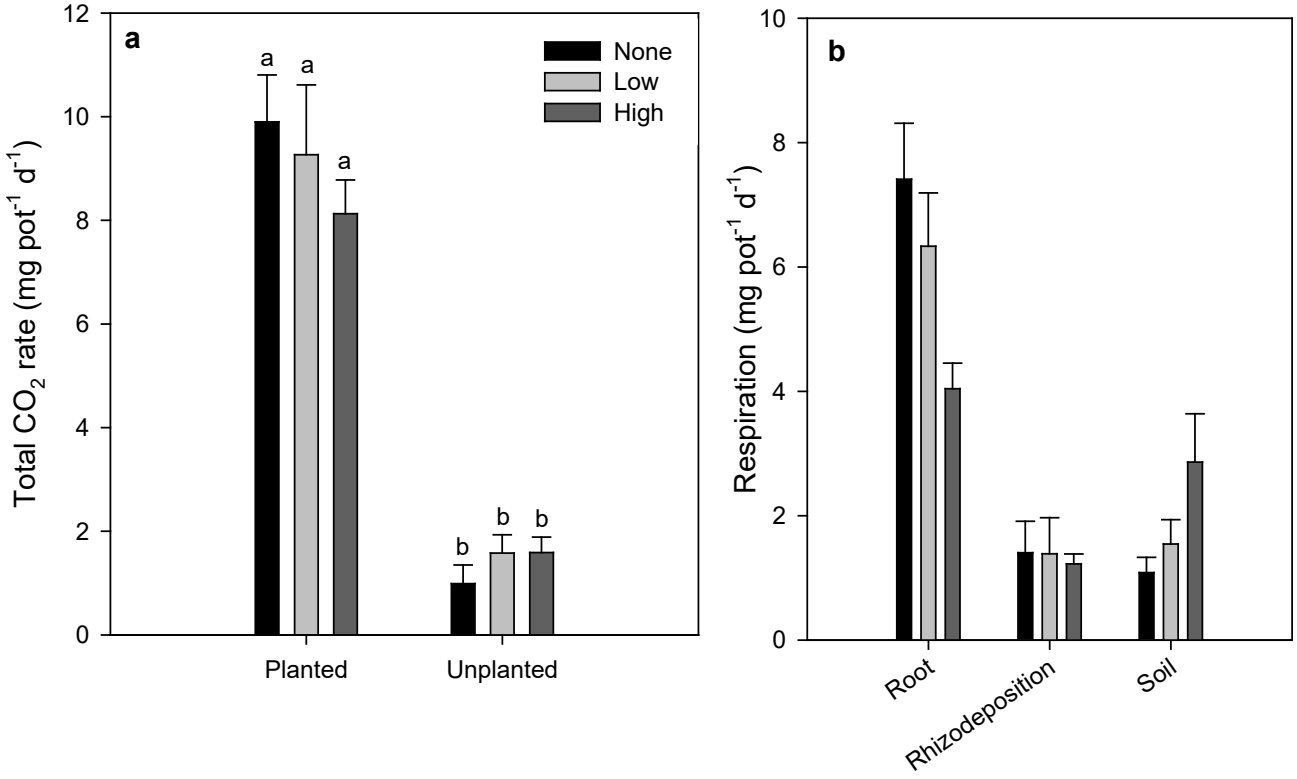

Figure 5.2. The total soil respiration rate from planted and unplanted pots (a) and respiration from roots, rhizodeposition, and soil (b) treated with no litter (None), low litter (Low), and high litter (High) treatments. Error bars represent standard error, (n=5). Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

In planted soils there was a significant RPE in the high litter treatment (i.e., RPE > 0, Figure 5.3), although statistically, we could not detect a significant effect of the litter addition treatment on the RPE.

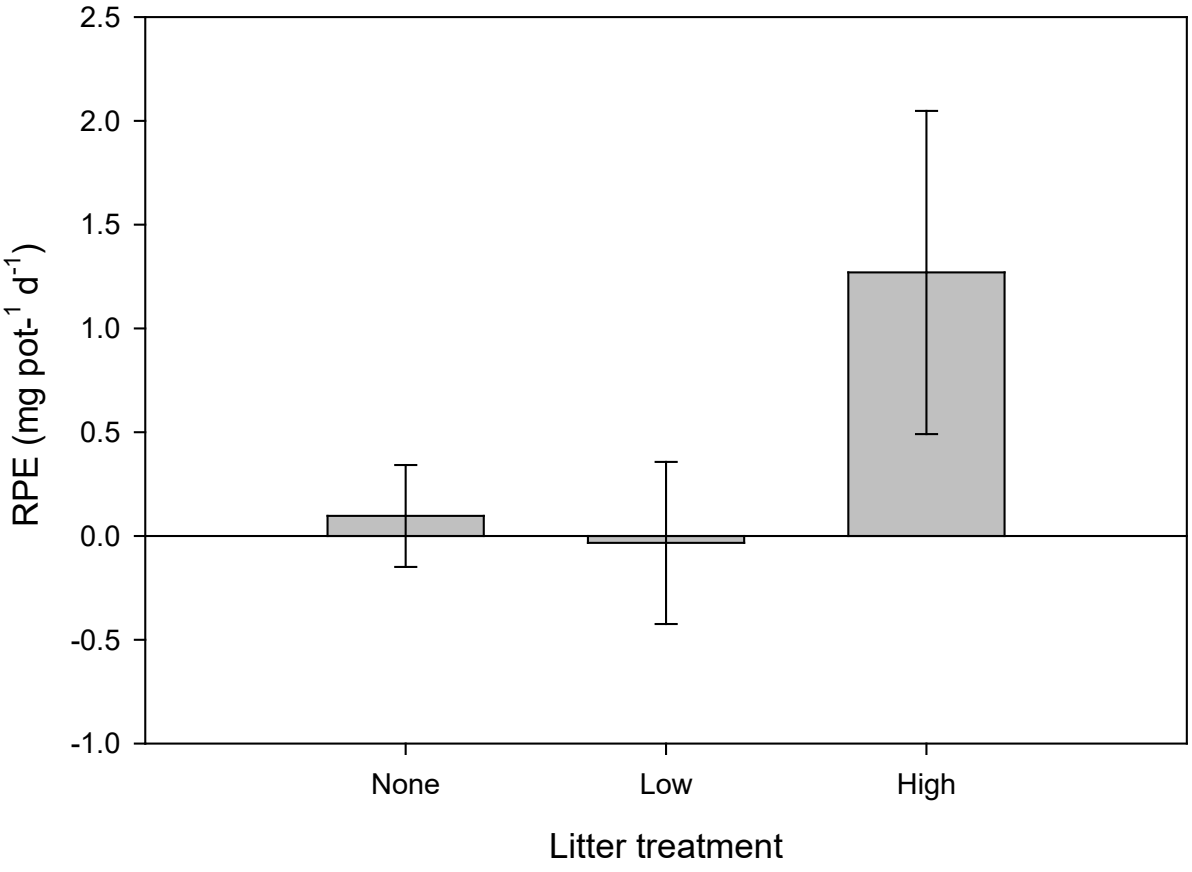

Figure 5.3. The rhizosphere priming effect (RPE) in planted soils with litter (none, low, and high) treatments. Error bars represent standard error, (n=5).

5.3.3 Microbial parameters

The microbial biomass C (MBC) significantly increased with the increase in litter addition rate, where planted soil resulted in a higher MBC (Figure 5.4a). Nevertheless, there was no significant difference between the None and Low litter treatment, both with and without plants. Both main effects of plant and litter treatments were significant on the MBC ($p<0.0001$), but the interactive effect of plant and litter treatments was marginally significant ($p=0.05$).

In planted soils MBC ^{13}C recovered in MBC increased with the addition of Low litter, then decreased with High litter addition, although the effect of litter treatment was not significant (Figure 5.4b).

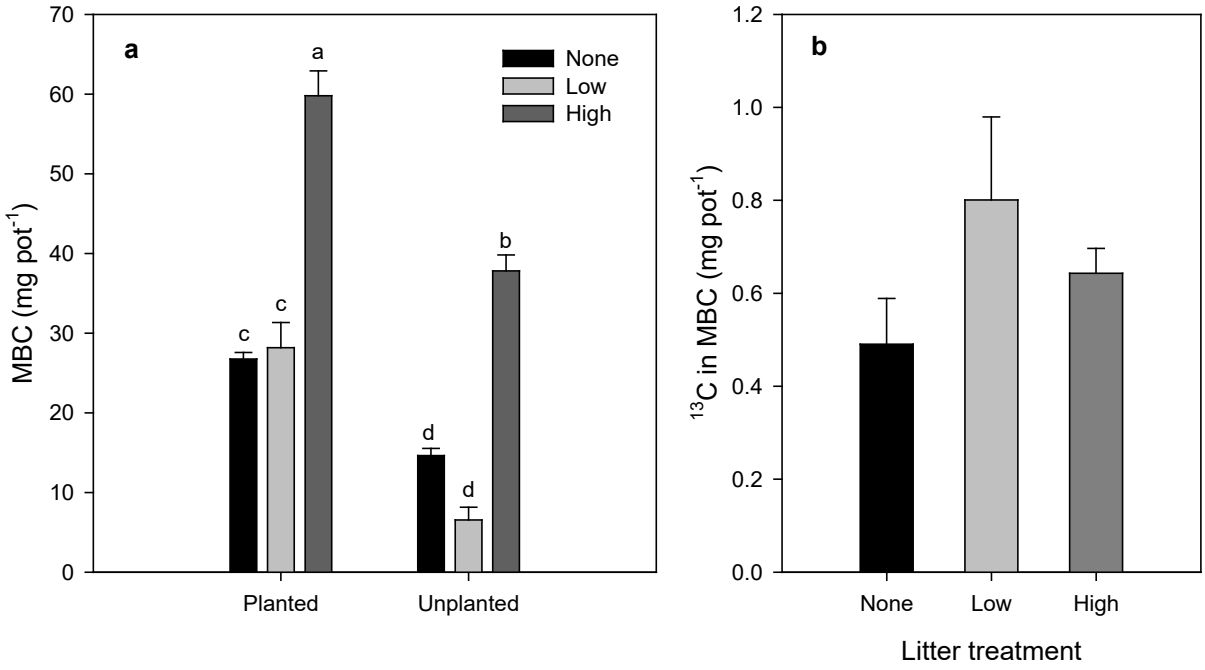

Figure 5.4. Microbial biomass carbon (MBC) in planted and unplanted treatments (a), ^{13}C recovered in MBC in planted soils (b) for litter treatments (None, Low, and High). Error bars represent standard error, (n=5). Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

5.3.4 Soil aggregates and MWD

The effect of soil plant and litter treatments on water stable soil aggregate fractions varied among the different aggregate size classes (Figure 5.5a, b, c). The increase in litter addition rate tended to increase the macroaggregate size class in planted soils, whereas it tended to decrease in unplanted soils (Figure 5.5a). Soil microaggregate and silt and clay fractions generally decreased in the absence of plants (unplanted treatments), but each aggregate fraction showed a different pattern across litter treatments (Figure 5.5b, c). While the treatments effect on the microaggregate fraction

was not significant, it showed a decrease after the addition of litter for both planted and unplanted soils (Figure 5.5b). The response from silt and clay fraction to plant and litter treatments was the opposite of the response of the soil macroaggregate fraction, where the effect from the plant treatment was found significant ($p=0.03$) (Figure 5.5c).

The mean weight diameter (MWD) showed similar patterns with the soil macroaggregate fraction results (Figure 5.5a, d), and no significant effect was found from either plant or litter treatments on the MWD (Figure 5.5d).

5.3.5 Soil Carbon

The total Soil C did not show significant differences between plant (planted, unplanted) or litter (None, Low, High) treatments, although it tended to an increase in planted pots with High litter treatment and tended to a decrease in both Low and High soil litter treatments in unplanted pots (Figure 5.6d).

Interestingly, the measured C in macroaggregates and silt and clay fractions generally showed the same trends as the recovered associated aggregate fractions measured in the same soil treatments (Figure 5.6a, c). However, C in microaggregates increased the most in the unplanted soil with high litter treatment.

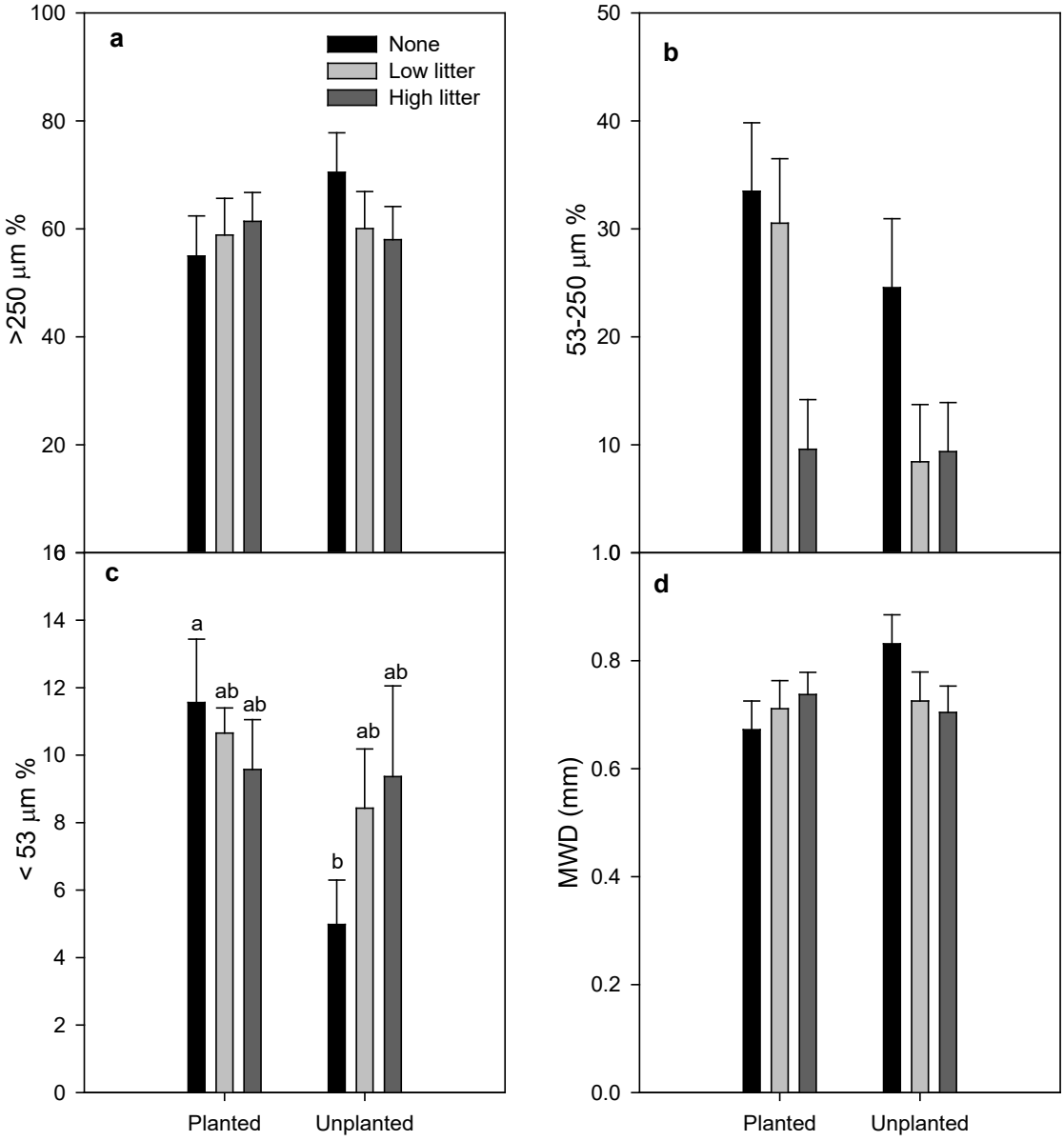

Figure 5.5: Soil aggregate fractions including macroaggregates (> 250 µm, a), microaggregates (53-250 µm, b), silt and clay fraction (<53 µm, c), and mean weight diameter (MWD, d) for plant (Planted, Unplanted) and litter (None, Low, and High) soil treatments. Error bars represent standard error, (n=5). Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

The plant treatment was only found marginally significant (p=0.05) on C in macroaggregates, but had no significant effect on the C in micro or silt and clay aggregate fractions. Surprisingly, there

was no significant effect from the litter treatment on C in any of the aggregate size fractions. However, the interactive effect between plant and litter treatments was found marginally significant ($p=0.07$) on the C in macroaggregates.

In planted soil, the ^{13}C recovered in soil aggregate fractions from the ^{13}C-CO_2 pulse labelling varied between the different aggregates across litter treatments (Figure 5.7). There were no significant differences in ^{13}C recovered in macro and microaggregates between litter treatments. However, the effect of litter treatment on the ^{13}C in the silt and clay fraction was significant ($p=0.0005$). The smallest ^{13}C recovery was observed in soil with the High litter treatment in all aggregate fractions, with the lowest observed in the silt and clay fraction.

5.4 Discussion

The ^{13}C-CO_2 pulse labeling method we used in this experiment resulted in significant increases in the ^{13}C signatures of the plant parts, that could be traced into soil respiration, MBC, and different soil aggregate size classes, and indicate that our ^{13}C pulse labeling was successful. Most of the ^{13}C label was recovered in shoots, but significant amounts of the assimilated C were transferred to roots and soil three days after labeling (Figure 5.1). We note that with time more of the ^{13}C label recovered in shoots could be transferred belowground (Bromand et al., 2001), and that our study only provides a snapshot of the dynamics of the recently photosynthesized C.

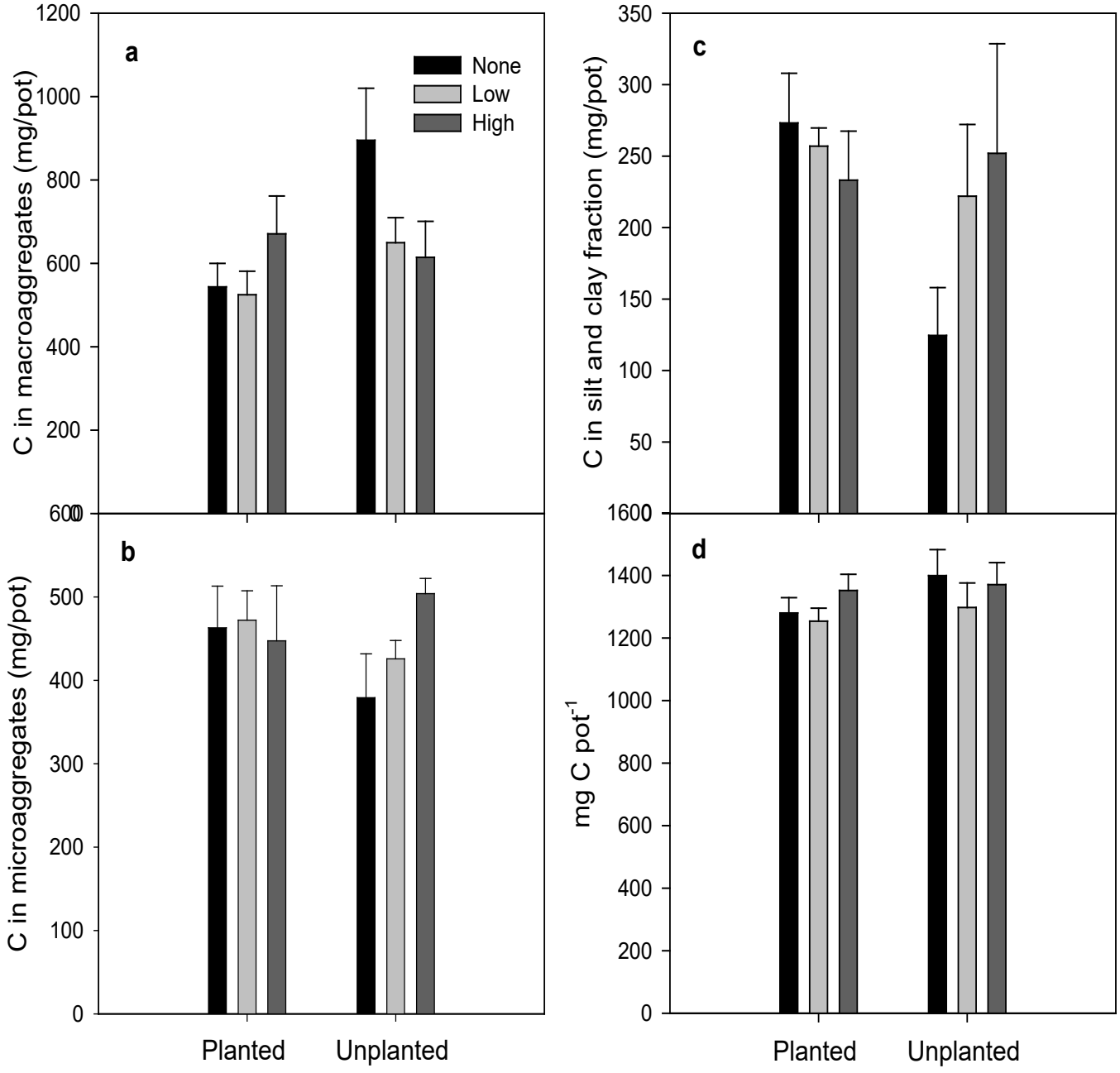

Figure 5.6. Carbon (C) recovered in macroaggregates (a), microaggregates (b), and silt and clay fraction (c), total soil C (d), for plant (Planted, Unplanted) and litter (None, Low, and High) treatments. Error bars represent standard error, (n=5).

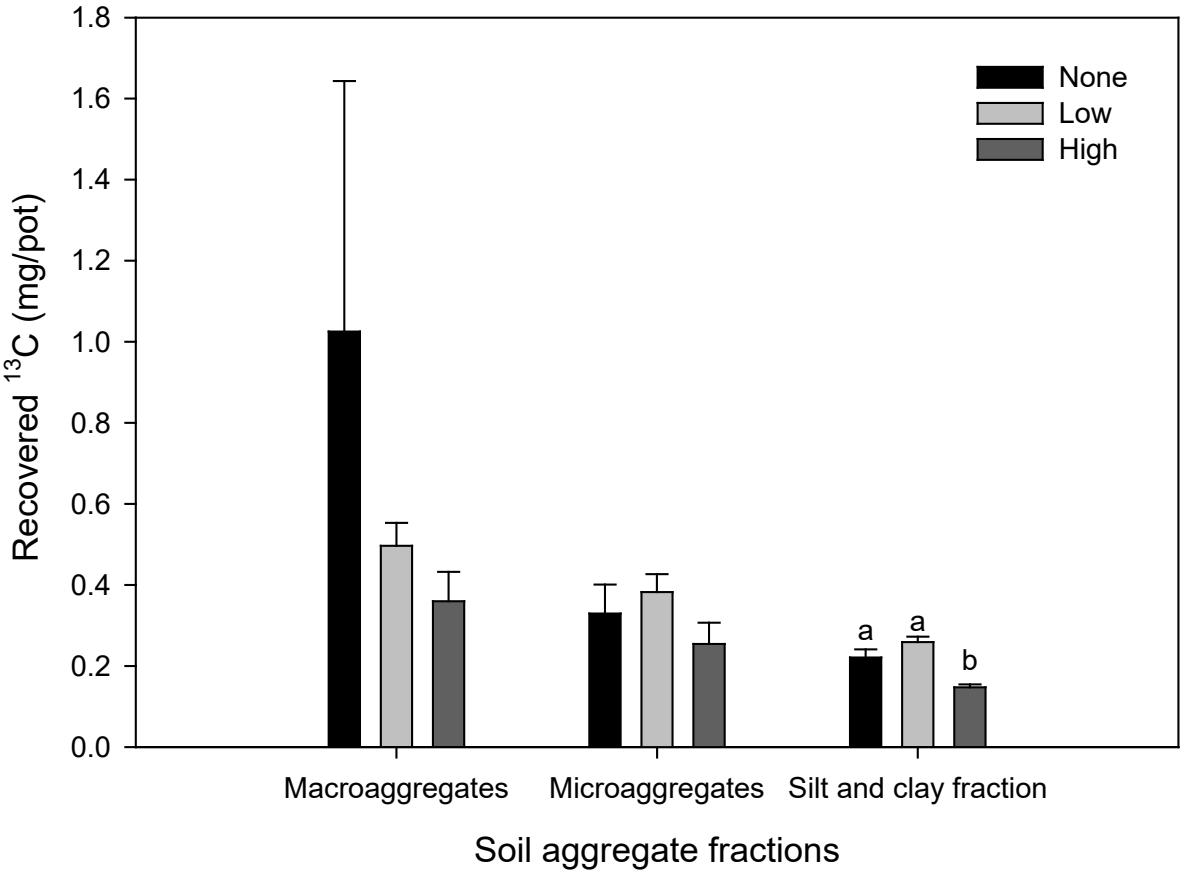

Figure 5.7. The ^{13}C recovered in aggregate fractions (macroaggregates, microaggregates, and silt and clay fraction) in planted soil with litter (None, Low, and High) soil treatments. Error bars represent standard error, (n=5). Bars with different letters are significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

5.4.1 The effect of rye grass rhizosphere on the decomposition of added rye grass litter, soil C and microbial biomass C

Planted soil resulted a higher total respiration than unplanted soils, largely because much of the total respiration in the planted treatment came from the root respiration and rhizodeposition (Figure 5.2). It is interesting to note that the contribution of rhizodeposition to the total respiration was as large as the contribution from soil C decomposition (including added litter), suggesting that rhizodeposition is an important component of soil respiration. Our results about rhizodeposition

respiration contribution to the total respiration are consistent with results from the review by Pausch and Kuzyakov (2018), where the ratio of rhizodeposition to root respiration was more than one in most of grass studies. Furthermore, the recovered ^{13}C in MBC from planted soil across all litter treatments (Figure 5.4b) indicates that rhizodeposition was incorporated in microbial biomass, and supports the above statement about the contribution of rhizodeposits in the total respiration (as a result of the soil microbial activity).

While rhizodeposition was not affected by litter addition, we observed an increase in soil C decomposition in planted pots with increased litter addition (Figure 5.2b). On the other hand, litter addition had no significant effect on soil C decomposition in unplanted pots (Figure 5.2a). This was somewhat surprising given that we added 0.2 and 2 g litter per pot (or 88 and 880 mg C pot^{-1}) in the Low and High litter treatment, respectively. Apparently, after 30 days, much of the litter was already decomposed, so that we observed no significant increases with litter addition anymore in the unplanted pots after 30 days. In other words, any litter that remained in the soil decomposed at such small rates that we were unable to detect it. In the planted pots we observed a stronger increase in soil C decomposition with litter addition, and possibly this was due to an increase in native soil C decomposition rather than an increase in litter decomposition. Unfortunately, in our study we were not able to separate C decomposition from native soil organic matter and litter. Regardless, our results provide support for our first hypothesis in that the presence of plants enhanced soil C decomposition, particularly with increased litter addition. In a long term study, Shahbaz, Kuzyakov and Heitkamp (2017) found a significant increase in soil C decomposition (including the decomposition of added litter) with increased litter addition regardless of the plant residue type and concluded that soil priming is highly dependent on the litter addition rate.

The increase in soil C decomposition in the presence of plants is frequently expressed as a rhizosphere priming effect (RPE). Although we did not observe a significant litter treatment effect on the RPE, only in the High litter treatment did we observe a significant positive RPE in the soil that was significantly larger than zero (Figure 5.3). As discussed above, we are unclear whether the positive RPE was due to an increase in litter decomposition, to an increase in native soil organic matter decomposition, or both. Possibly, the high litter addition may have stimulated microbial growth, so that when plants provided rhizodeposition, this may have enhanced decomposition of native soil organic matter, and possibly the added litter.

This idea is supported by our MBC results. The MBC was higher in planted than in unplanted treatments and was particularly high in the High litter treatment (Figure 5.4a), indicating the positive effect from the rhizosphere on soil microbial growth on the one hand, and addition of litter supporting microbial biomass on the other hand. However, we observed no significant litter effect on ^{13}C recovered in MBC (Figure 5.4b), indicating that there was no microbial preference of using relatively complex C sources, either from added litter or from native soil organic matter, *vs* using more labile C sources from the plant rhizodeposition when more litter is added to the planted soil. In a recent study Olagoke et al. (2022) reported that some bacteria species thrived in the soil when complex plant substrate (cellulose) was added more than when a simple plant substrate was provided in the soil. In two meta-analysis studies on the effect from the rhizosphere (Zhao et al., 2022) or from the addition of rhizosphere surrogates such as sugars and other low molecular organic compounds, that can mimic the effect of plant rhizodeposition on soil C dynamics (Yan et al., 2023), a positive effect on MBC, microbial enzymes, respiration and soil C decomposition was observed. In this study we also showed an increase in MBC and soil C decomposition with plant presence, but particularly when litter was added in high amounts.

5.4.2 The influence of rye grass rhizosphere and litter addition on soil aggregation and soil C content.

In contrast to our second hypothesis, plants had no significant effect on overall soil aggregation: the plant treatment had no effect on the MWD, although the presence of plants did increase the fraction of silt and clay (Figure 5.5). Likewise, litter addition had no significant effect on MWD, although it tended to increase MWD in planted pots and decrease MWD in unplanted pots (Figure 5.5d). A similar trend was observed for macroaggregates (Figure 5.5a). Given that not much litter was left in the soil after 30 days, we speculate that rhizodeposition fueled the high MBC present in the High litter treatment (Figure 5.4a) to produce microbial compounds that are a crucial component of forming macroaggregates (Marschner, 2023). It is unclear why macroaggregates and MWD show a decreasing trend with increased litter. However, treatment effects on soil aggregation were in general weak, and it is possible that our study did not last long enough to significantly alter soil aggregation.

The detection of ^{13}C in all aggregate size classes indicate that the plant rhizodeposition C was incorporated in soil aggregates despite the short experiment length (3 days after ^{13}C pulse labeling), and that root exudates are incorporated in all three investigated soil aggregate size fractions. Our results are consistent with previous research indicating the strong effect of plants on improving soil aggregation more than other factors such as soil properties (Xiao et al., 2020). Rhizodeposition was not affected by litter addition (Figure 5.2b), and consequently, litter addition in general did not affect incorporation of rhizodeposition in different aggregates. However, the ^{13}C recovered in the silt and clay fraction was significantly lower in the High litter treatment (Figure 5.7). This could suggest that the microbial CUE of using rhizodeposition decreased in the High litter treatment so that less of the rhizodeposition-derive C remained in the soil. Possibly, the microbial

community in the High litter treatment changed towards a community specialized in decomposing more complex substrates (Olagoke et al., 2022) that may tend to have a lower CUE (Keiblinger et al., 2010). In a meta-analysis Ma et al. (2023) concluded that the magnitude of rhizosphere effect on soil C was greater from woody than herbaceous plants (like the rye grass in this research). We further note that the short duration of our ^{13}C labeling study may have underestimated the significance of the role of plant rhizodeposition and plant litter application for the long-term aggregate formation and stability.

5.5 Conclusions

We conclude that planting soil with rye grass can significantly increase the MBC, particularly with increasing rye grass litter addition rate, and thereby increasing the soil priming effect. In contrast, the addition of high litter to soil planted with rye grass did not increase the soil macroaggregate formation or the MWD, likely because of the short duration of the experiment. The effect of rye grass and litter addition was therefore not significant on soil aggregation or on the incorporation of rhizodeposits in soil aggregates. Longer-term studies are needed to address the interactive effects on rhizodeposition and litter addition on soil aggregation and C stabilization.

5.6 References

Angers, DA & Caron, J 1998, 'Plant-induced changes in soil structure: Processes and feedbacks', in Van Breemen, N (ed.), *Plant-induced soil changes: Processes and feedbacks*, Springer Netherlands, Dordrecht.

Baldock, JA & Skjemstad, JO 2000, 'Role of the soil matrix and minerals in protecting natural organic materials against biological attack', *Organic Geochemistry,* vol. 31, no. 7, pp. 697-710.

Beck, T, Joergensen, R, Kandeler, E, Makeschin, F, Nuss, E, Oberholzer, H & Scheu, S 1997, 'An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C', *Soil Biology and Biochemistry,* vol. 29, no. 7, pp. 1023-1032.

Bromand, S, Whalen, JK, Janzen, HH, Schjoerring, JK & Ellert, BH 2001, 'A pulse-labelling method to generate 13C- enriched plant materials', *Plant and Soil,* vol. 235, no. 2, pp. 253-257.

Bucka, FB, Kölbl, A, Uteau, D, Peth, S & Kögel-Knabner, I 2019, 'Organic matter input determines structure development and aggregate formation in artificial soils', *Geoderma,* vol. 354, pp. 113881.

Burton, AJ, Pregitzer, KS, Ruess, RW, Hendrick, RL & Allen, MF 2002, 'Root Respiration in North American Forests: Effects of Nitrogen Concentration and Temperature across Biomes', *Oecologia,* vol. 131, no. 4, pp. 559-568.

Campbell, TP, Ulrich, DEM, Toyoda, J, Thompson, J, Munsky, B, Albright, MBN, Bailey, VL, Tfaily, MM & Dunbar, J 2022, 'Microbial Communities Influence Soil Dissolved Organic Carbon Concentration by Altering Metabolite Composition', *Frontiers in Microbiology,* vol. 12.

Canarini, A, Carrillo, Y, Mariotte, P, Ingram, L & Dijkstra, FA 2016, 'Soil microbial community resistance to drought and links to C stabilization in an Australian grassland', *Soil biology & biochemistry,* vol. 103, pp. 171-180.

Cayley, J, Hannah, M, Kearney, G & Clark, S 1998, 'Effects of phosphorus fertiliser and rate of stocking on the seasonal pasture production of perennial ryegrass-subterranean clover pasture', *Australian Journal of Agricultural Research,* vol. 49, no. 2, pp. 233-248.

Chakraborty, D, Mondal, S, Das, A, Paul, A, Thomas, P, Aggarwal, P, Behera, U & Sharma, A 2019, 'Tillage and Crop Rotation Effects on Mechanical Properties and Structural Stability of a Sandy Loam Soil in a Semi-arid Environment', *Journal of the Indian Society of Soil Science,* vol. 67, no. 2, pp. 174-182.

Franzluebbers, AJ 2022, 'Soil mean-weight diameter and stability index under contrasting tillage systems for cotton production in North Carolina', *Soil Science Society of America journal,* vol. 86, no. 5, pp. 1327-1337.

Geyer, K, Schnecker, J, Grandy, AS, Richter, A & Frey, S 2020, 'Assessing microbial residues in soil as a potential carbon sink and moderator of carbon use efficiency', *Biogeochemistry,* vol. 151, no. 2, pp. 237-249.

Guhra, T, Stolze, K & Totsche, KU 2022, 'Pathways of biogenically excreted organic matter into soil aggregates', *Soil Biology and Biochemistry,* vol. 164, pp. 108483.

Guo, LB & Gifford, RM 2002, 'Soil carbon stocks and land use change: a meta analysis', *Global change biology,* vol. 8, no. 4, pp. 345-360.

Hasibeder, R, Fuchslueger, L, Richter, A & Bahn, M 2015, 'Summer drought alters carbon allocation to roots and root respiration in mountain grassland', *The New phytologist,* vol. 205, no. 3, pp. 1117-1127.

Hernandez, T, Hernandez, MC & Garcia, C 2017, 'The effects on soil aggregation and carbon fixation of different organic amendments for restoring degraded soil in semiarid areas: Effect of amendments on soil aggregation and C fixation', *European journal of soil science,* vol. 68, no. 6, pp. 941-950.

Husain, H & Dijkstra, FA 2023, 'The influence of plant residues on soil aggregation and carbon content: A meta-analysis', *Journal of plant nutrition and soil science*.

Isbell, R 2002, *The Australian Soil Classification*, 2nd ed. edn, CSIRO PUBLISHING, Melbourne.

Keiblinger, KM, Hall, EK, Wanek, W, Szukics, U, Hämmerle, I, Ellersdorfer, G, Böck, S, Strauss, J, Sterflinger, K, Richter, A & Zechmeister-Boltenstern, S 2010, 'The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency', *FEMS Microbiology Ecology,* vol. 73, no. 3, pp. 430-440.

Kuzyakov, Y 2002, 'Review: Factors affecting rhizosphere priming effects', *Journal of Plant Nutrition and Soil Science,* vol. 165, no. 4, pp. 382-396.

Lu, J, Dijkstra, FA, Wang, P & Cheng, W 2019, 'Roots of non-woody perennials accelerated long-term soil organic matter decomposition through biological and physical mechanisms.(Report)', *Soil Biology and Biochemistry,* vol. 134, pp. 42.

Ma, Y, Yue, K, Heděnec, P, Li, C, Li, Y & Wu, Q 2023, 'Global patterns of rhizosphere effects on soil carbon and nitrogen biogeochemical processes', *CATENA,* vol. 220, pp. 106661.

Marschner, P 2023, 'Chapter 15 - Rhizosphere biology☆☆This chapter is a revision of the third edition chapter by P. Marschner, pp. 369–388. DOI: https://doi.org/10.1016/B978-0-12-384905-2.00015-7. © Elsevier Ltd', in Rengel, Z, Cakmak, I & White, PJ (eds.), *Marschner's Mineral Nutrition of Plants (Fourth Edition)*, Academic Press, San Diego.

Mcgee, K, Eaton, W, Shokralla, S & Hajibabaei, M 2019, 'Determinants of Soil Bacterial and Fungal Community Composition Toward Carbon-Use Efficiency Across Primary and Secondary Forests in a Costa Rican Conservation Area', *Microbial Ecology,* vol. 77, no. 1, pp. 148-167.

Mehnaz, KR, Corneo, PE, Keitel, C & Dijkstra, FA 2019, 'Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen

mineralization and nitrous oxide emission from soil', *Soil Biology and Biochemistry,* vol. 134, pp. 175-186.

Neumann, G & Ludewig, U 2023, 'Chapter 14 - Rhizosphere chemistry influencing plant nutrition☆☆This chapter is a revision of the third edition chapter by G. Neumann and V. Römheld, pp. 347–368. DOI: https://doi.org/10.1016/B978-0-12-384905-2.00014-5. © Elsevier Ltd', in Rengel, Z, Cakmak, I & White, PJ (eds.), *Marschner's Mineral Nutrition of Plants (Fourth Edition)*, Academic Press, San Diego.

Olagoke, FK, Bettermann, A, Nguyen, PTB, Redmile-Gordon, M, Babin, D, Smalla, K, Nesme, J, Sørensen, SJ, Kalbitz, K & Vogel, C 2022, 'Importance of substrate quality and clay content on microbial extracellular polymeric substances production and aggregate stability in soils', *Biology and Fertility of Soils,* vol. 58, no. 4, pp. 435-457.

Pausch, J & Kuzyakov, Y 2018, 'Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale', *Global Change Biology,* vol. 24, no. 1, pp. 1-12.

Rakshit, A, Sarkar, B & Abhilash, P 2018, *Soil Amendments for Sustainability : Challenges and Perspectives*, First edition. edn, CRC Press, Boca Raton, FL.

Sarker, TC, Zotti, M, Fang, Y, Giannino, F, Mazzoleni, S, Bonanomi, G, Cai, Y & Chang, SX 2022, 'Soil Aggregation in Relation to Organic Amendment: a Synthesis', *Journal of Soil Science and Plant Nutrition,* vol. 22, no. 2, pp. 2481-2502.

Shahbaz, M, Kuzyakov, Y & Heitkamp, F 2017, 'Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls', *Geoderma,* vol. 304, pp. 76-82.

Sindelar, M, Blanco-Canqui, H, Jin, VL & Ferguson, R 2019, 'Cover Crops and Corn Residue Removal: Impacts on Soil Hydraulic Properties and Their Relationships with Carbon', *Soil Science Society of America journal,* vol. 83, no. 1, pp. 221-231.

Six, J, Paustian, K, Elliott, ET & Combrink, C 2000, 'Soil Structure and Organic Matter I. Distribution of Aggregate-Size Classes and Aggregate-Associated Carbon', *Soil Science Society of America Journal,* vol. 64, no. 2, pp. 681-689.

Soares, M & Rousk, J 2019, 'Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry', *Soil Biology and Biochemistry,* vol. 131, pp. 195-205.

Sparling, G 1992, 'Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter', *Soil Research,* vol. 30, no. 2, pp. 195-207.

Tisdall, JM & Oades, JM 1982, 'Organic matter and water-stable aggregates in soils', *Journal of Soil Science,* vol. 33, no. 2, pp. 141-163.

Wang, C, Qu, L, Yang, L, Liu, D, Morrissey, E, Miao, R, Liu, Z, Wang, Q, Fang, Y & Bai, E 2021a, 'Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon', *Global change biology,* vol. 27, no. 10, pp. 2039-2048.

Wang, R, Bicharanloo, B, Shirvan, MB, Cavagnaro, TR, Jiang, Y, Keitel, C & Dijkstra, FA 2021b, 'A novel 13C pulse-labelling method to quantify the contribution of rhizodeposits to soil respiration in a grassland exposed to drought and nitrogen addition', *The New phytologist,* vol. 230, no. 2, pp. 857-866.

Xiao, L, Yao, K, Li, P, Liu, Y, Chang, E, Zhang, Y & Zhu, T 2020, 'Increased soil aggregate stability is strongly correlated with root and soil properties along a gradient of secondary succession on the Loess Plateau', *Ecological Engineering,* vol. 143, pp. 105671.

Yan, S, Yin, L, Dijkstra, FA, Wang, P & Cheng, W 2023, 'Priming effect on soil carbon decomposition by root exudate surrogates: A meta-analysis', *Soil Biology and Biochemistry,* vol. 178.

Zhao, X, Tian, P, Sun, Z, Liu, S, Wang, Q & Zeng, Z 2022, 'Rhizosphere effects on soil organic carbon processes in terrestrial ecosystems: A meta-analysis', *Geoderma,* vol. 412, pp. 115739.

Chapter 6. General discussion, conclusion, and future research

6.1 Discussion

Soil structure maintains soil functions in terrestrial ecosystems on earth. However, the needed organic carbon (C) as a key factor for the formation and stability of soil structure units (*i.e.,* soil aggregates) goes through continuous transformations by natural (plants, soil microbes, and climate) and anthropogenic (such as land management) factors. As soil microbes (such as bacteria and fungi) are considered as important players in the decomposition of soil organic C, in this thesis I focused on the microbial processes and plant-microbial interactive effects on soil C decomposition (priming effect), soil aggregation and soil C content.

In this thesis I addressed factors influencing the soil structure and the soil C content as influenced by soil microbes (particularly bacteria and fungi), land management such as plant residue amendment (type and addition rate), and vegetation (root trait and rhizosphere effect) (chapter 2). The effects of soil amendments with plant materials on soil respiration, microbial biomass C (MBC), the microbial carbon use efficiency (CUE) of added plant substrates, soil priming effect (PE), soil aggregation and substrate C incorporation into the MBC and into each soil aggregate size fraction (macroaggregate, microaggregate, and silt and clay fraction), and soil total C were also investigated (chapters 3, 4, and 5). In chapters 4 and 5, I used ^{13}C labeling techniques to be able to trace C allocations as affected by soil treatments in plant components (shoot and root) (chapter 5), total soil, soil aggregate fractions, MBC, and soil respiration (chapters 4 and 5). Here, the findings of my studies will be briefly discussed.

6.1.1 The microbial decomposition of organic C in the soil

Plant organic materials that enter the soil vary in the energy content and recalcitrance, consequently triggering different trends of microbial activity (enzyme production) in the soil (Ai et al., 2023). For instance, simple sugars like glucose (either secreted from live plant roots (rhizodeposition) or from the microbial decomposition of complex organic materials in the soil) are easy C-sources for activating bacteria and fungi in the soil (Gunina & Kuzyakov, 2015). Recalcitrant plant materials such as plant litter require more energy from soil microbes (i.e., slow decomposition) and these are mainly decomposed by soil fungi rather than soil bacteria (Ullah, Carrillo & Dijkstra, 2023). In **Chapter 2** I addressed the microbial processes that govern the decomposition of plant substrates with respect to soil geochemical factors affecting bacterial and fungal activities in the soil, the microbial CUE of added substrates to soil, and how these factors are related to C transformations and sequestration in the soil. The previous research results regarding the microbial decomposition processes of plant residue soil amendments indicated that the continuous addition of various types of plant residues to soil will result in successive transformations in the composition of soil microbial communities according to the type and quality of provided plant substrate. These transformations of the residing microbial communities in the soil will induce new patterns of microbial enzymatic activities, affecting the degree of substrate decomposability, microbial CUE, and C mineralization in the soil (microbial respiration). The soil C mineralization rate is related to the CO_2 emissions from the soil, challenging the human efforts of climate change mitigation. Based on the above, there is a need of continuous analysis of the plant residue amended soils to adopt a better land management practice to tackle the growing concerns regarding the soil contribution into the rising global warming problem due to the elevated concentration of atmospheric CO_2.

In **Chapter 4**, I examined the bacterial vs fungal decomposition of two types of added substrates to the soil: a simple-C (glucose) and a complex-C (wheat root litter). As expected, the decomposition of glucose was very rapid, compared to the decomposition of wheat litter biomass, which was relatively slow. Therefore, glucose was added several times during the incubation period. The suppression of both soil microbes (bacteria and fungi) using bronopol and captan significantly reduced the decomposition of added substrates, indicating the significant role of both bacteria and fungi for the decomposition of both types of plant substrates (labile and complex C) in the soil. However, soil bacteria appeared to be more important than soil fungi in the initial stages of glucose decomposition, but both bacteria and fungi appeared to be important for the decomposition of complex substrate (wheat root biomass).

With respect to the microbial decomposition of the soil native C (PE), the addition of either type of substrate (labile (glucose) or complex C (wheat root biomass) induced similar trends of PE across soil treatments. However, the lowest PE was observed from suppressing fungi in the soil, indicating that soil fungi is mostly responsible for a positive PE (*i.e.,* C loss from the soil) with the addition of labile or complex C substrate.

In **Chapter 5** I investigated the decomposition of two addition rates of rye grass (*Lolium perenne*) litter to the soil with and without the presence of rye grass live roots (the effect of rhizosphere on soil C decomposition will be discussed in a separate section below). In this study, the litter addition to soil was from the beginning of the experiment (along with sowing the rye grass seeds) whereas soil respiration was measured three days after the CO_2 pulse labeling (after 33 days of the start of the glasshouse experiment). Here, I believe that during the 30 days of the rye grass growth most of the added litter will be decomposed (before the respiration measurements were taken), especially with the presence of rye grass live root exudates (planted treatments) that may accelerate

the litter decomposition by enhancing the microbial activity in the soil. Consequently, the different addition rates of litter to soil did not appear to be influential on the soil respiration. The significant plant treatment effect on soil respiration is attributed to the high root respiration. The rhizosphere PE trend in the planted soil treatments appeared to be influenced by the increase in the addition rate of rye grass litter (although it was not significant).

In this study, it was not possible to separate soil respiration from the microbial decomposition of added rye grass litter from the soil native C. Another note here is that there was no separation of the of bacterial activity from the fungal activity in treated soils. I first attempted this with the addition of bronopol and captan, but these chemicals prevented germination of the rye grass seeds when they were added at the beginning of the experiment. Another attempt of adding the same biocides after the germination of rye grass seeds was not successful either, as the seedlings did not tolerate the biocide addition to soil, where they turned yellow, wilted and stopped growing. Therefore, the experimental design was changed to plant and litter addition treatments only without focusing on the specific bacterial and fungal processes as a response of the implemented soil treatments.

6.1.2 The effect of plant residue amendment on soil structure and soil C content

The addition of plant residue to soil has been proven to enhance the soil aggregation and soil C content (Sarker et al., 2022). However, the heterogeneity of the plant residue chemistry and related decomposition mechanisms for each substrate type (Ai et al., 2023) restrain our understanding of the extent of each residue type benefits to soil aggregation and soil C. In **Chapter 3**, through a meta-analysis study I investigated the significance of experimental factors (experimental duration, addition rate), soil factors (clay content, initial organic C, and pH), residue type (fresh and charred) and residue origin for soil aggregation and soil C content in response to plant residue soil

amendments. My findings indicated that soil amendments with fresh plant residue is more beneficial for soil aggregation than for soil C content, while soil amendments with charred plant residues is more favorable for the soil C content enhancement. However, the benefits of plant residue addition to the soil were found to be influenced by soil factors like the initial soil C content, where the soil can reach a saturation state of C content (*i.e.,* no more organic C can be sequestered in the soil) (Gulde et al., 2008). All investigated origins of fresh plant residue soil amendments resulted in a positive response of the MWD, where the highest positive response was from soil amendment with alfalfa residue and the lowest positive response was from wheat residue. However, in previous research it was emphasized that the positive effect of alfalfa residue on the MWD is valid on the short term only, where the stability of the MWD was considered dynamic on the long term when compared with the effect of barley residue amendment (Zhu et al., 2017). Nevertheless, my findings from the meta-analysis showed a lower response from barley residue soil amendment than from the alfalfa residue soil amendment, possibly due to the short duration of studies (weeks to few months) used in the meta-analysis for these two residue origins.

In **Chapter 4**, soil aggregation revealed a positive response towards the addition of substrates. However, the experimental duration (16 days) was not long enough to explain the long-term stability of the newly formed aggregates during the incubation time. Moreover, the measured aggregation represented by the mean weight diameter (MWD) was not related to the microbial growth nor CUE of the added substrates as was expected (**Chapter 2,** Figure 2.2), where the short duration of the study is suggested to be a possible reason for the absence of such relationship. The significance of biocide effect on MWD and the reduction of MWD when fungal activity was suppressed alone or along with bacterial activity suppression indicated that soil fungi are more influential for soil aggregation than soil bacteria.

In this study I found that the MBC was not significantly affected by the type of added substrate, but the effect from the biocide addition appeared to be significant, indicating that the type of soil microbial community is more influential than the type of substrate for the MBC. Qiao et al. (2019) found that the CUE (that depends on MBC) is dependent on the substrate type. However, the same study highlighted that glucose could influence changes in the CUE, where these changes were attributed to environmental factors like temperature.

In **Chapter 5**, I note that the addition of rye grass litter was coupled with the rye grass rhizosphere effect in planted soil treatments. The high rye grass litter addition soil treatment influenced an increase in the trend of MWD in the planted soil treatment, which is consistent with the findings of my meta-analysis regarding the positive response from the MWD with the increase in plant residue amount applied to soil (**Chapter 3**). The effect of rye grass addition on soil C content was not significant, possibly because of the short duration of my study.

6.1.3 The effect of plant rhizosphere on soil aggregation and soil C content

Plant root exudates in the rhizosphere activate the coexisting soil microbes such as bacteria and fungi and accelerate the microbial decomposition of soil organic C (including any added plant litter) (Kuzyakov, 2002). The variations in plant rhizodeposits into the soil rhizosphere can activate different soil microbes, and thus plant rhizodeposition mediate specific plant-microbial interactions in the rhizosphere microenvironment (Jones & Hinsinger, 2008). The plant litter addition to planted soil (with presence of rhizosphere) can increase the effect of rhizosphere on the decomposition of soil C (priming effect) through triggering an increased microbial production of decomposition enzymes (Ai et al., 2023). The rhizosphere effect can enhance soil aggregate stability and soil organic C in comparison with non-rhizosphere soil (Li et al., 2020).

In **Chapter 4**, I used glucose as a surrogate of plant rhizodeposition of simple sugars to investigate the effect of the plant rhizosphere on aggregation (macroaggregates, microaggregates, and silt and clay fraction) and MWD, and how it relates to bacterial and fungal activity in the soil. Here the effect of glucose addition was more apparent on the soil microbes than the soil structure, as the response from soil aggregates was not statistically significant when glucose was added to the soil. On the other hand, only the effect from biocide addition was significant on all investigated aggregate size classes, indicating the higher significance of soil bacteria and fungi for soil aggregation than the type of added organic C to the soil.

In **Chapter 5**, the rye grass rhizosphere effect was significant only for the silt and clay aggregate fraction, where the presence of rhizosphere (planted treatment) increased the silt and clay aggregate fraction. However, the addition of rye grass litter in planted soil decreased the proportion of silt and clay aggregate fraction with no significant difference when more litter was added. The MWD response to the presence of rye grass rhizosphere was opposite to the silt and clay fraction, but followed the same trend as macro and microaggregates.

I note here that the soil was sieved to destroy macroaggregates. Therefore, all separated macroaggregates are newly formed during the incubation period (**Chapter 4**) and the duration of glasshouse study (**Chapter 5**). This indicates that the formation of macroaggregates can be achieved in short periods such as a few weeks. However, the stability of newly formed aggregates because of litter or plant treatments on the long-term cannot be examined by these short-term studies. Research by Wang et al. (2022) noted that the change in soil aggregation is as slow as that it can only be observed after 5 years of changing the land use from bare into a grass land. In this thesis, the aim of the studies in **Chapters 4** and **5** was to investigate the initial aggregate and microbial processes in response to plant litter addition, rhizosphere presence or both.

Consistent with a meta-analysis finding by Zhao et al. (2022), the rye grass rhizosphere increased the MBC significantly, and the MBC increased more when the rhizosphere effect was coupled with high litter addition.

Based on the results and discussions presented in this thesis, it is recommended for agroecosystems to continue adapt the practice of plant residue soil amendments to enhance aggregation and soil C content. Despite the differences in the magnitude of the positive effect among plant residue origins on soil aggregation and C content, it seems more likely that farmers will prefer to continue using easily available local agricultural residues as a sustainable source of soil fertilizers and a cost-effective alternative as the data showed (see supplementary data, Chapter 3) a wide range of local agricultural residues in use as soil amendments. However, because of the growing demands of food security, energy, and industry plus climate change implications on C bio-transformations in the soil by microbial communities as key regulators of soil C (Chapter 4 and 5), it will be always a challenge to tackle these transformation/changes. Consequently, there is a great need to include the "biological perspective" to soil management policies (Lehmann et al., 2020) (see section 6.3).

6.2 Conclusions

The main conclusions I can draw from the studies I reported in this thesis are:

- The effect of plant residue soil amendments on soil aggregation and soil C depends on soil chemistry (such as soil initial C content and pH).
- The soil bacteria are more important than fungi for labile C (such as glucose) decomposition, while both microbes are important for the decomposition of complex C (such as wheat root biomass) decomposition.

- Soil fungi seem to be influential on soil priming (C loss), while high litter addition may increase soil priming.
- Rye grass plants increased soil aggregation, but did not increase the decomposition of added litter.

6.3 Future research

- Long-term studies (more than a year) are needed to investigate the long-term effect of plant residue soil amendments on soil aggregation and soil C content.
- There is a need for a continuous monitoring for the soil microbial communities as key players in the decomposition of soil organic C and influencers of soil aggregation and the C sequestration in the soil. Techniques such as phospholipid fatty acid analysis can be adapted to evaluate the changes in the soil microbial communities to detect the specific effects of soil management practices on functioning microbial communities in the soil (Nkongolo & Narendrula-Kotha, 2020).
- Future research should consider combining more than one type of plant residue to explore the best land management practices for soil structure conservation and soil C enhancement.

6.4 References

Ai, L, Wu, F, Fan, X, Yang, Y, Zhang, Y, Zheng, X, Zhu, J & Ni, X 2023, 'Different effects of litter and root inputs on soil enzyme activities in terrestrial ecosystems', *Applied Soil Ecology,* vol. 183, pp. 104764.

Gulde, S, Chung, H, Amelung, W, Chang, C & Six, J 2008, 'Soil Carbon Saturation Controls Labile and Stable Carbon Pool Dynamics', *Soil Science Society of America journal,* vol. 72, no. 3, pp. 605-612.

Gunina, A & Kuzyakov, Y 2015, 'Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate', *Soil Biology and Biochemistry,* vol. 90, pp. 87-100.

Jones, D & Hinsinger, P 2008, 'The rhizosphere: complex by design', *Plant and Soil,* vol. 312, no. 1, pp. 1-6.

Kuzyakov, Y 2002, 'Review: Factors affecting rhizosphere priming effects', *Journal of Plant Nutrition and Soil Science,* vol. 165, no. 4, pp. 382-396.

Lehmann, J, Bossio, DA, Kögel-Knabner, I & Rillig, MC 2020, 'The concept and future prospects of soil health', *Nature reviews. Earth & environment,* vol. 1, no. 10, pp. 544-553.

Li, J, Yuan, X, Ge, L, Li, Q, Li, Z, Wang, L & Liu, Y 2020, 'Rhizosphere effects promote soil aggregate stability and associated organic carbon sequestration in rocky areas of desertification', *Agriculture, Ecosystems & Environment,* vol. 304, pp. 107126.

Nkongolo, KK & Narendrula-Kotha, R 2020, 'Advances in monitoring soil microbial community dynamic and function', *Journal of applied genetics,* vol. 61, no. 2, pp. 249-263.

Qiao, Y, Wang, J, Liang, G, Du, Z, Zhou, J, Zhu, C, Huang, K, Zhou, X, Luo, Y, Yan, L & Xia, J 2019, 'Global variation of soil microbial carbon-use efficiency in relation to growth temperature and substrate supply', *Scientific Reports,* vol. 9, no. 1.

Sarker, TC, Zotti, M, Fang, Y, Giannino, F, Mazzoleni, S, Bonanomi, G, Cai, Y & Chang, SX 2022, 'Soil Aggregation in Relation to Organic Amendment: a Synthesis', *Journal of Soil Science and Plant Nutrition,* vol. 22, no. 2, pp. 2481-2502.

Ullah, MR, Carrillo, Y & Dijkstra, FA 2023, 'Relative contributions of fungi and bacteria to litter decomposition under low and high soil moisture in an Australian grassland', *Applied Soil Ecology,* vol. 182, pp. 104737.

Wang, F, Zhang, X, Neal, AL, Crawford, JW, Mooney, SJ & Bacq-Labreuil, A 2022, 'Evolution of the transport properties of soil aggregates and their relationship with soil organic carbon following land use changes', *Soil and Tillage Research,* vol. 215, pp. 105226.

Zhao, X, Tian, P, Sun, Z, Liu, S, Wang, Q & Zeng, Z 2022, 'Rhizosphere effects on soil organic carbon processes in terrestrial ecosystems: A meta-analysis', *Geoderma,* vol. 412, pp. 115739.

Zhu, Z, Angers, DA, Field, DJ & Minasny, B 2017, 'Using ultrasonic energy to elucidate the effects of decomposing plant residues on soil aggregation', *Soil & tillage research,* vol. 167, pp. 1-8.