CRANFIELD UNIVERSITY

Emily Dowdeswell-Downey

Climate impacts on soil susceptibility to erosion

School of Water, Energy and Environment

PhD
Academic Year: 2016 - 2020

Supervisor: Dr Robert Grabowski
Associate Supervisor: Professor Jane Rickson
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ABSTRACT

Soil erosion threatens soil sustainability and the provision of ecosystem services and is predicted to increase in the future with climate change. Soil erodibility, the susceptibility of soil to erosion, is often estimated as a constant variable but the best indicator of erodibility is aggregate stability, which is a dynamic soil property and has been observed to vary with changes in local climatic conditions. Aggregate stability is influenced by biological stabilisation and the soil microbial community are known to respond to changes in climatic conditions, yet whether aggregate dynamics can be explained by shifts in the soil microbial community has not been fully investigated. This thesis aims to investigate the influence of climatic conditions, in terms of soil temperature and moisture content, on aggregate stability, and thus soil erodibility, and whether these dynamics are explained by climate-induced changes in the soil microbial community. Environmental chambers and a rainfall simulator were used to examine the effects of climatic conditions and rainfall on aggregate stability and soil microbial properties as indicators of biological stabilisation in single-layer and multi-layered aggregate microcosms. The key findings show that temperature and moisture content significantly affected aggregate stability and the influence of soil temperature and moisture on soil microbial properties is soil texture dependent. Soil microbial properties were significant predictors of aggregate stability. Aggregate stability did not differ between climate scenarios in seasonal treatments but was significantly lower in seasonal treatments compared to constant seasons. Soil temperature and moisture significantly affected soil erodibility related to changes in aggregate stability and the soil microbial community. Rainfall significantly affected microbial properties in eroded soil and selectively mobilised a fungal-dominated component of the microbial community, influenced by preceding climatic treatments. The research highlights the further need to (i) recognise the role of climate-driven microbial shifts mediating aggregate stability mechanistically; and (ii) integrate knowledge on aggregate-scale mechanisms across larger spatial scales.

Keywords: aggregate stability, soil erodibility, climatic conditions, temperature, moisture content, soil microbial community

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LIST OF ABBREVIATIONS

EPS	Extracellular Polymeric Substances
PLFA	Phospholipid Fatty Acid
RABIT	Rapid Automated Bacterial Impedance Technique
CFE	Chloroform Fumigation Extraction
DOC	Dissolved Organic Carbon
PC	Principal Component
PCA	Principal Component Analysis

1 Introduction

1.1 Introduction

Soil is a unique material that is vital as a resource, providing numerous ecosystem services for humans and for ecological functions, including food provision, nutrient cycling and hydrological cycling (Haygarth & Ritz, 2009; Dominati *et al.*, 2010; Jónsson & Davíðsdóttir, 2016). Soil is a crucial finite and non-renewable resource. Whilst soil erosion, the removal of soil particles and associated nutrients and organic matter, is a natural process balanced by soil formation processes, accelerated soil erosion rates can lead to net soil depletion and soil degradation (Montgomery, 2007; Amundson *et al.*, 2015; Fernández-Raga *et al.*, 2017). Soil erosion presents environmental problems on-site where soil becomes degraded with a reduced capability and capacity to provide ecosystem services (Rickson *et al.*, 2015). Additionally, the erosion of soil generates negative off-site impacts, such as the pollution of water-bodies leading to decreased water quality and eutrophication, and sedimentation (Boardman, 2013; Rickson, 2014). The 'tolerable' rate of soil erosion in Europe is generally estimated to be around 1 t ha^{-1} yr^{-1}, yet actual erosion rates can be accelerated and are often much greater than the tolerable erosion rate due to human action and land management (Verheijen *et al.*, 2009; Benaud *et al.*, 2020). Climate change is also predicted to further accelerate soil erosion rates, with numerous studies estimating the effects of increased climatic erosivity on soil erosion (Nearing, 2001; Nearing *et al.*, 2004; Panagos *et al.*, 2017).

Soil erodibility, i.e. the susceptibility of soil to erosion processes, is an important component of soil erosion processes and rates (Middleton, 1930; Bryan, 1968, 2000). Despite evidence that soil erodibility is dynamic and has been observed to change seasonally and interannually (Dimoyiannis, 2009), it is often treated as a static soil property when predicting soil erosion rates. Soil erodibility was thought to be mainly influenced by physico-chemical properties, such as soil texture, which are slow to change over decades and so has often been treated as a constant soil property for a given soil type (Bryan, 2000; Deviren Saygin *et al.*, 2018). However, it has been recognised that soil erodibility is controlled by dynamic soil properties, such as aggregation and soil microbial properties, that are strongly influenced by climatic

conditions, but the implications of transient soil properties have not been fully considered for the prediction of soil erodibility and erosion response (Bryan, 2000; Ding & Zhang, 2016; Deviren Saygin *et al.*, 2018). Currently erosion models do not capture inter- and intra-annual variation in soil erodibility and rarely are the effects of changing climatic conditions on soil erodibility considered (Deviren Saygin *et al.*, 2018).

Soil erodibility is a function of multiple soil characteristics and can be measured by a number of soil erodibility indices, of which the most suitable indicator that is best correlated with soil loss, appears to be aggregate stability (Bryan, 2000). Aggregate stability has a strong inverse relationship with soil erodibility and is an important soil property that defines soil structure and influences multiple soil functions. The stability of aggregates, controlled by interparticle and intra-aggregate bonds, governs their resistance to external stresses and the physical integrity of the soil pore network by influencing pore-size and shape distribution, and pore-connectivity (Bryan, 1968; Barthès & Roose, 2002; Nciizah & Wakindiki, 2015). Aggregates are collections of soil particles that are more strongly bound together than surrounding particles and are collectively influenced by physical, chemical, and biological soil properties and stabilising mechanisms (Figure 1.1). Particle interaction forces and intra-aggregate bonds determine fundamentally aggregate stability and are influenced by soil physical and chemical properties, such as particle size and shape, soil texture (particle size distribution), clay content and mineralogy, organic matter content, soil pH and the presence of metal (hydr)oxides and multivalent cations, and further altered by biological bonding and stabilising mechanisms (Degens, 1997; Hu *et al.*, 2015; Regelink *et al.*, 2015). The microbial community has a positive stabilising influence on aggregates through the hyphal enmeshment of particles and the production of extracellular polymeric substances (EPS) which build cohesive and adhesive bonds between particles and aggregates, thereby determining aggregate stability (Tisdall & Oades, 1982; Tisdall, 1991; Degens, 1997; Lehmann & Rillig, 2015). A recent global meta-analysis of 279 studies found that soil biota had a strong significant positive effect on aggregate stability, with bacteria and fungi of the highest importance (Lehmann *et al.*, 2017). Soil microbial community composition, biomass, and activity, in terms of respiration, are closely associated with biological stabilising mechanisms

and are sensitive indicators of microbial responses to changes in soil status. These microbial properties encapsulate key changes in microbial functioning, and thus have been frequently measured in the literature as indicators of microbially mediated processes (Schloter *et al.*, 2003; Bending *et al.*, 2004; Ritz *et al.*, 2009; Truu *et al.*, 2009; Pulleman *et al.*, 2012).

Aggregate stability has been observed to change with local climatic conditions (Blackman, 1992; Cosentino *et al.*, 2006; Algayer *et al.*, 2014), yet empirical evidence is often contradictory and so a complete mechanistic explanation is lacking. Further information is required on the climate-sensitive stabilising mechanisms to elucidate climate-induced changes in aggregate stability and soil erodibility. This knowledge would help to refine knowledge of soil erodibility and erosion processes, and more accurately predict future soil erosion processes and rates. Soil temperature and moisture are known to affect aggregate stability directly through the physical disruption of intra-aggregate bonds. For example, an increase in moisture content can lead to aggregate breakdown by slaking and physico-chemical dispersion (Le Bissonnais, 1996, 2016). However, these processes do not fully account for the temporal variability of aggregate stability. The soil microbial community has long been known to alter soil aggregate stability and respond to climatic conditions, but despite this there has been limited research on how climatic conditions affect aggregate stability by influencing the soil microbial community and biological stabilisation.

Changes in aggregate stability may be explained by climate-driven shifts in the soil microbial community. Soil microbes are well known to respond rapidly to changes in climatic conditions, such as temperature and soil moisture (Figure 1.1). Temperature has been closely associated with microbial metabolic rates and is recognised as a key driver that affects the rate of microbial processes in the metabolic theory of ecology (Brown *et al.*, 2004; Clarke, 2006). Microbial respiration has exhibited a strong positive relationship with temperature, dependent on optimal temperature ranges, and oxygen and resource availability (Moyano *et al.*, 2013; Karhu *et al.*, 2014). Microbial respiration rates have also been reportedly influenced by soil moisture, causally linked to oxygen availability and solute diffusion (Manzoni *et al.*, 2011; Moyano *et al.*, 2013). Soil moisture content affects water and oxygen availability, osmotic pressure, and pore-water connectivity, and thus drives changes in the soil microhabitat (Or *et al.*, 2007b).

Soil microbes respond to changes in osmotic pressure by osmoregulation, however variations in microbial physiological cell traits and adaptations results in differences in resistance and survival to osmotic stress (Csonka, 1989; Wood *et al.*, 2001; Wood, 2015). Therefore, changes in soil moisture are expected to induce shifts in microbial community composition. Pore-water connectivity increases with soil moisture and increases bacterial motility and solute diffusion, thus increasing accessibility to resources and stimulating microbial activity and growth (Or *et al.*, 2007b; Wang & Or, 2010). Therefore, soil temperature and moisture can stimulate or limit microbial activity and growth and alter microbial community composition (Figure 1.1). The effects of temperature and soil moisture on soil microbes are relatively well examined, but continues to be a key topic for research with the challenges of high microbial diversity and varying microbial responses to climatic conditions, as well as the complexity of the soil microhabitat and interaction of multiple simultaneous processes. The consequences of climate-induced changes in the microbial community and the effects on microbial functions, such as decomposition and carbon cycling, have been recognised (Davidson & Janssens, 2006; Bardgett *et al.*, 2008; Conant *et al.*, 2011), though the implications for aggregate stability have received less attention. Changes in microbial properties, such as composition, biomass, and respiration, may indicate changes in the operation and effectiveness of biological stabilising mechanisms by influencing the allocation of resources for growth and production of exudates. The influence of climatic conditions on aggregate stability, possibly explained by the effects of climatic conditions on the soil microbial community and biological stabilisation, requires characterisation and is a critical knowledge gap and research need. This research will examine the effects of climatic conditions, soil temperature and moisture, on both aggregate stability and the microbial community, where previously only two components of climate-aggregate-microbe feedbacks have been considered (Manzoni *et al.*, 2011; Ebrahimi & Or, 2016; Ren *et al.*, 2017).

Climatic conditions follow seasonal patterns and some studies have observed a seasonal pattern in aggregate stability, with aggregate stability generally increasing over spring and summer and decreasing to a minimum in winter (Bullock *et al.*, 1988; Blackman, 1992; Dimoyiannis, 2009; Algayer *et al.*, 2014). This seasonal pattern of stability has been related to physical processes associated with seasonal climatic

conditions such as freeze-thaw and drying-wetting cycles (Bullock *et al.*, 1988; Cosentino *et al.*, 2006; Dagesse, 2013; Le Bissonnais, 2016), as well as the time of year when organic matter amendments and soil management practices are applied (Six *et al.*, 2000; Abiven *et al.*, 2009). However, the response of soil microbes to seasonal climatic conditions and the role of the soil microbial community in aggregate stabilisation may provide further explanation for seasonal aggregate stability dynamics. The effects of seasonal shifts in climatic conditions on the soil microbial community remain unclear and is complicated by interactions with soil properties such as texture. Evidence has shown that preceding climatic conditions influences the community composition and response of soil microbes to subsequent conditions (Fierer *et al.*, 2003; Evans & Wallenstein, 2012, 2014). It is therefore necessary to examine the influence of seasonal conditions on soil microbial properties and whether this can elucidate seasonal patterns in aggregate stability. Climate change will alter multiple aspects of climate and weather, of which two critical climatic conditions for soils are air temperature and precipitation (Bradley et al., 2005). Air temperature is closely related to soil temperature, so higher air temperatures in both summer and winters will cause an increase in soil temperature. Likewise, changes in the amount of rainfall and evaporation will alter soil moisture content. Therefore, climate change is expected to result in hotter, drier summers and warmer, wetter, winters (Jenkins *et al.*, 2009; Lowe *et al.*, 2018). Further information on the effects of predicted changes in temperature and soil moisture during summer and winter on microbial community properties and aggregate stability is a key research need, and the change in aggregate stability and response of the soil microbial community to future climatic scenarios must be examined.

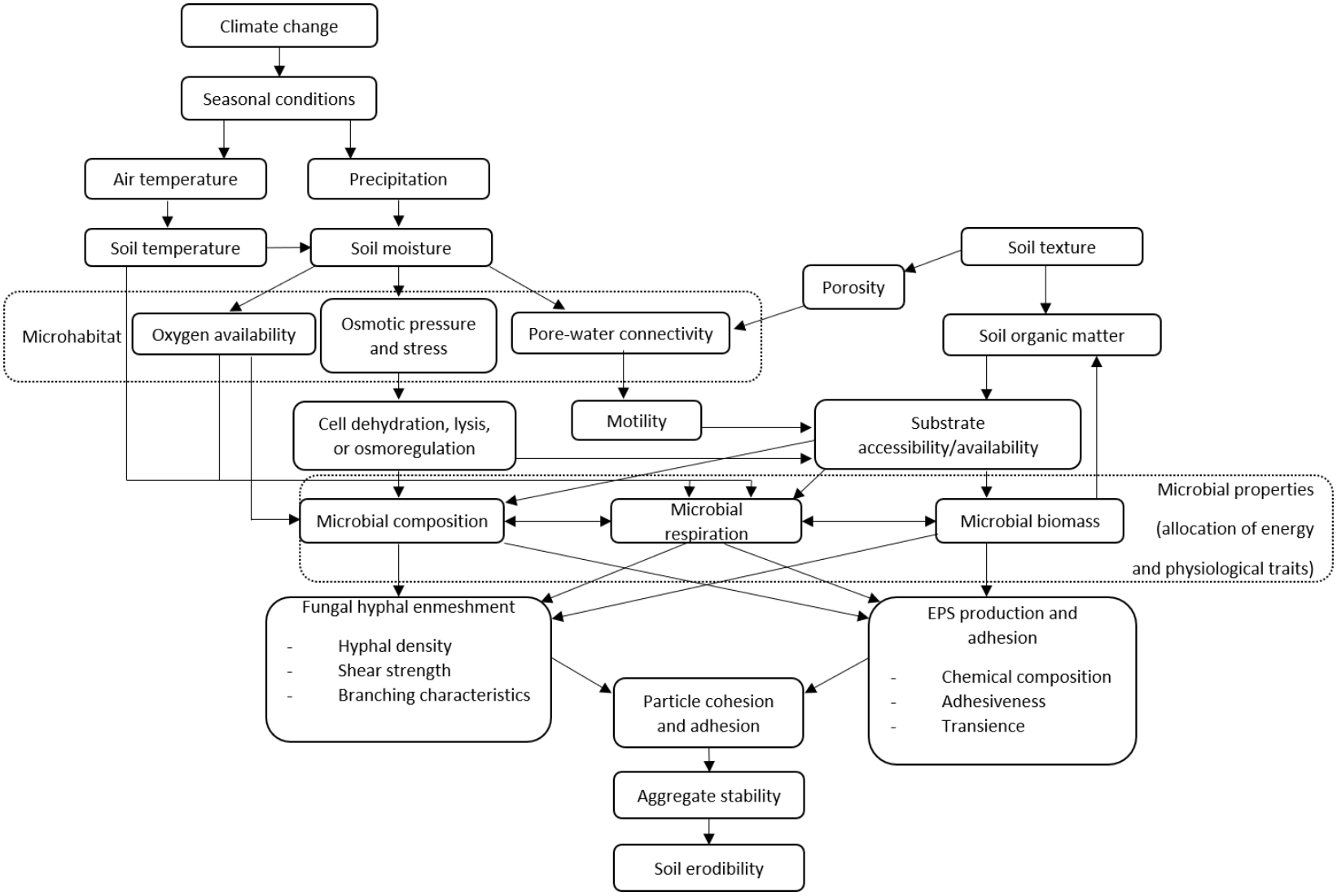

Figure 1.1: The effects of climatic conditions, in terms of soil temperature and moisture on the microbial community, biological stabilising mechanisms, and thus aggregate stability and soil erodibility.

The effects of climatic conditions on the soil system and erosion processes span multiple spatial scales (Imeson & Lavee, 1998; Vereecken *et al.*, 2016). Microscale heterogeneity and microbial processes upscale to influence landscape- and global-scale soil processes and ecosystem functions (Mueller *et al.*, 2013; Ebrahimi & Or, 2016; Baveye *et al.*, 2018). Here, climate-induced changes in the microbial community and biological stabilisation upscale to influence aggregate stability, and in turn aggregate stability upscales to affect soil erodibility. In order to understand the effects of climatic conditions on aggregate stability, it is necessary to examine the soil microbial community, and biological stabilisation at a scale relevant to the mechanisms in operation (Young *et al.*, 2008; Nunan, 2017; Bach *et al.*, 2018; Juyal *et al.*, 2019). Therefore, this research is initially focussed on the aggregate-scale. However, to interpret the effects of climatic conditions on soil erodibility it is necessary to upscale independent aggregate mechanisms to a system of aggregates, with interactions and processes operating across the aggregate matrix. In a multi-layered matrix of aggregates, there will be additional processes in operation with interactions across multiple interfaces with inter-aggregate connections and pore-space. Climate-induced changes in aggregate stability, provoked by changes in the soil microbial community and biological stabilisation, are expected to have a direct effect on soil erodibility. The relationship between climatic conditions, the soil microbial community, and aggregate stability could also influence hydrological processes by altering soil structure (Bronick & Lal, 2005; Vergani & Graf, 2016). Aggregate breakdown is expected to reduce porosity and result in the reduction of hydraulic conductivity, thus reducing infiltration rate and increasing runoff generation. Therefore climate-induced changes in the soil microbial community and aggregate stability may affect soil erosion two-fold by influencing soil erodibility and hydrological processes.

A rainfall event may directly affect the soil microbial community through i) changes in soil moisture, ii) the breakdown of aggregates, soil structure, and microhabitats, thereby redistributing microbes and resources and iii) the mobilisation of microbes and transportation from the soil matrix in aqueous

cultures in runoff and infiltrate (Cosentino *et al.*, 2006; Allton *et al.*, 2007; Manzoni *et al.*, 2011). Changes in the soil microbial community following a rainfall event have implications for subsequent microbial diversity and functioning (Le *et al.*, 2020). For example, rainfall events may induce changes in the soil microbial community due to the selective mobilisation of components of the microbial community, resulting in the potential loss of biomass or diversity in in-situ eroded soil. In turn the adjustment of the soil microbial community may alter subsequent biological stabilisation and aggregate stability, and thereby affect future soil erodibility and erosion rates. Studies have investigated the effects of soil moisture, and to an extent aggregate breakdown, on the soil microbial community, particularly microbial respiration with drying-wetting cycles (Birch, 1958; Moyano *et al.*, 2013; Meisner *et al.*, 2017). However, uncertainties concerning the microbial properties of soil post-rainfall remain, because the rainfall event not only affects soil moisture, but also affects aggregate breakdown and microbial redistribution and mobilisation. Despite evidence of the mobilisation of a component of the microbial community in runoff and infiltrate, few studies have investigated the transportation of soil microbes by soil erosion and have considered the implications of reduced biomass or diversity for the microbial functioning of post-rainfall soil (Allton, 2007; Le *et al.*, 2020). Studies that have considered the export of soil microbes in runoff are generally focused on faecal bacteria and the consequences for water contamination and water quality management (Tyrrel & Quinton, 2003). Preceding soil temperature and moisture may alter the effects of rainfall on the soil microbial community by affecting microbial community composition, biomass, and respiration, as explained above. Climatic conditions may also provoke changes in microbially-mediated aggregate stability, and thus influence aggregate breakdown during rainfall and affect microbial redistribution and susceptibility to mobilisation and transport (Allton *et al.*, 2007). Empirical research is needed to investigate the effects of rainfall itself on the soil microbial community and the potential for pre-rainfall climatic conditions to mediate the impact of rainfall.

1.2 Aim and objectives

Aim: The main aim of this research is to determine the effect of climatic conditions, in terms of soil temperature and moisture content, on aggregate stability and soil erodibility, and explore the role of the soil microbial community in mediating biological stabilisation of aggregates under changing climatic conditions.

Objective 1: Evaluate the effects of constant soil temperature and moisture content conditions on aggregate stability and the soil microbial community. Microbial properties will be considered as indicators of biological stabilisation at the aggregate-scale.

Objective 2: Investigate the influence of seasonal conditions (summer followed by winter), representing current and future climate scenarios, on soil aggregate stability and the microbial community.

Objective 3: Quantify the effects of soil temperature and moisture content on the soil microbial community and soil erodibility at the erosion-scale.

Objective 4: Determine the effects of rainfall on the soil microbial community incubated under different preceding climatic conditions.

1.3 Thesis outline

The thesis is written in the format of papers, an approved style for Cranfield University. Thus, the introduction, methods, results, and discussion sections are presented within each chapter. Due to similarity in the experimental approach, there is some unavoidable repetition of methods between chapters. The four experimental chapters (Chapters 3, 4, 5, and 6) stem from three laboratory-based experiments, with Chapters 3 and 4 focussed on two experiments at the aggregate-scale and Chapters 5 and 6 examining components from the same experiment at the erosion-scale (Figure 1.2). The papers presented here are organised around the above objectives (Figure 1.2) and contributions of authors

to each chapter are described in Table 1.1. General methodologies applied throughout the research are included as an appendix (Appendix A).

Chapter 2 provides a critical review of the literature concerning the physical, chemical, and biological soil properties that influence aggregate stability and stabilising (and destabilising) mechanisms as well as the effects of temperature and soil moisture on these mechanisms.

Chapter 3 presents an aggregate microcosm experiment, which investigates the effects of soil temperature and moisture on aggregate stability and on the soil microbial community which mediates biological stabilising mechanisms.

Chapter 4 examines the effects of climate change scenarios during a summer-winter cycle on aggregate stability and the soil microbial community in an aggregate microcosm experiment.

Chapter 5 investigates the effects of climate on soil erodibility and soil erosion, upscaling the mechanisms observed in Chapters 3 and 4 to soil trays.

Chapter 6 explores the effects of rainfall and the interaction with climatic treatment on eroded soil in comparison to non-eroded soil, and the selective mobilisation of a component of the microbial community.

Chapter 7 synthesises the experimental findings and recognises current limitations and future research opportunities.

Chapter 8 identifies the contributions to knowledge made by this research and summarises the key conclusions.

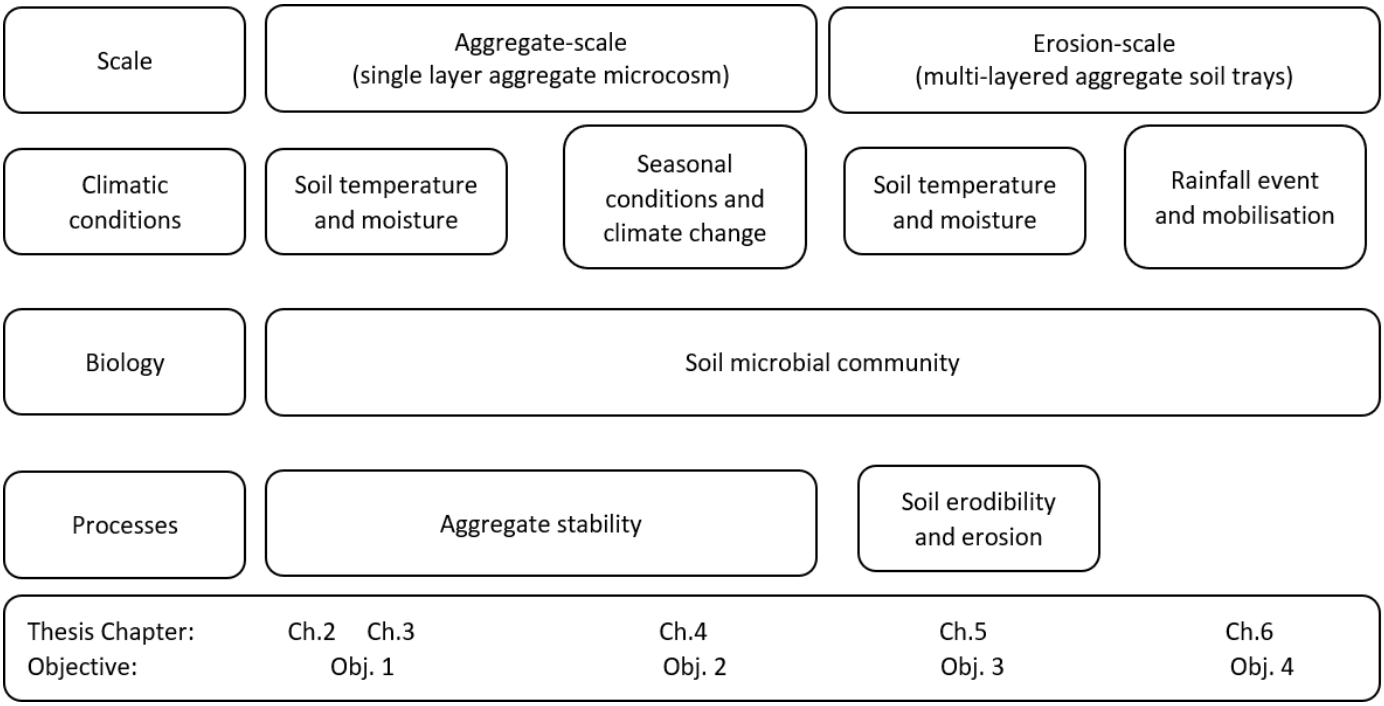

Figure 1.2: Conceptual schematic of scales, climatic conditions, biological variables, and processes (indicated in row labels on the left) considered in each chapter within the thesis.

Table 1.1: Contributions of authors to each chapter and objectives within the thesis.

	Chapter 1	Chapter 2	Chapter 3	Chapter 4	Chapter 5	Chapter 6	Chapter 7	Chapter 8
		Objective 1	Objective 1	Objective 2	Objective 3	Objective 4		
		Intent is to submit to Earth Science Reviews	Currently in review process with the European Journal of Soil Science (EJSS)	Intent is to submit to Geoderma	Intent is to submit to Earth Surface Processes and Landforms (ESPL)	Intent is to submit to a peer-reviewed journal		
E. Dowdeswell-Downey	Structure, and writing	Literature review, synthesis, structure, and writing	Data collection and analysis, methodology development, discussion, structure, and writing	Data collection and analysis, methodology development, discussion, structure, and writing	Data collection and analysis, methodology development, discussion, structure, and writing	Data collection and analysis, methodology development, discussion, structure, and writing	Discussion, structure, identification of knowledge gaps, limitations and future research, and writing	Discussion, structure, identification of contributions to knowledge and key conclusions, and writing
R.C. Grabowski	Guidance on structure, editing	Guidance on structure, editing	Guidance on methods, structure, editing	Guidance on methods, structure, editing	Guidance on methods, structure, editing	Guidance on methods, structure, editing	Guidance on structure, editing	Guidance on structure, editing
R.J. Rickson	Guidance on structure, editing	Guidance on structure, editing	Guidance on methods, structure, editing	Guidance on methods, structure, editing	Guidance on methods, structure, editing	Guidance on methods, structure, editing	Guidance on structure, editing	Guidance on structure, editing

2 Soil erodibility under changing climatic conditions: the role of aggregate stability and the dynamics of stabilising mechanisms

Abstract

Soil is a valuable resource but is widely degraded by accelerated rates of soil erosion. Climate change is predicted to increase soil erosion rates due to increased rainfall erosivity. Soil erodibility, i.e. the susceptibility of soil to erosion, is an important factor determining the rate of soil erosion but is often treated as a static soil property, despite evidence that soil erodibility varies over time. Aggregate stability is well accepted as a suitable indictor for soil erodibility and is a crucial soil property mediating several soil processes and functions. Aggregate stability is controlled by multiple stabilising and destabilising mechanisms and has been observed to vary in line with local climatic conditions.

This article presents a critical review of the stabilising and destabilising physical, chemical, and biological soil properties which govern aggregate stability. Significant relationships between climatic conditions and these soil properties have been observed previously, here the implications of these climate-sensitive stabilising and destabilising mechanisms for aggregate stability are discussed. Finally, future lines of enquiry are proposed to identify key knowledge gaps and opportunities for research.

2.1 Introduction

Soil is the foundation for terrestrial life on Earth and plays a critical role in delivering many ecosystem services, including food production, water provisioning, nutrient cycling and climate regulation (De Groot *et al.*, 2002; Haygarth & Ritz, 2009; Dominati *et al.*, 2010). Soil forms the basis for 99.7% of our food (Pimentel & Burgess, 2013), and is the planet's third largest pool of carbon after the geological and oceanic pools, storing more carbon than both the atmosphere and living biomass (Batjes, 1996; Oelkers & Cole, 2008). Soil is therefore an essential resource and is effectively non-renewable due to slow formation rates (Blum, 2005). However, despite the importance of soil, in the UK an estimated 2.2 million tonnes of topsoil are lost each year and soil degradation costs approximately £1.2billion annually (POSTnote 265, 2006; Graves *et al.*, 2015; POSTnote 502, 2015). Soil erosion has a negative impact on the long-term sustainability of agricultural productivity through the loss of soil as a rooting medium, and associated nutrients and organic matter that are necessary for crop growth (Lal, 1998; Pimentel, 2006; Pimentel & Burgess, 2013). Soil erosion also causes pollution in and sedimentation of waterbodies with eroded soil and associated nutrients and pesticides, increased risk of flooding and negative impacts on aquatic ecosystems (Gorlach *et al.*, 2004; Boardman, 2013; Rickson, 2014) and depletes the soil organic carbon pool (Lal, 2003). Many factors influence soil erosion, yet there is a paucity of research that has focused on soil erodibility, the intrinsic susceptibility of soil to erosion processes.

Soil aggregates are groups of soil particles bound to each other more strongly than adjacent soil particles through cohesive and adhesive stabilising processes. The degree of soil aggregation, aggregate size distribution and aggregate stability are fundamental determinants of soil structure and influence the architecture and stability of soil pores, the flow of gases and water, and the storage of organic matter and nutrients (Díaz-Zorita *et al.*, 2002). As such, aggregates influence many soil properties, including soil aeration and soil hydraulic characteristics (Brady, 1990), and have great influence over soil functions such as organic matter and carbon cycling, infiltration and root growth.

Driven by cohesive and adhesive forces, aggregate stability is inversely correlated to soil erodibility, and thus is often used as an indicator of soil structural stability (Bryan, 1968; Le Bissonnais, 1996, 2016; Barthès & Roose, 2002; Almajmaie *et al.*, 2017). Aggregate stability and soil erodibility are a function of multiple physical, chemical, and biological soil properties. Intrinsic physical and chemical properties, such as soil texture (the proportion of sand, silt, and clay particles) change relatively slowly over decadal timescales, whilst biological soil properties exhibit much higher temporal variability and respond more rapidly to changing climatic conditions (Bryan, 2000; Song, 2005; Deviren Saygin *et al.*, 2018).

Soil aggregates are a highly dynamic and complex soil characteristic, and are greatly affected by climatic conditions, sometimes displaying systematic variation with seasonal climatic changes (Blackman, 1992; Bryan, 2000; Sanchis *et al.*, 2008). There are multiple mechanisms of aggregate formation, stabilisation, and breakdown acting simultaneously. Despite sustained and focused research on aggregates by soil scientists, a complete understanding of how and why aggregates change temporally and spatially is still lacking. Evidence has indicated that aggregate stability increases with increasing aggregate size, possibly due to the incorporation of cementing agents such as organic matter (Vaezi *et al.*, 2018). It is suggested that soil is more susceptible to erosion if the soil has a greater proportion of smaller aggregates (<2 mm), although, larger aggregates (>2 mm) are more likely to breakdown by raindrop impact than smaller aggregates (Martínez-Mena *et al.*, 1999). Aggregate dynamics are crucial to understanding the changing nature of soil structure and susceptibility to erosion (Bryan, 1968; Blanco-Canqui *et al.*, 2009; Nciizah & Wakindiki, 2015; Ding & Zhang, 2016).

Despite research highlighting the importance of aggregates in many aspects of soil systems, the dynamics of aggregate stabilising and destabilising mechanisms remain ill-defined. There are several reasons why shifts in aggregate stability have not been clearly explained:

i. physical, chemical, and biological aggregation mechanisms operate simultaneously, with interaction effects, to influence aggregate stability (Tisdall & Oades, 1982; Amézketa, 1999),
ii. mechanisms operate at multiple scales, including scales such as the microbial, pore, micro/macro-aggregate, soil matrix, soil horizons, and soil profile (Baveye *et al.*, 2018; Juyal *et al.*, 2019),
iii. the heterogeneity of soil leads to variability and case-specific results (Zádorová *et al.*, 2011; Wanzek *et al.*, 2018),
iv. there are multiple methodologies used to define aggregate stability, each considering different mechanisms of aggregate breakdown (Beare & Bruce, 1993; Amézketa, 1999; Le Bissonnais, 2016; Almajmaie *et al.*, 2017), see Section 2.1.1.2 for further information on aggregate stability methods,
v. experimental studies investigate a limited range of soil variables, which vary between studies, hindering cross-comparison (see Almajmaie *et al*., 2017; Amézketa, 1999).

Critically, aggregate stability, and soil on a wider basis, is holistically a complex product of physical, chemical and biological processes, with intricate interactions and feedbacks and must be considered in an interdisciplinary manner (Brevik *et al.*, 2015), as presented in this review.

It is now widely recognised that climate is changing globally, and human activities are influencing global warming (IPCC, 2014; Li & Fang, 2016; Lowe *et al.*, 2018). Surface temperatures are predicted to rise globally with more frequent and longer hot extremes, and precipitation patterns are predicted to shift globally with changes in rainfall intensity and duration and more frequent extreme precipitation events (IPCC, 2014). Shifts in temperature and precipitation regimes are projected to increase the risk and severity of soil erosion (Nearing, 2001; Li & Fang, 2016), though there are uncertainties across regions due to close feedbacks between land-use management, vegetation cover and biogeochemical responses (Nearing *et al.*, 2004). Notwithstanding the importance of aggregates for many critical soil functions, aggregate stability dynamics are largely unexplored when considering future climate scenarios. Yet aggregate stability is a responsive soil property, strongly affected by climatic conditions, and has been found to vary seasonally (Perfect *et al.*, 1990b; Mulla *et al.*, 1992; Cosentino *et*

al., 2006). It is essential to understand aggregate stability and breakdown, and associated changes in soil structure, to fully analyse the implications for soil erosion and soil functions. For example, studies modelling soil erosion rates under climate change have largely focussed on the effects of changes in rainfall erosivity (Pruski & Nearing, 2002; Segura *et al.*, 2014). However, the impact of changing climatic conditions on aggregate stability in determining soil erodibility and future soil erosion rates remains largely unexamined.

This review aims to critically assess the soil properties and climate-sensitive stabilising and destabilising mechanisms that affect aggregate stability with the following objectives i) consolidate and update the scientific understanding of the physical, chemical, and biological soil properties that control aggregate stability (Section 2.2), ii) assess the response of stabilising and destabilising mechanisms to changes in climate conditions such as temperature and precipitation (Section 2.3), and iii) present a framework of interactions between physical, chemical, and biological stabilising and destabilising mechanisms in the context of changing climatic conditions, and identify lines of enquiry and research gaps for further investigation (Section 2.4). This review will build on previous reviews on the physical, chemical, and biological properties that govern aggregate stability (Amézketa, 1999; Bryan, 2000; Six *et al.*, 2004; Young *et al.*, 2008), to consider the impact of climate change and interactions between mechanisms and identify gaps in knowledge between specific research areas.

2.1.1 Definitions

2.1.1.1 Soil erodibility, aggregates and aggregation theories

The evaluation of soil erodibility in the field can often be time-intensive and costly (Barthès & Roose, 2002; Nciizah & Wakindiki, 2015), therefore studies have focussed on using soil properties, such as aggregate stability, as an indicator to evaluate erodibility. This research employs the definition of soil aggregates as collections of particles with stronger bonds than surrounding particles (Nimmo, 2004a). Aggregate stability is closely related to soil erodibility and is widely accepted as the most suitable indicator (Bryan, 1968; Barthès & Roose,

2002).There are numerous empirical reports of high correlation and a significant negative relationship between aggregate stability and soil erodibility. Bryan (1968) conducted laboratory tests with 90 English soils by measuring soil loss induced by intense rainfall simulation and comparing to 17 proposed indices of soil erodibility. This study concluded that aggregate stability was the most reliable indicator of erodibility. Barthès and Roose (2002) also tested soil erodibility in the field with a range of soil types across several scales using comparative measurements of runoff, soil loss and aggregate stability. The research reported that runoff and soil loss were significantly correlated with macroaggregate stability (>0.2mm, assessed by the wet-sieving approach) with r=-0.95 and r=-0.9, respectively. Therefore, it was concluded that aggregate stability was closely related to soil erodibility. Similarly, Cantón *et al.* (2009) reported a significant negative relationship between aggregate stability, measured by water-drop, and observed runoff (R^2=0.68) and erosion rates (R^2=0.9) under natural rainfall in a semi-arid environment, concluding that aggregate stability is a valuable indicator for soil erodibility, runoff, and erosion. The use of soil aggregates in laboratory experiments allows the examination of physico-chemical and biological processes that determine the degree of aggregation. Whilst there remains some debate on the trade-off between realism and extraction of aggregates from in-situ soil (Kravchenko *et al.*, 2019), soil aggregates have been useful to advance knowledge of aggregate-scale processes and the operation of soil systems (Hallett *et al.*, 2013; Yudina & Kuzyakov, 2019).

Numerous theories on the process of aggregate formation have been proposed (reviewed in further detail in Six et al., 2004 and Lal and Shukla, 2004). The most prominent concept is the aggregate hierarchy which defines aggregate formation by spatial scale-dependent mechanisms of formation for microaggregates (<250µm) and macroaggregates (>250µm) (Tisdall & Oades, 1982). The aggregate hierarchy model postulates that different binding agents (categorised as transient, temporary or persistent binding agents) act at different scales of aggregates. The smallest microaggregates (<0.2µm) are formed through physical and chemical stabilising mechanisms as a result of particle interaction forces

(Bryan, 2000). The aggregate hierarchy model proposes that primary particles (<20µm) and these smallest microaggregates are then bound together by persistent binding agents to form other microaggregates (<250µm), which in turn are bound together by temporary and transient binding agents to form macroaggregates (>250µm) (Tisdall & Oades, 1982). Microaggregates are thought to form within macroaggregates, where the organic matter occluded within the macroaggregate forms the nucleus for the microaggregate (Oades, 1993; Jastrow, 1996). The high organic matter content encapsulated within macroaggregates can lead to areas of high microbial activity with microbial communities formed within the macroaggregates and on the surface (Flemming & Wingender, 2010; Kuzyakov & Blagodatskaya, 2015). As such macroaggregates are often associated with biological stabilising mechanisms (see Section 2.2.3). Microaggregates and macroaggregates can also be formed as the resulting fragments of breakdown processes, discussed in Section 2.1.1.2. Loosely bound macroaggregates are more vulnerable to disaggregation under wetting stresses, particularly raindrop erosion, than the denser and more resistant microaggregates (Martínez-Mena *et al.*, 1999; Bryan, 2000).

2.1.1.2 Aggregate breakdown mechanisms and tests for aggregate stability

There are four main mechanisms of aggregate breakdown including slaking, differential swelling and microcracking, raindrop impact and physico-chemical dispersion (Le Bissonnais, 1996). Wetting is known to physically disrupt aggregates through a number of these breakdown mechanisms including slaking, swelling and microcracking, and dispersion. The process of slaking occurs when an aggregate cannot withstand the internal stresses from the compression of entrapped air caused by a rapid influx of water (Le Bissonnais, 1996). Slaking causes significant aggregate breakdown and the release of microaggregate fragments (Le Bissonnais, 1996). Wetting rate has a strong influence on slaking; the faster the wetting rate, the stronger the internal pressures and so a greater proportion of aggregates undergo slaking (Ben-Hur *et al.*, 2009). The effect of wetting rate on slaking and aggregate stability is dependent on the initial moisture

content of the soil. Upon rapid wetting, moist aggregates are less susceptible than dry aggregates to slaking, as the volume of entrapped air is greater in dry aggregates than in moist aggregates (Le Bissonnais, 1996; Lado *et al.*, 2004). Differential swelling of aggregates is dependent on the clay particle characteristics (discussed further in Section 2.2.2.2), and leads to microcracking and aggregate breakdown with the release of macroaggregates and microaggregates (Le Bissonnais, 1996; Haraguchi & Nakaishi, 2008). While slaking decreases with increasing clay content, breakdown by differential swelling can increase (Le Bissonnais, 1996). Physico-chemical dispersion is caused by the response of particle interaction forces (see Section 2.1.1.3) to wetting and releases primary particles, though it is highly dependent on cation (Le Bissonnais, 1996). Raindrop impact causes aggregate breakdown and detaches primary particles and fragments as small microaggregates <100µm (Le Bissonnais, 1996). Aggregate breakdown under rainfall is predominately caused by raindrop impact, though wetting of the aggregates can also combine the effects of slaking, differential swelling, and physico-chemical dispersion.

Numerous methods have been developed to test aggregate stability, due to these multiple processes causing aggregate breakdown (Amézketa, 1999). Soil aggregate stability has often been measured using the wet-sieving approach, based on early work by Kemper and Koch (1966) and Kemper and Rosenau (1986) which submerges aggregates in water and separates aggregates based on size by sieving and changes in aggregate mass determine water-stable aggregates. Though wet-sieving has been commonly used, there are large variabilities in its application, such as sample pre-treatment, the number of sieves, the timing and frequency of wetting, and the liquid used for immersion, which are likely to affect the results and impedes comparison across studies (Beare & Bruce, 1993; Amézketa, 1999). The wet-sieving approach has also attracted criticism for considering the combined influence of potential breakdown mechanisms, without separating mechanisms of aggregate breakdown and overemphasising the process of slaking (Le Bissonnais, 1996; Almajmaie *et al.*, 2017). Subsequently, Le Bissonnais (1996) developed the unified framework for

stability assessment which combines three treatments; fast wetting, slow wetting and stirring after pre-wetting and measures the resultant fragment size distribution from each method of breakdown. The application of these three treatments enables the differentiation and comparison of aggregate breakdown mechanisms with slaking caused by fast wetting, swelling and microcracking induced by slow wetting, and the mechanical breakdown of aggregates through stirring (Le Bissonnais, 1996; Legout *et al.*, 2005). However, the methods applied to induce aggregate breakdown are highly mechanical, while the treatments provide information on the forces responsible for breakdown, they are not realistic of field conditions and so cannot fully represent naturally occurring processes (Amézketa, 1999; Almajmaie *et al.*, 2017). The unified framework also does not take in to account the effect of raindrop impact and dispersion is often not measured (Almajmaie *et al.*, 2017). Meanwhile rainfall simulation incorporates the effect of raindrop bombardment and in a recent review, Almajmaie *et al.,* (2017) consider rainfall simulation to best represent the disaggregation processes that occur in the field when aggregates are exposed to rainfall. Therefore, to best represent field conditions the rainfall simulation method was selected for this investigation.

2.1.1.3 Particle interaction forces

Aggregates are clusters of particles bonded together by particle interaction forces, as such a clarification of these forces is relevant to the discussion of aggregate stabilisation and destabilisation. Soil internal forces have a fundamental influence on aggregate stability (Santamarina, 2003; Rengasamy *et al.*, 2016), some of these forces are repulsive (such as electrostatic and hydration forces), encouraging aggregate breakdown, whilst others are attractive (such as van der Waals forces and cation bonding), inferring structural stability (Lal & Shukla, 2004; Hu *et al.*, 2015). These forces are influenced by particle electrostatic forces and polarisation, particle spacing and distance between particles and clay particle characteristics (Li *et al.*, 2013b; Xu *et al.*, 2015; Hu *et al.*, 2018).

Soil internal forces can be categorised as intramolecular and intermolecular forces. Intramolecular forces hold atoms together within individual molecules. There are two types of intramolecular forces; ionic bonds and covalent bonds, which can be either nonpolar or polar. Ionic (also known as electrostatic) bonding occurs where an electrostatic attractive force exists between atoms with incomplete outer electron shells, meaning the atoms are ions with positive or negative charges (Panchuk, 2019). Covalent (also known as molecular) bonds are formed between atoms which share electrons (Panchuk, 2019). Two atoms with equal electronegativity will share the electron equally. However, if the bonded atoms have a different electronegativity, then the electrons are shared unequally; the atom with higher electronegativity attracts the shared electron closer to itself. If the electron distribution is unequal, a polar bond is formed, creating a two-pole condition called a dipole, and this generates a vector force directed toward the higher electronegative atom called a dipole moment. The polarisation of molecules influences particle interaction between molecules.

Intermolecular forces act between molecules and govern the nature of particle cohesion. The most prominent intermolecular forces are van der Waals forces, which are distant dependent forces that form due to electrostatic interactions between particles and can be attractive or repulsive (Huang, 1980; Quirk & Murray, 1991; Liang *et al.*, 2007). There are three types of van der Waals forces: dipole-dipole forces, hydrogen bonding, and London dispersion forces. Dipole forces occur in polar molecules, where they have an unequal distribution of electrons and so have positively and negatively charged sides. Polar molecules will therefore interact due to these slight differences in charges; meaning a positive side of one molecule will naturally orientate itself with a negative side of another molecule. Hydrogen bonding is an electrostatic attraction between a hydrogen atom, which carries a weak positive charge, and an electronegative atom. London dispersion forces are the weakest van der Waals force and exist between nonpolar molecules when they temporally induce dipoles.

In soils, clay minerals and organic matter often exhibit electrostatic charges, and so the resultant interparticle attraction or repulsion influences organo-mineral

interactions and adsorption. Surface tension is also an important factor in soils due to intermolecular forces, where at a liquid-gas interface, water molecules are hydrophilic and have a greater attraction to liquid molecules than gas molecules creating tension of the surface film of water. Surface tension of soil solution is influenced by temperature, pH metal ions and solute effects such as the presence of hydrophobic organic matter compounds (Anderson *et al.*, 1995; Yates & von Wandruszka, 1999) and has been found to influence capillary pressure, aggregate wettability and slaking (Bachmann & Van Der Ploeg, 2002; Goebel *et al.*, 2005).

2.2 Stabilising and destabilising properties governing aggregate stability

This section provides a summary of the main stabilising and destabilising properties governing aggregate stability, through physical (Section 2.2.1), chemical (Section 2.2.2) and biological properties (Section 2.2.3).

2.2.1 Physical properties controlling aggregate stability

Various soil physical properties influence aggregation mechanisms such as particle size and shape, particle density, porosity, and soil texture (particle size distribution). A number of physical aggregation mechanisms operate simultaneously, with different processes dominating in different soils. It is this complexity, plus the manifestation of multiple scales at which processes can operate, that makes investigation of these processes challenging.

2.2.1.1 Particle size and shape (particle density and porosity)

Attributes of the mineral component of soil have a profound influence on aggregate stability through variations of particle size and shape (specific surface area), which affect soil physical properties such as particle density and porosity, as well as influencing chemical properties by affecting the number of exchange sites for chemical interactions. The influence of particle size and shape characteristics is manifested across multiple scales. At the particle scale, the size and shape of particles influences the packing density of particles (particle density)

and therefore void space between particles (porosity). At the aggregate scale, particle size and shape affect the arrangement and bonding of primary particles in the formation of aggregates, which define soil structure and structural porosity. Particle density and pore space govern the movement and storage of water and air through the soil. For example, sand has large pore spaces between the particles and therefore has very low water holding capacity. Particle density and porosity affect aggregate stability physically by modifying internal air pressure the storage and movement of water, and thus influences slaking, an aggregate breakdown process caused by the compression of entrapped air during wetting (Dal Ferro *et al.*, 2012; Jakšík *et al.*, 2015). Porosity is also a very important property which defines the microhabitat for the microbial community (Young *et al.*, 1998) and is discussed further in Section 2.2.3. Increasing particle size and shape irregularity limits the dense packing of particles and increases void space (Santamarina & Cho, 2004; Cho *et al.*, 2006). The irregularity of particle shape also influences particle reorientation and rearrangement, slippage and contact breakage which extends to influence macroscale processes such as compressibility and shear stress response (Cho *et al.*, 2006) and therefore aggregate stability.

Particles are often classified based on particle size; sand has a particle size range of 2000 - 63µm, silt ranges between 63-2µm, and clay minerals are less than 2µm, according to the British Soil Classification System (BS 1377-1: 2016, 2016), though sometimes sand particles are split into fractions representing coarse, medium and fine sand (Lal & Shukla, 2004). Textural fractions, including fine sand and silt, have previously been used as erodibility indices, closely related to the K factor used in erosion models (Duiker *et al.*, 2001; Ahmadi *et al.*, 2010; Vaezi *et al.*, 2018). Particle shape can be described by measuring sphericity (particle form and symmetry), roundness (curvature of surface features) and surface smoothness (surface texture) (Santamarina & Cho, 2004). Sand and silt particles are generally spherical; however, the angularity and surface roughness are dependent on the extent of physical weathering.

Clay minerals are plate-like in structure, as they are fine assemblages of tetrahedral and octahedral sheets. As a result of their layered sheet structures, clays have a large specific surface area (a large surface area compared to weight). Clays also carry a negative charge and so with their large specific surface area, clays are a highly reactive soil fraction with high adsorption of ions (Huang, 2004) and thus clay content has important implications for chemical soil properties which influence aggregate stability (discussed further in Section 2.2.2.2). Clays notably influence several soil physical properties including hydration characteristics, and shrinking and swelling capacity, and mechanisms of cohesion such as electrostatic bonding. Clays with low shrink-swell capacity enhance aggregate stability as a result of this cohesion (Ahmadi *et al.*, 2010). There are different types of clay minerals defined by their formation and structure, which control how clays interact with other soil components, as such the significance of variations in clay mineralogy for aggregation is discussed in Section 2.2.2.2.

2.2.1.2 Soil texture (proportion of sand, silt, and clay particles)

Soil texture, the composition of the mineral component of soil and the proportion of textural fractions (sand, silt, and clay), has a strong influence on the nature of particle interaction forces and thus affects aggregate stability and soil erodibility. Soil texture is defined by the proportion of sand, silt, and clay minerals, i.e. the particle size distribution. Systems of soil classification vary slightly in the boundaries of soil textural classes, generally clay soils have more than 25% clay, while sandy soils are mainly sand (above 40%) with less than 25% clay. Silty soils are comprised mainly of the intermediate size particles and loams are a mixture of sand, silt, and clay. Generally, clay soils are the least susceptible to erosion because of the high cohesion between clay particles, sandy soils also have a relatively low erodibility as the larger grain size requires greater energy for detachment and transport, whilst silt soils are the most susceptible to erosion with low clay content and small particle size (Hjulstrom, 1955; Wischmeier & Mannering, 1969).

2.2.2 Chemical properties controlling aggregate stability

Various soil chemical properties influence aggregation processes and aggregate stability, including organic matter content, clay content and mineralogy, soil pH, metal (hydr)oxides and multivalent cations. In general, rates of chemical aggregation increase with increasing organic matter content, clay surface area, and cation exchange capacity (the amount of positive charge that can be exchanged per mass of soil) (Bronick & Lal, 2005). In soil with high organic matter or clay content these components exert strong influence over chemical aggregation processes, however in soil with low organic matter and clay content the chemical aggregation processes are more dominated by cation behaviour (Bronick & Lal, 2005). There are close interactions between chemical soil properties, as such the rate and stability of aggregation is often an amalgamation of chemical processes and specific to soil type. For example, soil pH affects the charge characteristics of clay particles, particularly 1:1 clays which are associated with variable charge, and therefore the formation and strength of bonds between minerals and organic matter, which directly influences aggregate stability (see Section 2.2.2.3). Furthermore, the large microscale heterogeneity in soil due to changes in porosity and particle surfaces leads to variability in chemical activity and biogeochemical processes (Wanzek *et al.*, 2018), and therefore variations in chemical bonds which affect aggregate stability.

2.2.2.1 Organic matter

Increasing organic matter content has been shown to enhance aggregate stability (Puget *et al.*, 2000; Cosentino *et al.*, 2006). Organic matter can promote soil aggregation through the influence on interparticle forces and thus adsorption with soil particles and formation of organo-mineral assemblages (Tisdall & Oades, 1982; Yu *et al.*, 2017). Soil organic matter also influences various soil properties, including porosity, water holding capacity, infiltration capacity, and hydrophobicity (Haynes & Naidu, 1998; Zheng *et al.*, 2016). Soil organic matter encompasses numerous types of complex organic compounds, including carbohydrates, celluloses, lignins, proteins, fats, humic acids, and polysaccharides (Regelink *et al.*, 2015). Organic substances exhibit great complexity in their composition and

heterogeneity across different scales within the soil system. Studies have described organic matter by the substances' composition of compounds, location within the soil matrix (e.g. inter- and intra- aggregate, particulate, occluded; Six, Elliott and Paustian, 2000), and residency time in soil (e.g. transient, temporary, labile, persistent; Tisdall and Oades, 1982). The composition of organic matter, interaction with soil particles, location within the soil matrix, and rate of accumulation and decomposition all affect aggregate stabilisation. Whilst soil organic matter can improve soil aggregation, aggregates also protect organic matter from biodegradation through the physical protection and sequestration of organic matter within aggregates. There is also a strong interaction between soil organic matter content and the microbial community; decomposition is the physical breakdown and chemical transformation of organic matter and is a microbially-mediated process. The carbon and nitrogen polymers which constitute organic matter are complex molecules (e.g. cellulose, chitin, and lignin), which are broken down into smaller components by microbes during decomposition via enzyme activity and depolymerisation (Burns *et al.*, 2013). The smaller organic components can then be mineralised for microbial uptake. Therefore, the activity of the microbial community strongly affects the amount and chemical structure of organic matter.

2.2.2.2 Clay content and mineralogy

As discussed in Section 2.2.1, clays have a large specific surface area and an electrostatic charge and so are a highly reactive soil fraction (Weil & Brady, 2017) which influence aggregate stability by affecting particle interactions and organo-mineral assemblages. Clay minerals can be broadly classified into three broad groups: kaolinites, smectites (aka the montmorillonite group) and illites. Each group is associated with differing sheet structures which influence properties significant for aggregate stability such as shrink-swell capacity, cation exchange capacity, charge density and interactions with organic matter (Amézketa, 1999; Bronick & Lal, 2005; Regelink *et al.*, 2015). Kaolinites have a 1:1 structure (meaning structurally kaolinites consist of one tetrahedral sheet and one octahedral sheet), low shrink-swell capacity and low cation exchange capacity

(Millot, 1970, 2013). Smectites have a 2:1 structure (meaning the structure has one octahedral sheet between two tetrahedral sheets), a high swelling potential as water can penetrate the clay sheet lattice and a high cation exchange capacity. Illites also have a 2:1 structure but are non-swelling clay minerals because interlayer cations prevent water breaching the lattice. Illites have a cation exchange capacity higher than kaolinites but much lower than smectites (Millot, 1970, 2013). Smectites are the most erodible group due to the expansion of water within the clay matrix can weaken chemical bonds (Millot, 1970, 2013) and therefore smectitic soils are more dispersible with lower aggregate stability, and thus more erodible, than clay soils dominated by illites and kaolinites (Wakindiki & Ben-Hur, 2002; Reichert *et al.*, 2009). Though clay soils dominated by illites or kaolinites may still be dispersible with a small amount of smectite clays (Stern *et al.*, 1991; Reichert *et al.*, 2009). The presence of clay minerals and their mineralogy affects aggregation processes and aggregate breakdown mechanisms by influencing shrinking and swelling (see Section 2.1.1.2) and interactions with cations and organic matter. However, the effects of specific mineralogical groups on aggregate stability have been difficult to assess because soil often contains a mixture of clay minerals. Additionally, clay content is highly correlated with a number of variables, therefore understanding the causal mechanisms related to clay content is complicated by confounding effects of other soil properties (Bosatta & Ågren, 1997).

Clay minerals also influence aggregate stability through interaction with organic matter, influencing the formation of organo-mineral assemblages, decomposition of organic matter, and dispersibility of clay. Clay content and mineralogy affects the establishment of organic-mineral bridges, which act to bind particles together, due to the electrostatic interaction of clay minerals and organic matter (Huang, 2004). The interaction between clay minerals and organic matter can be influenced by soil pH, solution chemistry, and the concentration of ions, e.g. Calcium, Sodium, Magnesium, Iron (Murphy *et al.*, 1994; Arnason & Keil, 2000; Huang, 2004; Feng *et al.*, 2005), which are discussed further in Sections 2.2.2.3 and 2.2.2.4. The interaction of clay minerals and organic matter affects the

decomposition rate of organic matter as clay mineralogy influences particle surface area, charge density, and cation exchange capacity (Regelink *et al.*, 2015). These physico-chemical soil properties, mediated by clay characteristics, affect the adsorption of organic matter and therefore influence the formation of organo-mineral assemblages as well as the accessibility of organic matter for soil microbes. Complex feedbacks occur where clay mineralogy affects the decomposition rate of organic matter, thereby influencing the products of decomposition, and in turn the soil organic matter and decomposed products affects clay characteristics, such as surface charge, organic coatings and interlayer clay-organic complexes (Huang, 2004), which also affects dispersibility and wetting rate of clays (Chenu *et al.*, 2000). Therefore, organic matter has been reported to increase and decrease the dispersibility and wetting rate of clays, dependent on the type of organic substance and clay mineralogy. Increasing the dispersibility of clays can weaken interparticle bonds, leading to a reduction in aggregate stability and increased soil erodibility.

2.2.2.3 Soil pH

The logarithmic soil pH scale represents the relative concentration and ratio of H^+ and OH^- ions. Soil pH is primarily dependent on the balance between cations associated with soil particles and the balance of H^+ and OH^- ions in soil solution (Weil & Brady, 2017). Generally aggregate stability has been reported to increase with decreasing pH (Regelink *et al.*, 2015). Soil pH influences multiple soil properties and processes which in turn affect aggregation, such as the charge characteristics and dispersibility of clays, the adsorption properties of organic matter, and the solubility of metal (hydr)oxides and multivalent cations (Arnason & Keil, 2000; Feng *et al.*, 2005; Nguetnkam & Dultz, 2011). Metal (hydr)oxides and multivalent cations, as well as the influence of soil pH, are discussed further in Section 2.2.2.4.

Soil pH influences the dispersibility of clays by affecting the charge of clay particles, as increasing pH increases the net negative charge of clay soils thereby increasing clay dispersion (Chorom *et al.*, 1994). The adsorption of organic matter

and clay minerals decreases with increasing soil pH (Feng *et al.*, 2005). As higher pH increases the negative surface charge on clay particles, this also increases electrostatic repulsive forces, thereby limiting particle adhesion. Alternatively, decreasing pH reduces electrostatic repulsive forces, thereby increasing the interaction of organic and mineral particles and creates organo-mineral associations with thicker coatings of organic matter on mineral surfaces (Murphy *et al.*, 1994; Arnason & Keil, 2000; Feng *et al.*, 2005; Weng *et al.*, 2008; Regelink *et al.*, 2015). Therefore, in general decreasing pH increases the chemical aggregation of organic matter and minerals, and consequently increases aggregate stability (Regelink *et al.*, 2015).

Soil pH, as an indicator of the ionic nature of the soil, is closely related to chemical processes which influence multivalent cations, metal (hydr)oxides, and organo-mineral bonds. For example, soil pH affects the dissolution of metal (hydr)oxides, such as iron (Fe) and aluminium (Al) (hydr)oxides, and therefore affects the concentration of metal ions in soil solution; their role in aggregate stability is presented in Section 2.2.2.4. Soil pH is also a key environmental factor that affects the soil microbial community and biological processes by mediating the chemistry of biochemical reactions (Husson, 2013). It is suggested that fungi are fairly versatile whilst intermediate and high pH conditions are more optimal for bacterial communities (Weil & Brady, 2017). For further information on the effects of soil pH, and reduction-oxidation conditions (discussed in Section 2.3.2), on the microbial community readers are referred to the review by Husson (2013).

2.2.2.4 Multivalent cations and metal (hydr)oxide minerals

Exchangeable multivalent cations and metal (hydr)oxide minerals can enhance soil aggregation through their influence on interparticle interaction forces and the formation of organo-mineral bonds (Ding *et al.*, 2019). Cations are released during the dissolution of particles during weathering processes and can oxidise or form associations with clay minerals and organic compounds (Scheinost, 2005), therefore ionic composition and concentration are influenced by weathering processes and changes in electrolyte concentration. Ionic

concentration and cationic type affect aggregate stability through their influence on numerous properties including clay swelling and dispersion, pore-size distribution, and soil infiltrability (Ding *et al.*, 2019). Metal (hydr)oxide minerals exhibit strong interactions with organic matter and are generally thought to improve aggregate stability, though research on the role of metal (hydr)oxides, particularly iron hydroxides, in the formation of organo-mineral assemblages has been focussed on oxide-rich soils, so their role is less clear in soils with lower concentrations (Regelink *et al.*, 2015). Iron (hydr)oxides have been observed to have a variable effect on aggregate stability (Duiker & Rhoton, 2003), with positive effects on soil aggregation reported through inorganic binding and the formation of organo-mineral associations (Tisdall & Oades, 1982), whilst others have reported no effects (Borggaard, 1983). The aggregating capability of hydroxides is influenced by pH, ionic composition of the soil solution, and interactions with organic matter. It is also possible that differences in hydroxide crystallinity can mediate the reactive surface area, perhaps causing some of the contrasting experimental observations (Duiker & Rhoton, 2003).

Soil pH has a large effect on the solubility and mobility of multivalent cations (Bronick & Lal, 2005). Decreasing pH increases the concentration of Al^{3+} and Fe^{3+} cations due to dissolution of Fe- and Al-(hydr)oxide minerals. Also, in calcareous soils increasing pH decreases the concentration of Ca^{2+} by controlling the solubility of $CaCO_3$. Therefore in general, with decreasing pH, the increase in the concentration of multivalent cations (eg. Al^{3+}, Fe^{3+}, and Ca^{2+}) increases aggregate stability as a result of the formation of organo-mineral assemblages. However, the overall influence of multivalent cations on aggregation is dependent on the cation composition, cation exchange capacity, and the soil solution chemistry. The concentration of multivalent cations and specific cation properties influences clay dispersion, carbonate coatings, oxide formation, and organic matter incorporation. For a more detailed review of the effects of specific ions (e.g. Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}) and a review of the metal oxides present in soil readers are referred to Bronick and Lal (2005) and Scheinost (2005) respectively.

2.2.3 Biological properties controlling aggregate stability

The high biological diversity of soils results in a broad range of biological stabilising mechanisms. Fungi and bacteria can influence soil aggregation and aggregate stability. There are existing reviews investigating the microbial nature of soils addressing: the physical constraints on microbes (Or *et al.*, 2007b), biofilms and extracellular polymeric substances (Sutherland, 2001a; b) and the relationship between soil biota, organic matter, aggregates and aggregate stability (Degens, 1997; Six *et al.*, 2004). In terms of the direct effects of microbes on aggregate stability, bacteria and fungi have been identified as of the highest importance (Lehmann *et al.*, 2017). Therefore, the effects of higher tropic organisms, such as protists and earthworms, is outside the scope of this research. Geisen et al., (Geisen *et al.*, 2017, 2018) provide a thorough overview of soil protistology and identify key knowledge gaps in protist function and response to global change. For information on the role of earthworms readers are directed to reviews on the direct effects of earthworms on soil structure (Shipitalo & Le Bayon, 2004) and indirect effects through ecological interactions (Liu *et al.*, 2019). Plants are well known to reduce soil erosion by slowing overland flow and reducing raindrop impact and thereby reducing aggregate breakdown. Above-ground plant biomass is a key source of organic matter through the incorporation and decomposition of phytomass. Below-ground, roots encourage aggregation through the enmeshment of soil particles and can promote cohesion by re-orientating clay particles (Tisdall & Oades, 1982; Six *et al.*, 2004). Roots can also improve aggregate stability by increasing particle adhesion through the production of exudates and mucilages (Oades, 1993), which also affects hydraulic properties of soil in the rhizosphere (Carminati *et al.*, 2017; Kroener *et al.*, 2018). Roots support a rhizosphere microbiome, supplying a source of food for soil inhabitants, including mycorrhizal fungi and bacteria. The numerous soil-plant-microbial feedbacks create complex interactions in the rhizosphere which remain challenging to disentangle and interpret. The focus of this research is on bare soil as this represents the most vulnerable soil system to erosion processes and the influence of vegetation and soil-plant-microbial interactions on soil

erosion processes have been extensively reviewed elsewhere (Angers & Caron, 1998; Gyssels *et al.*, 2005; Zuazo & Pleguezuelo, 2009; Meurer *et al.*, 2020), so will not be examined in this research.

2.2.3.1 Fungi

Soil fungi have been shown to significantly affect aggregate stability with the enmeshment of soil particles by filamentous hyphae which instate structural stability (Degens, 1997; Rillig & Mummey, 2006) and through the production of exudates which encourage particle adhesion (Wright & Upadhyaya, 1998; Tisdall *et al.*, 2012). Fungal hyphae promote aggregate stabilisation through the compression and enmeshment of soil particles and have been reported to influence physical and chemical bonds through the movement and alignment of soil particles (Tisdall & Oades, 1982; Tisdall, 1991; Rillig & Mummey, 2006). Hyphal branching expands the hyphal network, and the branching characteristics of hyphae (branching angles, number of growing tips, hyphal surface area) determines the amount of contact surface area between soil particles and hyphae, and therefore the degree of particle compression and enmeshment, and thus fungal-mediated aggregation (Deacon, 1997; Lehmann & Rillig, 2015). Previous studies have quantified the contribution of fungi to aggregate stability by measuring the effects of stimulating and suppressing the fungal community on aggregates. For example, one study reported that by stimulating fungi with glucose and straw amendments, the resultant growth in hyphal length led to an increase in the amount of dry-stable aggregates by 63-147% (Degens *et al.*, 1996). Studies have suppressed the fungal community with the application of fungicide and have consistently reported a subsequent reduction in aggregate stability (Beare *et al.*, 1997; Bossuyt *et al.*, 2001; Tang *et al.*, 2011). Most fungi are mycelial, meaning they grow a network of hyphae (Deacon, 1997), and so the mechanisms of hyphal-mediated aggregation will apply for many fungal species. However, the hypha diameter and length, wall thickness and composition, degree of branching and growth rate are all characteristics which differ between fungi species and influence the capacity for particle enmeshment and aggregate stabilisation (Lehmann *et al.*, 2020). The movement of hyphae

through the soil can also act as a disruptive force destabilising aggregates by pushing soil particles away from each other and creating lines of weakness vulnerable to water influx (Ritz & Young, 2004). Fungal hyphae have also been observed to penetrate and disrupt aggregates through existing points of weakness to access occluded organic matter as a carbon source (Lehmann & Rillig, 2015).

Fungi can also stabilise soil aggregates via the production of exudates (e.g. exopolysaccharides, mucilages, glycoproteins). These exudates such as glomalin (a specific well-studied exudate) encourage the adhesion of soil particles and electrostatic interactions between fungal exudates and soil particles can build organo-mineral assemblages, strengthening aggregate interparticle bonds (Wright & Upadhyaya, 1998). Fungal exudates are produced to chemically adjust the soil microhabitat and to obtain energy through decomposition, but simultaneously affect soil water repellency and aggregate stability (Wright & Upadhyaya, 1998; Morales *et al.*, 2010). Many fungi produce extracellular amylases to breakdown starches into usable sugars. For example, fungal saprotrophs obtain their nutrients from dead organic matter by the production of degradative enzymes and decomposition. Therefore, the impact on aggregation is two-fold; a reduction in organic matter by decomposition can destabilise aggregates, meanwhile the organic matter decomposed sustains fungal hyphae growth and the production of exudates increases soil particle adhesion, which in turn stabilises aggregates. Furthermore, fungal species produce a variety of exudates and the amount and composition of exudates secreted may influence the potential for particle adhesion or aggregate destabilisation though interactions with organic matter (Lehmann *et al.*, 2020). For example, the composition and activity of degradative enzymes influences the organic matter that is targeted and the efficiency and rate of decomposition. There are many different species of fungi, with various morphological characteristics that may influence the ability of fungi to stabilise and destabilise aggregates, though direct experimental evidence is sparse, and largely remains hypothetical (Rillig & Mummey, 2006; Lehmann & Rillig, 2015).

2.2.3.2 Bacteria

Bacteria affect aggregate stability through their production of extracellular polymeric substances (EPS), which adhere to soil particles, promoting particle adhesion, the formation of organo-mineral associations and therefore stabilising aggregates (Tisdall & Oades, 1982; Costa *et al.*, 2018). There are multiple mechanisms of adsorption for the adhesion of bacteria and EPS to soil particles, which are dependent on the active electrostatic interparticle forces and soil hydration status (Hermansson, 1999; Huang, 2004). Therefore, different adsorption mechanisms are likely to operate at various soil interfaces (e.g. air-water and liquid-solid interfaces), which may be altered with changing climatic conditions. Bacteria produce EPS for several reasons, though EPS production is energetically expensive, and EPS fulfils multiple ecological functions, including adhesion, hydration, nutrient and carbon source and genetic material transfer (Flemming & Wingender, 2010; Costa *et al.*, 2018). EPS acts as an interface between the bacterial community and the physico-chemical environment and so the primary function of EPS is thought to be the modification of the bacterial microhabitat and offer protection by adjusting the chemical habitat and buffering bacteria against desiccation (Roberson & Firestone, 1992).

EPS is generally thought to be a transient material due to the relatively rapid decomposition within the soil matrix (Tisdall & Oades, 1982; Amézketa, 1999; Redmile-Gordon *et al.*, 2020), but little is known about the quantitative estimation of EPS or rate of EPS production due to methodological limitations and bias in extraction procedures, and lack of methods for in situ monitoring. At present extraction procedures for EPS are harsh and often result in contamination from intracellular material and co-extractants, though research is refining extraction techniques to resolve these difficulties (Redmile-Gorden *et al.*, 2014; Redmile-Gordon *et al.*, 2020). Despite methodological limitations in the extraction of EPS studies have endeavoured to quantify the role of the bacterial population in aggregate stability by measuring microbial biomass carbon and hot-water extractable carbon, as well as investigating the effects of suppressed bacterial biomass by bactericide. Both biomass carbon and extractable carbon have been

significantly correlated with aggregate stability in numerous studies (see review by Degens, 1997), whilst the suppression of the bacterial population has been contrastingly reported to have reduced aggregate stability (Tang *et al.*, 2011), and have no effect (Bossuyt *et al.*, 2001).

2.3 The responsive nature of stabilising and destabilising mechanisms to climatic conditions

The role of aggregate stability dynamics in determining soil erodibility is influenced by how and why aggregate stability varies through time and under seasonal patterns of climatic conditions. Aggregate stability has been observed to vary seasonally and inter-annually, with aggregate stability generally lowest in the winter and increasing over spring and summer (Cosentino *et al.*, 2006; Sanchis *et al.*, 2008; Dimoyiannis, 2009). For example, Blackman (1992) observed a reduction in aggregate stability of around 30% during winter months, and a rise to around 80% during spring and summer months in the UK. These seasonal variations in stability have been linked to changes in precipitation and temperature, and aggregate stability has reportedly been sensitive to shifts in temperature by as little as 2°C - 4°C (Lavee *et al.*, 1996). This magnitude of change in temperature is what is predicted for many temperate regions with climate change, thus highlights the need to study how aggregate stability responds to changing climatic conditions. Climatic conditions affect soil temperature and moisture and thus influence fluctuations in aggregate stability directly by affecting physical and chemical stabilising mechanisms (Sections 2.3.1 and 2.3.2). Soil temperature and moisture may also influence aggregate stability indirectly by inducing changes in the microbial community and therefore limiting or stimulating biological stabilising mechanisms. However, previous studies have not mechanistically determined how climatic conditions and seasonal patterns affect aggregate stability provoked by changes in the physical, chemical, and biological stabilising mechanisms and their interactions. Therefore, in this section, we revisit the properties presented in Section 2.2, focusing on mechanism by which a changing climate could affect aggregate stability.

2.3.1 Climate sensitive physical mechanisms

Aggregates are exposed to seasonal changes in climatic conditions and experience multiple cycles of drying and wetting, as well as freezing and thawing. These processes disrupt the particle bonds in aggregates, thereby reducing aggregate stability (Bullock *et al.*, 1988). However, these processes can also reorientate particles, increasing particle associations and thereby increasing aggregate stability (Bullock *et al.*, 1988; Amézketa, 1999). The physical response of soil aggregates to changes in soil temperature and moisture is influenced by soil texture, clay content, aggregate size distribution, organic matter content, initial moisture content and history of climatic conditions. Therefore, the numerous confounding factors that influence aggregate stability raises a high context dependency within each experimental study and reported contradictory results has made synthesising trends complicated (Denef *et al.*, 2001). The appearance of conflicting results may be due to varying experimental methodologies and varying initial sample conditions which may impede formulating a consensus of results (Henry, 2007). Therefore, the disruptive or reformative effects of changing climate conditions on physical stabilisation of aggregates remains unclear.

Drying-wetting cycles alter aggregate stability and enhance aggregate turnover by initialising aggregate breakdown and reformation (Denef *et al.*, 2001). The drying of soil and reduction in soil moisture content is influenced by external environmental factors such as humidity, temperature, wind and transpiration (Borken & Matzner, 2009). During drying, capillary action via the retraction of minisci around particles can act to reorientate particles and increase interparticle bonds (Bronick & Lal, 2005; Totsche *et al.*, 2018). As discussed in Section 2.1.1.2 wetting physically disrupts aggregates and can lead to aggregate breakdown through slaking, differential swelling and physico-chemical dispersion. Soils high in clay content can swell and shrink dependent on clay mineralogy (Section 2.2.2.2) with wetting and drying cycles. Expansive clay soils, particularly smectite clays, have a high shrink-swell capacity, swelling with increased moisture content and shrinking with decreased moisture content. As clay soil dries, the amount of

water held within the clay matrix is reduced, causing micro-fissures and cracking alongside an increase in bulk density and reduction in the volume of inter-aggregate pores. Differential swelling of aggregates also causes microcracking of aggregates and is also strongly related to clay shrink-swell characteristics; swelling increases with increasing clay content (Le Bissonnais, 1996). Physico-chemical dispersion is the reduction in strength of interparticle bonds leading to the release of primary particles from aggregates (Le Bissonnais, 1996) and often occurs alongside slaking and differential swelling. Dispersion is related to clay characteristics, organic matter, and multivalent cation concentration (discussed further in Sections 2.2.2 and 2.3.2).

Freeze-thaw cycles disrupt soil structure through aggregate breakdown, but its longer-term impact on aggregate stability is debatable. Expanding ice crystals in freezing soils create shearing forces, developing planes of weakness and disrupting particle bonds in aggregates (Bullock *et al.*, 1988; Lehrsch *et al.*, 1992). Soil moisture content at the time of freezing is important; numerous studies report that moisture content at time of freezing is inversely proportional to aggregate stability (Bullock *et al.*, 1988; Lehrsch *et al.*, 1992; Dagesse, 2013). Furthermore, the disruptive effect of freeze-thaw cycles has been observed to accumulate over successive cycles under controlled laboratory conditions (Oztas & Fayetorbay, 2003). However, other studies suggest that thawing periods allow time for aggregate reformation and stabilisation with an increase in aggregate stability following freezing and thawing due to the compression of aggregates by growing ice crystals, the reorientation of particles, and the precipitation and redistribution of bonding agents on particle surfaces (Lehrsch *et al.*, 1992; Dagesse, 2013). The results of these studies may differ for the reasons outlined earlier, including differing soil types and the application of varying methodologies.

2.3.2 Climate sensitive chemical mechanisms

Changing climatic conditions, such as air temperature and precipitation, influence soil temperature and soil moisture content respectively, which in turn have a great effect on chemical processes by altering evaporation, soil drying, soil hydration,

and oxygen status. These physical changes alter soil solution chemistry and affect the chemical properties of interparticle interactions such as organo-mineral bonds and particle charge characteristics. For example, during wetting clays with a tendency to swell disrupt clay particle bonds, thus reducing aggregate stability, though during drying shrinking clays reform interparticle bonds as the clay particles are brought closer together, thus stabilising aggregates (Bronick & Lal, 2005).

Oxidation and reduction are important soil chemical processes, closely linked to changes in soil moisture content which mediates soil aeration status (Weil & Brady, 2017). Oxidation and reduction act together simultaneously (redox) in the transfer of electrons. Redox reactions adjust the solubility of elements, produce redox compounds, and alter soil solution chemistry. Interpreting the state of redox reactions in soil is challenging due to the highly variable nature of the soil matrix and is associated with methodological difficulties due to high pH dependence and complicated microbial consumption of oxygen, which decreases redox potential (Husson, 2013; König *et al.*, 2020). Within the soil system there are micro-sites where oxygen diffusion is limited, e.g. water-filled micropores and the inside of aggregates, and the distribution of microbes and organic matter, and therefore decomposition and oxygen consumption, is highly heterogenous. Shifts in the redox status of soil due to climatic conditions alters aggregate stability by influencing soil pH, cationic concentration and organic matter interactions (De-Campos *et al.*, 2009) and thereby adjusting chemical stabilising and destabilising mechanisms (Section 2.2.2). A wetter climate due to climate change will result in higher soil moisture and more oxygen limited soils, thereby influencing redox reactions and chemical stabilising mechanisms.

Changes in soil moisture content during drying and wetting cycles are known to cause changes in the availability and leaching of soil nutrient elements, such as phosphorus (P), carbon (C) and nitrogen (N) (Borken & Matzner, 2009) and influence ionic concentrations. Changes in the solution chemistry of soil and ionic compositions and concentrations influence the formation of mineral-mineral bonds and organo-mineral assemblages, in turn altering aggregate stability. Soil

moisture content can therefore influence aggregate stability by altering the concentration of multivalent cations, soil pH, and the adsorption and desorption of nutrients altering their availability. The drying of soil causes a decrease in the adsorption capacity of amorphous hydroxyl-Fe and Al, attributed to crystallisation, and decrease in surface area for adsorption (Haynes & Swift, 1985). Therefore, drying and wetting affects the nature and strength of interparticle bonds, which directly affects aggregate stability. Additionally, aggregate breakdown during rewetting may cause an increase in the number of adsorption sites, thereby influencing the location of cations following aggregate turnover.

Interactions between soil organic matter and changes in temperature are often considered in terms of changes in the rate of microbial activity and decomposition, though there are multiple processes through which warming can affect soil organic matter (Pold *et al.*, 2017). The adsorption of organic matter with soil particles is a function of chemical conditions and processes (Conant *et al.*, 2011). It has been suggested that increasing temperature causes an increase in desorption relative to adsorption rates and so releases organic matter, thereby increasing substrate availability (Conant *et al.*, 2011). Additionally, nutrient release and carbon flush following drying-wetting events greatly affect biological processes but are also mediated by soil chemical conditions. After drying and rewetting a flush of nutrients occurs due to the physical release of occluded organic matter by aggregate breakdown (Denef *et al.*, 2001), the microbial release from cell lysis (Bottner, 1985; Van Gestel *et al.*, 1993) and the microbial exportation of intracellular osmoregulatory solutes (Halverson *et al.*, 2000). The flush in nutrients, such as P, C, and N, has implications for the chemical nature of the soil matrix and chemical configuration of the soil solution, therefore affecting interparticle bonds and chemical stabilisation of aggregates. The nutrient flush succeeding rewetting also drives increased mineralisation by microbes and is discussed in further detail in Section 2.3.3.

2.3.3 Climate sensitive biological mechanisms

There are excellent recent reviews on microbial responses to changing climatic conditions, such as: the effects of water stress on soil microbial communities (Manzoni *et al.*, 2011); the impact of climatic changes on interactions between saprotrophic fungi and invertebrates (A'Bear *et al.*, 2014); the impacts on arbuscular mycorrhizal fungi (Mohan *et al.*, 2014; Lenoir *et al.*, 2016; Millar & Bennett, 2016). In this section, the effects of climatic conditions on the soil microbial community are expanded to consider the implications for aggregate stability.

Changes in soil temperature or moisture conditions can stimulate or disrupt the microbial community, thereby altering biological stabilising mechanisms, but the impact of climate-induced microbial changes for aggregate stability and soil structure is rarely considered. Research has explored the influence of climate conditions on the microbial community in the interest of understanding feedbacks with microbially-mediated processes such as C-cycling and gas regimes, nutrient cycling, and plant growth (Compant *et al.*, 2010). There is very little research on the impact of soil temperature and moisture content on the effectiveness of biological stabilising mechanisms and particularly for seasonal aggregate stability dynamics, due to methodological constraints which have limited direct measurements. The biological stabilising mechanisms are expected to be closely related to microbial community composition, microbial biomass, and respiration. Microbial community composition determines the relative abundance of microbial groups and thus the activity of their associated biological mechanisms. For example, increased fungal abundance is closely associated with increased hyphal enmeshment and therefore aggregate stability (Degens *et al.*, 1996; Deacon, 1997; Lehmann *et al.*, 2017). Changes in microbial biomass and respiration are also indicative of changes in microbially-mediated processes (Bending *et al.*, 2004; Truu *et al.*, 2009; Pulleman *et al.*, 2012) such as biological stabilisation by influencing resource availability and the allocation of energy to hyphal growth or EPS production. Here it is examined how soil temperature and moisture affect soil microbial community composition and microbial activity (in

terms of respiration and biomass), how patterns of climatic conditions can shape soil microbial responses and the influence of the soil microhabitat.

Changes in temperature will affect microbial community composition. Soil bacteria and fungi have differing optimal conditions and respond differently to changes in soil temperature and moisture content, due to physiological differences in cell structure, growth, and resource strategy. Temperature alters microbial metabolic rates, thereby affecting microbial processes, such as decomposition and biomass synthesis (Brown *et al.*, 2004; Fierer *et al.*, 2006; Koch *et al.*, 2007; Conant *et al.*, 2011). In warmer temperatures fungal abundance has been observed to increase (Castro *et al.*, 2010).

As discussed in Section 2.3.1 and 2.3.2, changes to soil moisture can affect soil physico-chemical properties that directly alter interparticle forces, but these changes can also indirectly affect aggregate stability through biological mechanisms. In particular, soil moisture strongly affects microbial composition by influencing the microhabitat and altering matric and osmotic pressure, oxygen concentration and pore-water connectivity, in turn affecting osmoregulation, microhabitat continuity, bacterial motility and ultimately internal cell processes and resource accessibility (Or *et al.*, 2007b). Moisture content regulates matric and osmotic pressure, the diffusion of solutes and oxygen diffusion, and therefore directly affects physiological microbial processes. It is well documented that in order to withstand osmotic stress, microbes undergo osmoregulation (Csonka, 1989; Wood, 2015). With a rapid increase in moisture content, external osmotic pressure decreases, causing an influx of water to the cell and cell swelling due to an increase in cytoplasmic volume and potential cell lysis. Decreasing moisture content leads to an increase in external osmotic pressure and cell dehydration. Microbial cells regulate changes in osmotic pressure by synthesising or releasing solutes, however tolerance to osmotic stress and shock varies between microbes. It has been suggested that dry conditions may favour gram-positive bacteria over gram-negative bacteria due to a thicker cell wall and therefore an associated greater resistance to osmotic and turgor pressure (Csonka, 1989). Fungi have been reported to be more tolerant than bacteria to drying and can

remain viable even under very dry soil conditions (Treseder *et al.*, 2010; Yuste *et al.*, 2011) due to more resilient cell structures and the growth of hyphae from the tip. Hyphal growth through apical extension enables fungal hyphae to continuously move into new areas searching for nutrients and bridge pore-spaces despite dry conditions (De Boer *et al.*, 2005; Schimel *et al.*, 2007). However, it has also been reported that fungi are more sensitive to fluctuations in temperature and moisture content due to their preferential location in soil macropores, whilst bacteria occupy micropores and can be protected inside aggregates (Chenu *et al.*, 2001).

Changes in soil temperature and moisture conditions influence microbial activity, (respiration and biomass synthesis) as well as microbial composition. For example, as drying reduces soil water potential (the tendency of water to move from one area to another), generally the metabolic activity of soil microorganisms also decreases, and solute and enzyme mobility is also reduced (Manzoni *et al.*, 2011). Subsequent rewetting therefore enhances metabolic and enzyme activity and can mobilise substrate, resulting in an increase in microbial activity and respiration (Fierer & Schimel, 2003). Drying and rapid rewetting can increase nutrient availability as a result of the release of previously occluded organic matter with aggregate breakdown, microbial cell lysis, and the release of microbial intracellular solutes to regulate osmotic stress (Halverson *et al.*, 2000; Fierer & Schimel, 2002; Fierer *et al.*, 2003). The rapid increase in nutrient availability causes enhanced mineralisation and a pulse in CO_2 production, known as the Birch effect (Birch, 1958; Fierer & Schimel, 2002). The implications of this pulse in microbial activity for aggregate stability remain unclear but this relationship is likely to influence the microbial ability to stabilise aggregates. Desiccation with low soil moisture is generally much slower than rewetting, allowing microbes more time for physiological adaptations, such as the adjustment and accumulation of intracellular solutes to manage osmotic stress (Keift *et al.*, 1987). Bacteria have been reported to increase EPS production in response to desiccation, thereby modifying the conditions of the inhabited microenvironment and enhancing survival under desiccation (Roberson &

Firestone, 1992; Lennon, 2012). In the instances where EPS production is stimulated under drought conditions to avoid desiccation, this microbial response may act to increase aggregate stabilisation, or at least limit aggregate destabilisation, during drying by increasing particle adhesion between EPS and soil particles. Thus, soil moisture content strongly affects microbial activity and EPS production, in turn affecting aggregate stability (see Section 2.2.3 for information on the relationship between EPS and aggregate stability). To address this gap in knowledge and explain aggregate response to changing climate conditions, it is imperative to investigate the response of the microbial community and aggregate stability simultaneously.

Changes in climate conditions can act as a perturbation and cause shifts in the composition of the microbial community. There is evidence that the previous climatic conditions (e.g. soil temperature, moisture, number of drying-wetting cycles), influence the response and development of the microbial community (Henry, 2007; Castro *et al.*, 2010; Evans & Wallenstein, 2012, 2014). Experimental research has shown that bacteria and fungi exhibit varied responses to changes in climatic conditions (Manzoni *et al.*, 2011), and responses may vary within fungal and bacterial populations (Evans & Wallenstein, 2014). Some fungal species utilise various adaptive mechanisms to withstand stressful conditions, such as the increased production of specific carbohydrates, proteins, and lipids. For more details on the various stress-resistance mechanisms readers are directed to the review by Lenoir *et al.,* (2016). Under stress microbial community composition will likely change with the success of adaptations and the loss of non-resistant species that cannot withstand disturbances from perturbations (Millar & Bennett, 2016). Changes in EPS production and composition due to such adaptations may influence the biological stabilisation of aggregates but this has not yet been investigated.

The effects of soil temperature and moisture are also modulated by micro-scale variations in soil physico-chemical properties (which are largely influenced by soil texture), which shape the microhabitat for fungi and bacteria (Nunan, 2017). Bacteria are often found in biofilms adhered to soil particles but can also be motile

in soil solution, or occluded within soil aggregates (Flemming & Wingender, 2010), whilst fungi are often associated with larger macroaggregates and hyphal networks can stretch across pore spaces. The microhabitat governs many microbial processes by influencing nutrient accessibility and availability, predation risk and aeration (oxygen content). Microhabitats are defined by soil architecture (textural and pore properties; pore connectivity, size, shape, gas regimes and wetting regimes), and the distribution of nutrients within the soil matrix. The properties of microhabitats vary and respond to changes in temperature and soil moisture, and can create hotspots of microbial activity (Kuzyakov & Blagodatskaya, 2015; Ebrahimi & Or, 2016). Research has linked increases in EPS production to microbial hotspots (Benard *et al.*, 2019) and so suggests that these microscale variations and processes may be critical to soil functions.

2.4 Lines of enquiry on responsive properties and aggregate stability

Firm conclusions on the effects of soil temperature and moisture on aggregate stability are difficult to synthesise and so the current knowledge on the response of aggregates and the stabilising mechanisms to changing climatic conditions requires further refinement. Aggregate stability is the collective result of the confounding effects of the multiple physical, chemical, and biological stabilising and destabilising mechanisms and their interactions, as discussed in Sections 2.2 and 2.3. Few studies simultaneously consider and encapsulate the physical, chemical, and biological mechanisms occurring at the aggregate scale. Furthermore, despite the observed temporal shifts in aggregate stability, often related to local climatic conditions, there are very few studies reporting explanatory empirical evidence to elucidate the operation and feedbacks of stabilising mechanisms. Developing a holistic knowledge of aggregate stability by considering co-occurring and interacting mechanisms would enable better prediction of aggregate stability dynamics. A framework to map how stabilising mechanisms interact to affect aggregate stability is presented (Figure 2.1) and

possible lines of enquiry are demonstrated by three examples where researchers have investigated links within the framework.

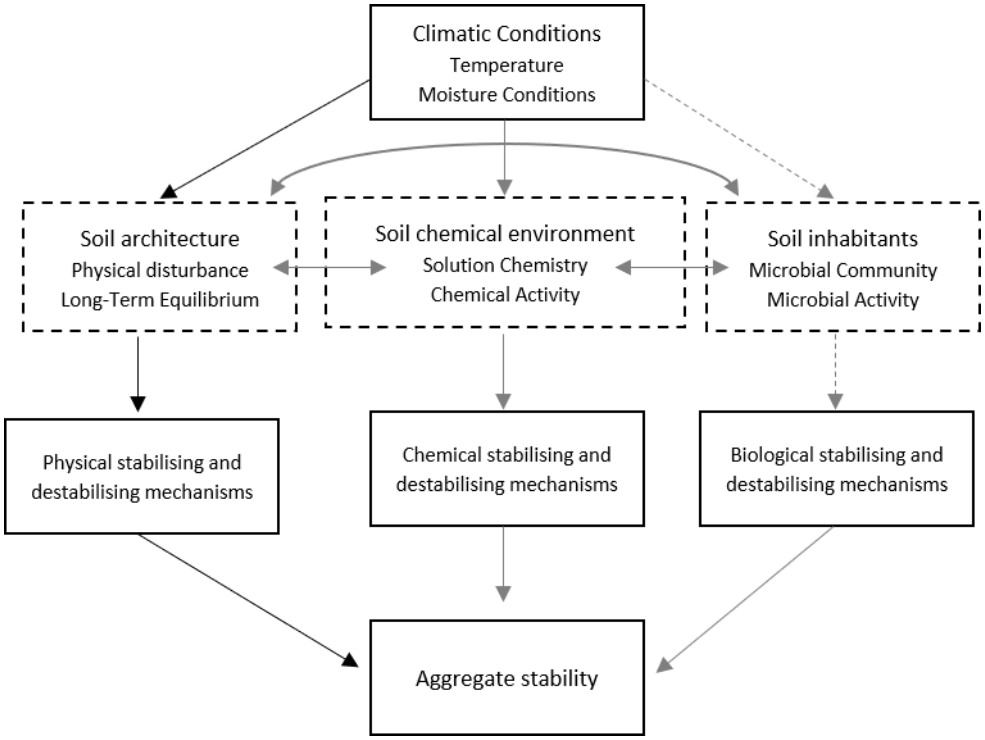

Figure 2.1: Framework presenting the links between environmental drivers (perturbations), soil architecture, soil chemical environment and soil inhabitants, stabilising and destabilising mechanisms, and aggregate stability. Black continuous connectors show the pathway of interactions discussed in Example 1. The dashed connectors indicate the relationships between climatic conditions, soil inhabitants, and biological stabilising mechanisms considered in Example 2. Dashed boxes represent the soil properties and their interactions discussed in Example 3.

The framework shows that climatic conditions act as an environmental driver influencing soil architecture, the chemical environment, and soil inhabitants as discussed in Section 2.3. The soil properties grouped as soil architecture, the soil chemical environment, and soil inhabitants alter the operation of physical, chemical, and biological stabilising and destabilising mechanisms which govern soil aggregate stability, as discussed in Section 2.2. The lines of enquiry identify current knowledge gaps and routes for future research.

2.4.1 Example 1: climatic conditions affect physical soil properties mediating aggregate stability

Climatic conditions induce changes in physical soil conditions altering soil architecture and potentially leading to the physical stabilisation or destabilisation of aggregate stability (Figure 2.1, Example 1). The effects of changes in climatic conditions, in terms of drying and wetting cycles and freeze-thaw cycles, on physical interparticle forces and therefore soil architecture, physical stabilising (and destabilising) mechanisms, and aggregate stability have been well-researched and are summarised in Section 2.3.1. However, consensus on the effects of climatic conditions on physical stabilising and destabilising mechanisms and aggregate stability has not yet been achieved due to methodological challenges giving rise to contradictory results (Denef *et al.*, 2001; Henry, 2007). Some studies have generalised the influence of seasonal climatic conditions on physical stabilising and destabilising processes and aggregate stability (Bullock *et al.*, 1988), but do not fully consider the role of the microbial community.

2.4.2 Example 2: climatic conditions drive microbial changes and biological stabilisation

In terms of the framework presented here, the link between changing climatic conditions and soil inhabitants is well-researched and continues to be a thriving research area as the complex dynamics are still not fully quantified (Figure 2.1). Shifts in the composition and activity of the soil microbial community have been observed in response to climatic conditions as discussed in Section 2.3.3. Soil ecologists have often interpreted microbial changes in terms of resistance, resilience and functional redundancy (Allison & Martiny, 2008), though often not including the influence of soil inhabitants on biological stabilising mechanisms and aggregate stability.

The influence of the soil microbial community on soil stability at the aggregate scale is summarised in Section 2.2.3. A great challenge within the presented framework (Figure 2.1) is the relationship between the microbial community and biological stabilising mechanisms. Recent work has begun to investigate the

influence of physiological traits of specific fungal species on aggregate stabilising function (Lehmann & Rillig, 2015; Lehmann *et al.*, 2020). Further research is needed to examine the influence of the microbial community on EPS properties and elucidate the relationship between taxonomy and function in terms of aggregate stabilisation but is currently restricted largely due to methodological limitations. Further information on the influence of physiological traits of microbial species on aggregation will be necessary for an eventual high-resolution mechanistic framework of microbially-mediated aggregate stabilisation, but challenges remain in identifying microbial diversity and monitoring the influence of morphological traits. Therefore, advancing knowledge of the biological stabilising mechanisms should consider the broader influence of the microbial community on aggregate stability. Soil microbial properties are often used as indicators of microbial processes, assuming that changes in the soil microbial community composition or activity are closely related to the operation of microbially-mediated processes (Schloter *et al.*, 2003; Bending *et al.*, 2004; Ritz *et al.*, 2009; Truu *et al.*, 2009; Pulleman *et al.*, 2012). There is a close association between these microbial properties and function in terms of biological stabilisation, as these properties reflect the allocation of resources and energy for growth and the relative investment in biological stabilising mechanisms. As such, these properties have previously been significantly correlated with aggregate stability (Perfect *et al.*, 1990a; Degens, 1997).

With great influence on multiple soil processes, the microbial community serves many functions. Where the response of the soil microbial community to climatic conditions has been documented, it has often not been related to the consequences for biological stabilising mechanisms and aggregate stability. Instead where research has taken the extra step of considering the implications of a changing microbial community and therefore the potential shifts in microbial processes and functioning, it is often under the context of C and N cycling (Ren *et al.*, 2017) or gas flux (Ebrahimi & Or, 2016), rather than aggregate stability. Therefore, future research must work to resolve the key knowledge gap on the

influence of climatic conditions on the microbial community in terms of aggregate stability.

2.4.3 Example 3: Physical, chemical, and biological feedbacks

There are complex interactions between soil physical and chemical properties and the microbial community (Figure 2.1). Microscale variations in soil physical, chemical, and biological conditions influence the composition and activity of the microbial community, in turn microbes mediate the conditions of soil microhabitats, affecting physical and chemical soil conditions and leading to a soil system with complex feedbacks (Young *et al.*, 1998; König *et al.*, 2020). Variability in physical properties including soil particle size, surface area and surface roughness, as well as pore-size, shape and connectivity in the pore network leads to a diverse microenvironment. These physical properties influence microbial adhesion and therefore microbial distribution (König *et al.*, 2020). This multi-way interaction between soil microbes and physico-chemical soil properties has been widely acknowledged (Nunan *et al.*, 2003; Young & Crawford, 2004). Yet studies must go further and jointly consider the physical, chemical, and biological domains in an integrated holistic approach, in order to examine the changes in associated stabilising mechanisms and aggregate stability. More explanatory empirical research is needed to resolve the lateral relationships and feedbacks between the soil inhabitants and the soil architecture and chemical environment at the aggregate scale.

As discussed in Section 2.3, soil physico-chemical conditions change temporally with shifts in climatic conditions such as temperature, precipitation, and evaporation, which affect soil hydration and oxygen status and processes of drying and wetting. The microbial community also exhibit multifaceted responses to the direct effects of changing climatic conditions on their physico-chemical microhabitat. To advance knowledge of the framework of interactions influencing aggregate stability, studies must holistically consider the feedbacks between changing climatic conditions, the physical, chemical, and biological components

of the soil and their associated stabilising and destabilising mechanisms and aggregate stability.

2.5 Conclusion

This review has summarised the multiple physical, chemical, and biological stabilising and destabilising mechanisms that mediate soil aggregate stability and demonstrated the influence of changing climatic conditions on these processes. In order to analyse the collective effects of changing climatic conditions on aggregate stability a holistic approach to advance knowledge of the feedbacks between climatic conditions, stabilising and destabilising mechanisms, and aggregate stability is critical. Studies investigating the response of aggregate stability to changing climatic conditions need explanatory empirical research, exploring the highly dynamic and responsive nature of stabilising and destabilising mechanisms and feedbacks which influence aggregate stability. This mechanistic framework then must be upscaled and incorporated into the understanding of soil erodibility and soil erosion under future climate change.

3 Do temperature and moisture conditions impact soil aggregate stability and the microbial community?[1]

Abstract

The role of soil erodibility, i.e. the susceptibility to erosion, must be considered when predicting future erosion rates associated with climate change. Soil erodibility is highly dependent on the stability of soil aggregates and is anticipated to change because of the effects of altered temperature and moisture on aggregate stability, which in turn is dependent on physico-chemical particle interactions and biological stabilising agents. This study aimed to investigate the effects of temperature and moisture conditions on aggregate stability and whether this could be explained by changes in the microbial community. Using an experimental approach with laboratory microcosms, aggregates from sandy loam and clay soils were incubated at three temperatures (5°C, 15°C, and 30°C) and three moisture conditions (representing wet, intermediate, and dry) in a fully factorial experimental design. Aggregate stability was quantified using rainfall simulation. Microbial properties of the aggregates, including microbial community composition, biomass carbon and respiration, were measured as proxies for microbial-induced stabilisation. Temperature and moisture content significantly affected aggregate stability and the microbial community. In the sandy loam soil, aggregate stability decreased significantly by approximately 10% with increasing moisture content. In the clay soil, aggregate stability increased significantly by approximately 5% with increasing temperature. In both soil textures, temperature and moisture content affected microbial respiration and microbial community composition. Regression analysis indicated that microbial properties were significant predictors of aggregate stability. The results show that temperature and moisture content affect aggregate stability and the microbial community, dependent on soil texture, suggesting an interconnectivity and potential

[1] This chapter has been prepared for publication and is currently submitted under review to the European Journal of Soil Science.

feedbacks between aggregate stability, physico-chemical soil properties, and microbial stabilising mechanisms.

3.1 Introduction

Climate is a main driver of soil erosion processes, as such ongoing global warming is predicted to impact the frequency, severity, and extent of soil erosion and undermine soil sustainability (Nearing *et al.*, 2004; Mullan, 2013). Whilst the potential impacts of climate change on rainfall erosivity have been well studied (Nearing, 2001), few studies have considered the effects on soil erodibility, i.e. the susceptibility of soil to erosive forces (Favis-Mortlock & Boardman, 1995). Soil erodibility is determined by a combination of several soil properties (e.g. soil texture, organic matter, biological activity) and has been found to be highly correlated inversely with aggregate stability, which is therefore widely considered as a suitable indicator of soil erodibility (Bryan, 1968; Sanchis *et al.*, 2008). The stability of aggregates governs their resistance to external stresses and the physical integrity of the soil pore network. Thus, aggregate stabilising mechanisms at the aggregate scale are important for soil erodibility, and in the context of this study, especially those influenced by climatic conditions such as temperature and soil moisture. Soil aggregate stability is influenced by numerous soil properties which influence the internal forces that bind soil particles and aggregates (Six *et al.*, 2004; Bronick & Lal, 2005; Regelink *et al.*, 2015). Soil microbes can aid aggregation through the physical enmeshment of particles by fungal hyphae (Degens *et al.*, 1996; Lehmann & Rillig, 2015), and by the production of extracellular polymeric substances (EPS), which promote particle adhesion (Tisdall & Oades, 1982; Ritz & Young, 2004; Blankinship *et al.*, 2016).

Despite investigations of the physical, chemical, and biological influences on aggregation, understanding temporal changes in aggregate stability has often proven difficult. A primary component of this challenge is the interpretation and quantification of the multiple stabilising and destabilising mechanisms which often interact and exhibit confounding effects and feedbacks (Amézketa, 1999). Furthermore, the complex influence of changing climatic conditions on the

microbial community (Compant *et al.*, 2010; Auffret *et al.*, 2016), and thus microbially mediated aggregation is a major source of uncertainty when interpreting the response of aggregate stability to climatic conditions (Cosentino *et al.*, 2006). Despite several proposed relationships between seasonal conditions and aggregate stability (e.g. Bullock et al., 1988), there is still considerable debate on the effects of changing climate conditions on aggregate stability. Aggregate stability has been reported to vary seasonally with changes in climatic conditions (Lavee *et al.*, 1996; Amézketa, 1999; Cosentino *et al.*, 2006; Sanchis *et al.*, 2008). Numerous contradictory results have been reported where aggregate stability has been observed to both increase and decrease after fluctuations in temperature and moisture content (Denef *et al.*, 2001; Cosentino *et al.*, 2006). Climatic conditions have a substantial influence on soil aggregation and breakdown, particularly temperature and moisture content through the occurrence of drying-wetting cycles, cooling and warming cycles, and freeze-thaw conditions. Whilst some of the differences between studies could be explained by different methodologies and soil properties (Amézketa, 1999), research has not fully considered the potential for changes in aggregate stability to be associated with microbial responses to climatic conditions, which in turn affects the biological stabilising mechanisms of aggregate stability. Physico-chemical aggregate stabilising mechanisms are often considered individually, but the potential rapid adaptation of microbial mechanisms to changing climate, and interactions between mechanisms, could influence overall aggregate stability.

Little research has investigated the indirect effects of temperature and moisture content on aggregate stability via the potentially rapid stabilising and destabilising effects brought about by the microbial community responding to climate conditions. Whether changes in the microbial community composition, activity and biomass influence the efficacy of biological stabilising mechanisms remains underexamined. Soil temperature and moisture can affect aggregate stability with the stimulation or limitation of microbial respiration or biomass carbon, dependent on microbial metabolic activity, optimal temperature ranges, available oxygen, resource availability and accessibility, microbial motility and soil microstructure

connectivity (Franzluebbers, 1999; Or *et al.*, 2007b; Moyano *et al.*, 2013). Soil temperature and moisture can also affect aggregate stability by influencing the composition of the microbial community (Evans & Wallenstein, 2014; Supramaniam *et al.*, 2016). The effects of climatic conditions on microbial groups, including fungi, gram-positive bacteria, and gram-negative bacteria, varies due to physiological differences, such as hyphal connectivity to resources and cell wall structure (Nazih *et al.*, 2001; Uhlířová *et al.*, 2005; Schimel *et al.*, 2007). The variation in the effects of climatic conditions on microbial groups affects ecological strategy and resource allocation, and in turn modifies hyphal growth and EPS production and characteristics (Roberson & Firestone, 1992; Evans & Wallenstein, 2014). Thus, shifts in the abundance of fungi, gram-positive, and gram-negative bacteria associated with climatic conditions may have a varying effect upon aggregate stability.

The aim of this study is to investigate whether aggregate stability response to temperature and moisture conditions is associated with changes in the microbial community. A laboratory experiment was designed to test the following hypotheses; i) aggregate stability will increase with temperature because of increased microbial metabolic rates and associated microbially-mediated aggregate stabilisation, ii) aggregate stability will increase with intermediate moisture content due to the stimulation of the microbial community with increased motility and resource accessibility, though aggregate stability will decrease with high soil moisture due to oxygen-limited conditions impeding the microbial community and microbially mediated stabilisation, iii) temperature and moisture content will invoke a unimodal response in microbial biomass and respiration, and will cause a shift in the microbial community composition, and iv) the influence of temperature and moisture content on the microbial community will affect microbial stabilisation mechanisms, thus altering aggregate stability.

3.2 Methods

3.2.1 Soil collection and preparation

Two surface soils (depth 0 – 150 mm) were used in this experiment both classified as Cambisols (WRB, 2007); a sandy loam and a clay collected from the Silsoe Experimental Farm (Bedfordshire, England, National Grid Reference TL075356/TL075351). The sandy loam is from the Bearsted series (6% organic matter, 69% sand, 20% silt, 11% clay) and the clay is from the Evesham series (6% organic matter, 42% sand, 15% silt, 44% clay). The soils were collected post-harvest in September 2017 to avoid freezing conditions and exceptionally dry or wet soil moistures (Le Bissonnais, 1996). Two soil textures were used because particle size distribution and clay content have been shown to alter numerous physical and chemical soil properties (e.g. porosity, soil pH, hydration characteristics and organic matter and cation interactions), which may affect the responses of the microbial community to temperature and moisture and influence soil aggregation processes (Bronick & Lal, 2005; Regelink *et al.*, 2015). On collection, the field moist soil was gently broken apart by hand following planes of least resistance, taking care to avoid compaction and smearing. Soils were then air-dried for 72 hours away from direct sunlight and sieved to obtain aggregates between 2 – 5.6 mm. Any plant material retained was gently removed by hand.

The sieved aggregates (25g of 2 – 5.6 mm) were assigned to microcosms, which consisted of a 2 mm aperture mesh (Wondermesh) fitted across a hoop and enclosed in an air-tight container (185 x 130 x 45 mm, l x w x d) with a cotton wool wetting bed to facilitate soil moisture content through capillary rise. To minimise the level of initial disruption and the risk of slaking caused by the methodological addition of moisture, the wetting bed within the microcosms was designed to generate a slow rate of initial wetting. The aggregates were spread across the microcosm mesh in a single layer with minimal contact between aggregates to enable the investigation of single aggregate mechanisms. Nine treatments were prepared for each soil type in a 3 x 3 multifactorial design, with

three levels of temperature (5°C, 15°C, and 30°C) and three levels of moisture content representing wet, intermediate and dry conditions. Temperature was controlled by placing the microcosms in environmental chambers, while moisture content was controlled by adjusting the wetness of the wetting bed. For the wet treatment, 80 ml of deionised water was added to the wetting bed, and aggregates reached a moisture content of 32% (sandy loam) and 44% (clay) moisture content. In the intermediate treatment, 60 ml of water was added, and aggregates reached a moisture content of 14% (sandy loam) and 20% (clay). No water was added for the dry treatment. To evaluate the effects of incubation duration, microcosms were held under treatment conditions for one week, two weeks or four weeks. For each treatment, paired microcosms were analysed for: aggregate stability by rainfall simulation; microbial community composition by phospholipid fatty acid (PLFA) analysis (Frostegård *et al.*, 1993, 2011); microbial respiration rate by rapid automated bacterial impedance technique (RABIT, Don Whitley Neil, UK; Ritz *et al.*, 2006); and microbial biomass carbon by chloroform fumigation extraction (Vance *et al.,* 1987). Microcosms were paired for the different destructive laboratory analyses, and there were three sets of replicates per treatment.

3.2.2 Aggregate stability and rainfall simulation

There are numerous methods used to measure aggregate stability, with many methodologies based on the wet-sieving approach (Kemper & Koch, 1966; Le Bissonnais, 1996; Amézketa, 1999). Whilst variants of this method enable the distinction of aggregate breakdown mechanisms, the process is highly mechanical and far removed from natural erosion processes. Comparatively, rainfall simulation has been reported to be the most realistic method for measuring aggregate stability (Almajmaie *et al.*, 2017) and so this method was selected here to determine aggregate stability (Figure 3.1). Aggregate stability was tested by subjecting the microcosms to a rainstorm generated by a 9 m gravity-fed hypodermic needle rainfall simulator (Allton *et al.*, 2007; Jeffery *et al.*, 2010). Before each rainfall event, all microcosms were air-dried for 24 hours (to standardise moisture conditions at the time of rainfall application to less than 1%)

and weighed. The microcosms were subjected to a simulated rainfall event of 33 mm h^{-1} for 5 minutes, which represents a gentle rainfall event with a return period of less than six months (NERC, 1975). After the rainfall simulation, samples were air-dried for 48 hours and weighed.

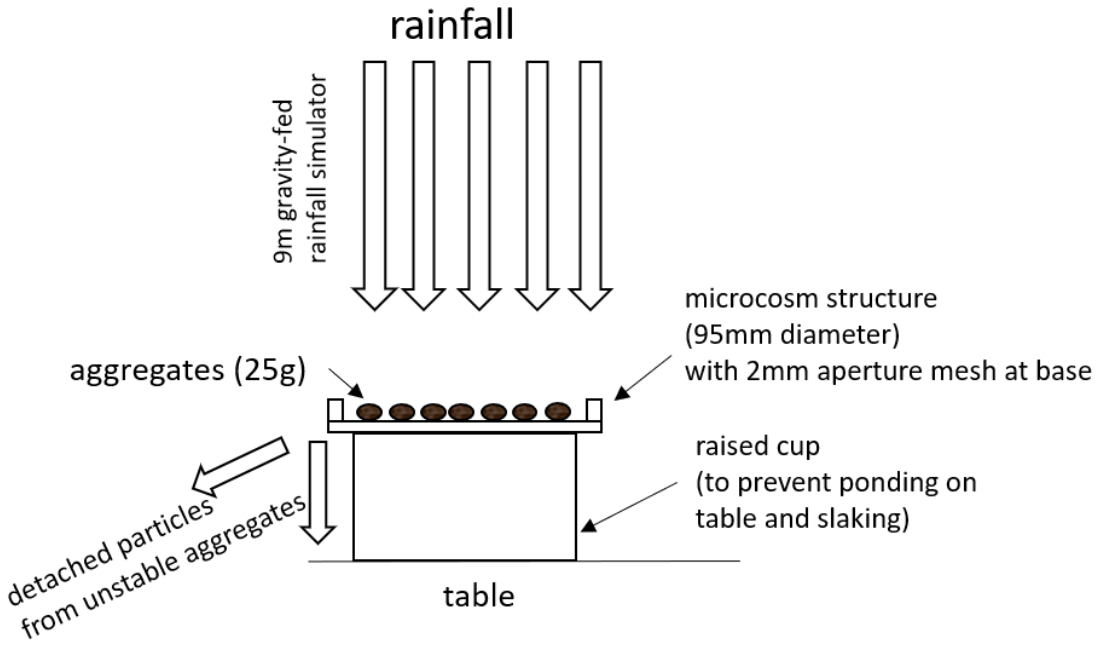

Figure 3.1: Schematic diagram of experimental set up for aggregate stability testing by rainfall simulation. Not drawn to scale.

The percentage of stable aggregates was calculated using the following equation (Almajmaie *et al.*, 2017):

$$Aggregate\ stability\ (\%) = \frac{(dry\ weight\ of\ aggregates\ post\ rainfall - microcosm\ structure)}{(dry\ weight\ of\ aggregates\ pre\ rainfall - microcosm\ structure)}\ x\ 100$$

Percentage data for aggregate stability was normalised using an arcsine transformation for statistical analyses.

3.2.3 Phospholipid Fatty Acid Analysis

Phospholipid Fatty Acid (PLFA) analysis was used to characterise microbial community composition. PLFAs were extracted based on the procedures by Frostegård *et al.* (1993), Frostegård and Bååth (1994) and Bardgett *et al.* (1996), based on the method developed by Bligh and Dyer (1959). Aggregates for PLFA analysis were frozen at -20°C and freeze-dried. PLFAs were extracted from 10g of aggregates. Fatty acid methyl esters (FAMEs) were identified by gas chromatography (GC) retention time. Results were expressed as a percentage of the total area of the identified peaks on the chromatogram, which was then used to assess relative abundance of fungi, gram-negative bacteria, and gram-positive bacteria. PLFAs 16:1ω5, 18:2ω6, cis18:1ω9 were used as an indicator for fungal biomass (Frostegård *et al.*, 1993, 2011; Frostegård & Bååth, 1994). Trans16:1ω11, cis16:1 ω7, cyc17:0iso, cis17:1ω8, cis17:0, trans17:1ω8, cis19:0 represented gram-negative bacteria. Finally, i15:0, ai15:0, i16:0, 17:0 i17:0 and ai17:0 represented gram-positive bacteria (Bardgett *et al.*, 1996; Zelles, 1999; Ruess & Chamberlain, 2010). Other PLFAs determined were 14:0, 15:0, i16:1, 16:0, Me17:0iso, Me17:0iso2, cyc17:0iso. 17:0br, 17:1ω 7, 17:0(12Me), 18:1ω7t, 18:1ω13, 18:0, 19:1ω6, 18:0(Me), 20:4(5,8, 4, 11,14), 20ω5(3), 20:0.

3.2.4 Microbial respiration rate

Microbial respiration rate was calculated based on the indirect impedance technique and rapid automated bacterial impedance technique (RABIT; Don Whitley Neil, UK) according to Ritz et al. (2006). This method measures the decrease in conductance of alkaline agar over time as the agar absorbs microbially produced CO_2 (Ritz *et al.*, 2006; Butler *et al.*, 2011, 2012). First, RABIT cells were created by immersing electrodes in 1ml of potassium hydroxide agar. Next, 1g of soil was weighed into a glass boat and sealed inside the RABIT cell. The basal microbial respiration rate was determined between two and four hours incubation at 25°C. Temperature has a strong influence on electrical conductivity and so during respiration measurements was held constant for all incubation treatments for consistent operational conditions. Respiration rates

were corrected for dry mass of soil to account for soil moisture conditions consistent with the incubation treatment at the time of measurement. For each sample, three methodological replicates were included. Falsely terminated tests were removed from the dataset, as were outliers (identified as 1.5 times the interquartile range above or below the upper and lower quartiles). Microbial respiration rate was measured after the treatment incubation rather than during incubation due to the destructive nature of sampling and measurement. As such the measurement of microbial respiration reflects the response of the microbial community to the consistent temperature during RABIT measurements post-incubation mediated by previous thermal stress conditions during the incubation and substrate availability. Thus, a low respiration rate suggests the potential depletion of substrate due to high activity during the treatment and a high respiration rate reflects induced activity post-incubation potentially due to low activity during the incubation and remaining substrate availability. Microbial biomass carbon

3.2.5 Microbial biomass carbon

Microbial biomass carbon was estimated for three replicates for each treatment, based on the chloroform extraction procedure developed by Jenkinson (1976) and Vance *et al.,* (1987). A subsample equivalent to 12.5g air-dry weight was placed in a 50ml Duran bottle and fumigated for 24 hours, while another subsample was not fumigated as a control. Dissolved carbon was extracted from both samples by shaking the subsamples in 50ml of 0.5M K_2SO_4 for 30 minutes and filtering through Whatman filter papers. Concentrations of dissolved organic carbon were measured using a segmented flow analyser. Carbon flush was assessed as the concentration of DOC from fumigated samples minus the concentration of DOC from the non-fumigated samples. Biomass carbon was estimated using a conversion factor of 0.45 (British Standards Institution, 1997).

3.2.6 Statistical analysis

Analysis of variance (ANOVA) and Tukey's HSD post-hoc tests were used to assess differences in aggregate stability, microbial biomass C and microbial respiration rate between treatments. Principal component analysis (PCA) was also used to visualise PLFA data and examine relationships. Pearson correlation analysis was used to test correlative associations between variables including aggregate stability, temperature, soil moisture, and microbial properties. Multiple regression analysis for both soil types was completed using the stepwise method. R3.2.5 was used for statistical analysis (R Core Team, 2018), and results were considered significant at $p \leq 0.05$.

3.3 Results

In both sandy loam and clay samples, the duration of incubation (one week, two weeks or four weeks) had no significant on aggregate stability ($p>0.05$, data not shown), therefore later statistical analyses were completed on pooled results as a single dataset. Furthermore, as there were no significant observed interaction effects between temperature and moisture content on aggregate stability (ANOVA, $p>0.05$), the main effects of the climatic conditions are considered individually with the main effects of temperature considered across all moisture contents and conversely.

3.3.1 Aggregate Stability

The sandy loam and clay aggregates responded differently to the temperature and moisture content treatments (Figure 3.2). For the sandy loam, aggregate stability was significantly different between moisture treatments ($p <0.001$), with the highest aggregate stability in the dry treatment (Figure 3.2B), but there was no significant difference in aggregate stability between temperature treatments ($p >0.05$, Figure 3.2A). Meanwhile in the clay samples, aggregate stability significantly increased with increasing temperature ($p <0.005$; Figure 3.2C), but there was no significant effect on aggregate stability for the moisture content treatments ($p >0.05$; Figure 3.2D).

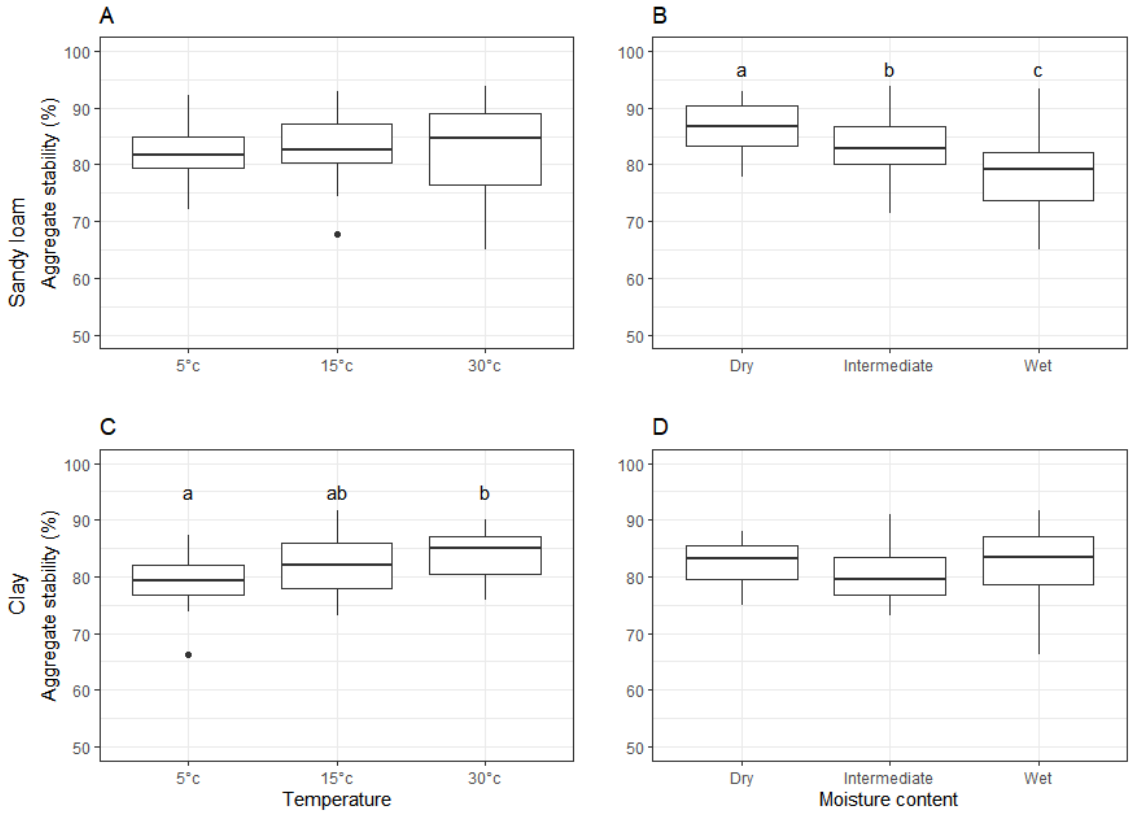

Figure 3.2: Aggregate stability by (A, C) temperature and (B, D) moisture content for (A, B) sandy loam and (C, D) clay. Different letters signify statistically significant difference for arcsine transformed percentage aggregate stability.

3.3.2 Microbial variables

3.3.2.1 Microbial response to temperature and moisture content (and incubation duration)

Respiration rates were significantly higher under the 5°C treatment than at the 15°C treatment and increased with increasing moisture content in the sandy loam (Figure 3.3A, B) and clay aggregates (Figure 3.4A, B). Microbial biomass carbon was not significantly affected by temperature or moisture content in the sandy loam (Figure 3.3C, D). However, in the clay microbial biomass carbon significantly increased with increasing moisture content (Figure 3.4D).

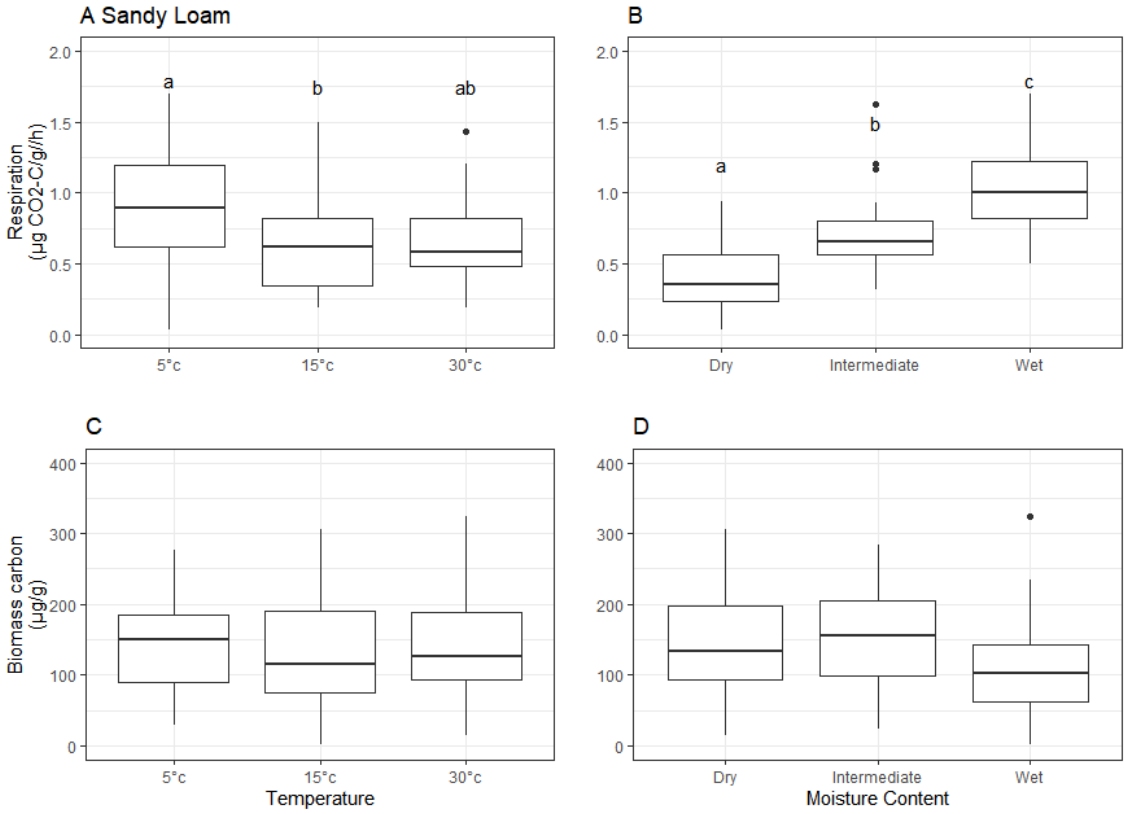

Figure 3.3: Microbial respiration (A, B) and microbial biomass carbon (C, D) by temperature and moisture content for sandy loam aggregates. Different letters signify statistically significant differences.

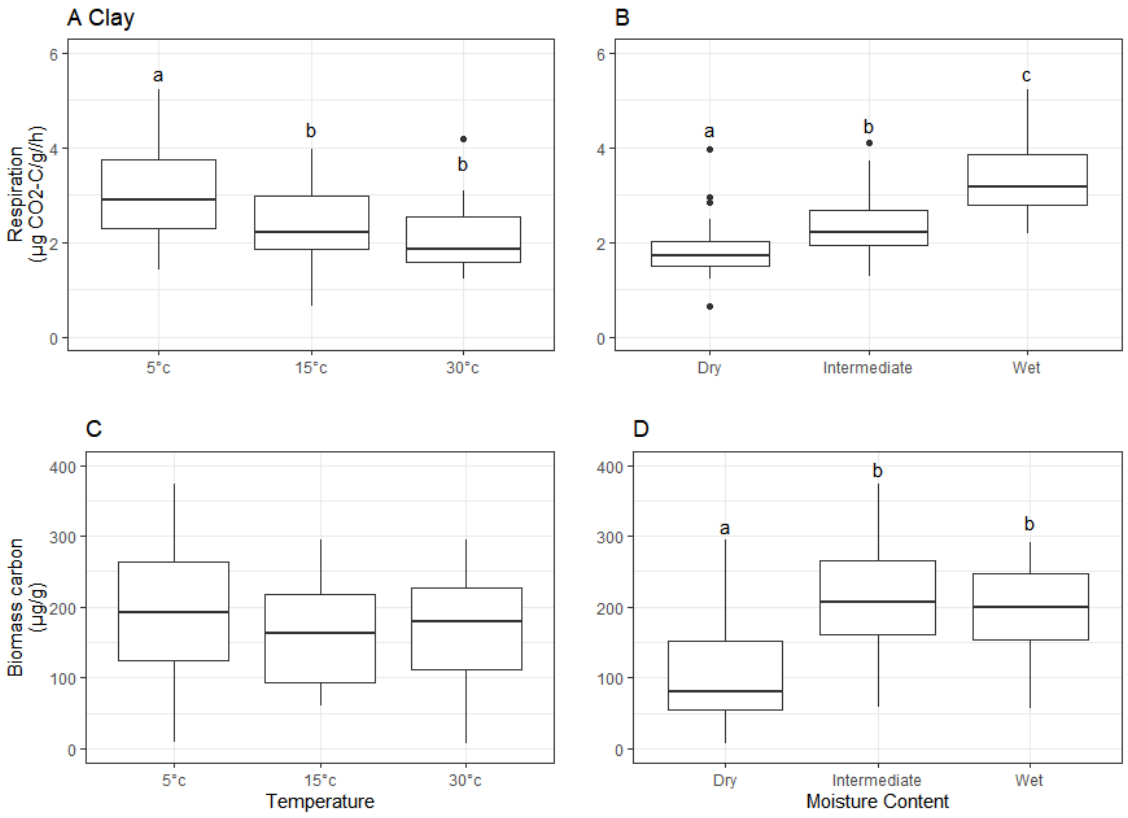

Figure 3.4: Microbial respiration (A, B) and microbial biomass carbon (C, D) by temperature and moisture content for clay aggregates. Different letters signify statistically significant differences.

The relative abundances of gram-positive and gram-negative bacteria were not significantly affected by temperature or moisture content in the sandy loam (Figure 3.5A - D). In the clay, increasing temperature significantly increased gram-positive bacteria abundance, with the relative abundance of gram-positive bacteria significantly higher at 30°C (Figure 3.6A). In contrast, gram-negative bacterial abundance decreased with increasing temperature, as the relative abundance of gram-negative bacteria was significantly higher at 5°C and decreased with increasing temperature (Figure 3.6C). In both soils, moisture content did not significantly influence the relative abundance of gram-positive or gram-negative bacteria (Figures 3.5, 3.6). In the sandy loam, the relative abundance of fungi was significantly higher in the driest treatment, but fungal abundance was not significantly affected by temperature (Figure 3.5E, F). In the

clay samples, temperature and moisture content conditions did not significantly influence fungal abundance (Figure 3.6E, F).

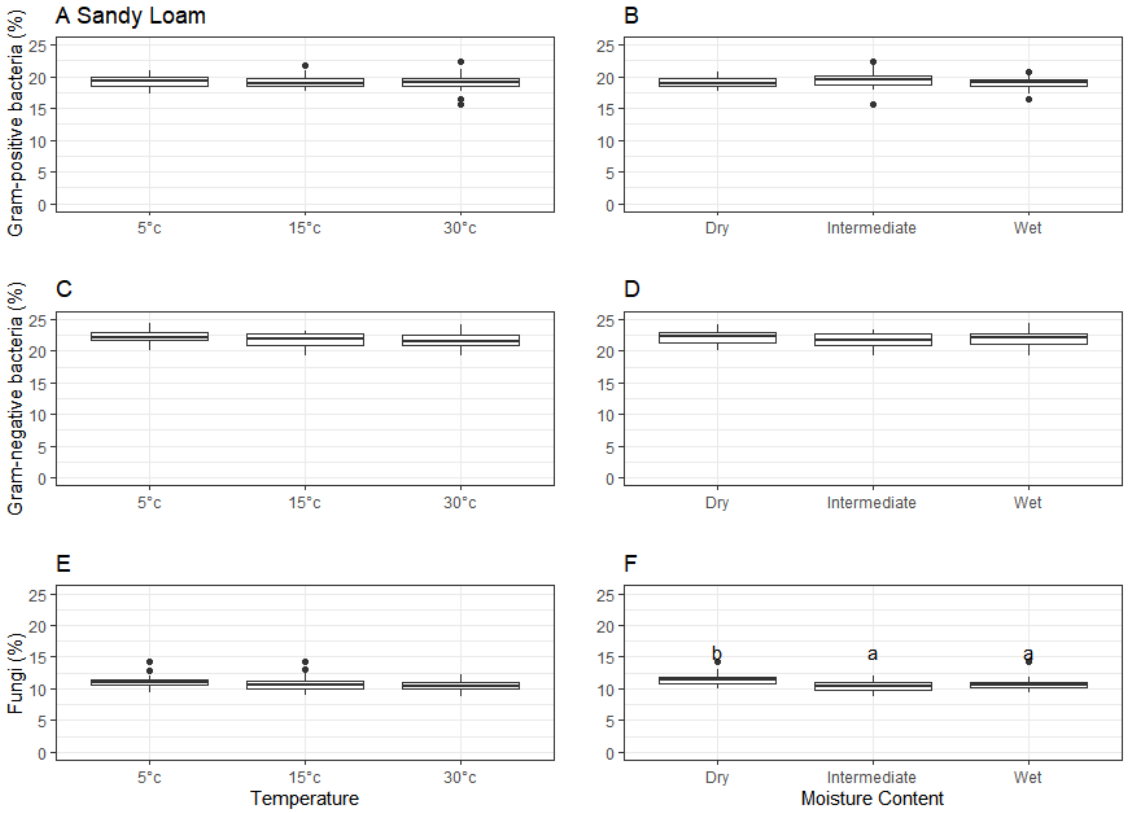

Figure 3.5: The relative abundance (%) of gram-positive bacteria (A, B) gram-negative bacteria (C, D), and fungi (E, F) by temperature and moisture content for sandy loam aggregates. Different letters signify statistically significant differences.

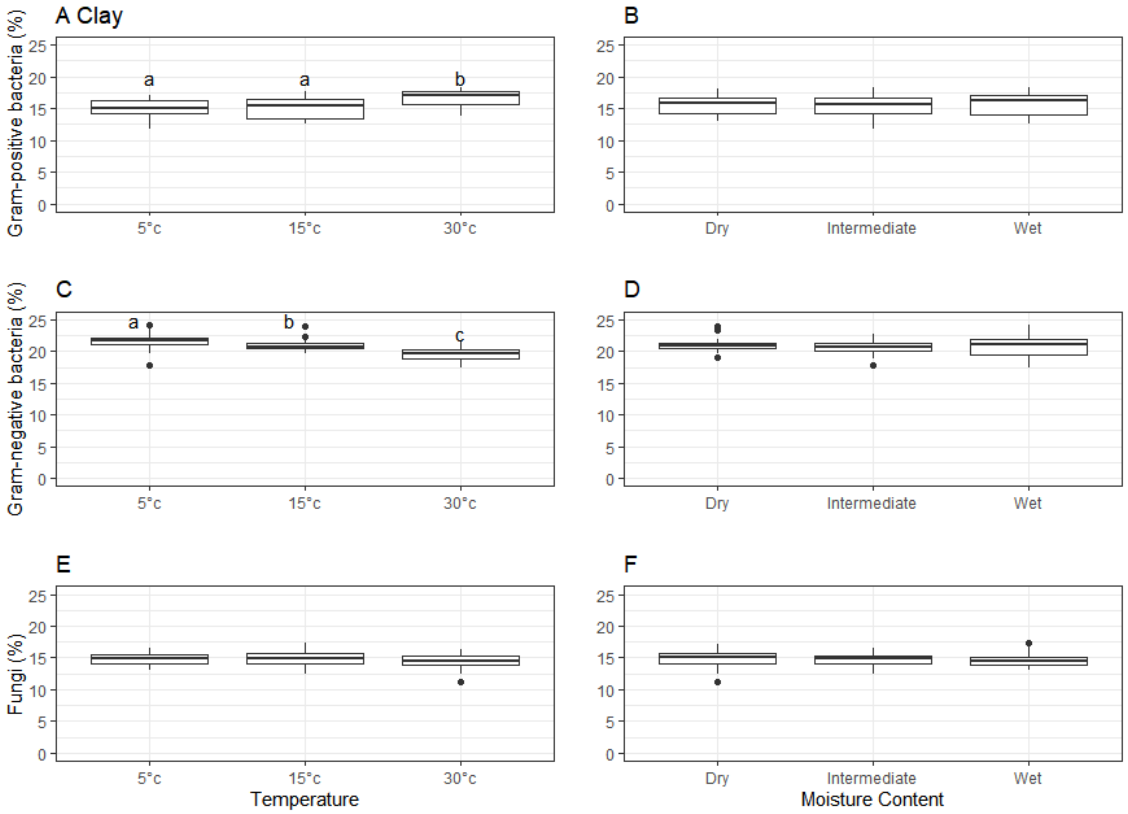

Figure 3.6: The relative abundance (%) of gram-positive bacteria (A, B) gram-negative bacteria (C, D), and fungi (E, F) by temperature and moisture content for clay aggregates. Different letters signify statistically significant differences.

3.3.2.2 Correlation of temperature and moisture content with aggregate stability and microbial parameters

For the sandy loam, aggregate stability was significantly negatively correlated with moisture content, but had no significant correlation with temperature (Table 3.1). Temperature was significantly negatively correlated with the relative abundance of fungi. Moisture content was significantly positively correlated with microbial respiration and significantly negatively correlated with the relative abundance of fungi. Aggregate stability showed significant positive correlations with microbial biomass carbon and relative abundance of gram-negative bacteria.

Table 3.1: Pearson correlation analysis for sandy loam aggregates.

	Aggregate stability (arcsine)	Temperature	Moisture content	Incubation duration	Microbial respiration (µg CO_2-C g^{-1} h^{-1})	Microbial biomass carbon (µg g^{-1})	Gram-positive bacteria (mol%)	Gram-negative bacteria (mol%)
Temperature	0.065							
Moisture	**-0.529*** **	-						
Incubation duration	0.018	-	-					
Microbial respiration (µg CO_2-C g^{-1} h^{-1})	**-0.304** **	-0.213	**0.655*** **	0.192				
Microbial biomass carbon (µg g^{-1})	**0.253***	-0.001	-0.171	0.065	-0.049			
Gram-positive bacteria (mol%)	-0.070	-0.036	-0.050	0.115	-0.157	-0.013		
Gram-negative bacteria (mol%)	**0.273***	-0.112	-0.115	**-0.296** **	0.068	-0.022	-0.151	
Fungi (mol%)	-0.070	**-0.250***	**-0.314** **	0.057	**-0.220***	-0.102	**-0.229***	-0.155

Pearson correlation analysis on the clay showed a significant positive correlation between aggregate stability and temperature (Table 3.2). Contrary to the sandy loam results however, aggregate stability was not correlated with moisture content. Temperature had a significantly negative correlation with microbial respiration and the relative abundance of gram-negative bacteria, but temperature was significantly positively correlated with the relative abundance of gram-positive bacteria. Moisture content had a significant positive correlation with microbial respiration and microbial biomass carbon. Aggregate stability was also shown to be significantly negatively correlated with the relative abundance of gram-negative bacteria.

Table 3.2: Pearson correlation analysis for clay aggregates.

	Aggregate stability (arcsine)	Temperature	Moisture content	Incubation duration	Microbial respiration (µg CO_2-C g^{-1} h^{-1})	Microbial biomass carbon (µg g^{-1})	Gram-positive bacteria (mol%)	Gram-negative bacteria (mol%)
Temperature	**0.373*** **							
Moisture	0.031	-						
Incubation duration	-0.112	-	-					
Respiration (µg CO_2-C g^{-1} h^{-1})	-0.138	**-0.430*** **	**0.657*** **	-0.001				
Microbial biomass carbon (µg g^{-1})	-0.133	-0.104	**0.396*** **	0.056	**0.380*** **			
Gram-positive bacteria (mol%)	0.099	**0.412*** **	0.036	-0.008	-0.130	-0.154		
Gram-negative bacteria (mol%)	**-0.275***	**-0.603*** **	-0.165	-0.059	0.163	0.007	**-0.518*** **	
Fungi (mol%)	-0.047	-0.213	-0.027	0.180	0.098	**0.240***	**-0.522*** **	-0.115

3.3.2.3 Regression analyses

Multiple regression analysis showed that several microbial properties were significant predictors of aggregate stability (Table 3.3). However, there were substantial differences between the sandy loam and clay minimally-adequate models. Aggregate stability was best predicted by microbial respiration, biomass carbon and gram-negative abundance in the sandy loam aggregates (stepwise-regression, adjusted $R^2 = 0.209$, $p < 0.001$), meanwhile the relative abundance of gram-negative bacteria was the sole significant predictor in the clay aggregates (stepwise-regression, adjusted $R^2 = 0.064$, $p = 0.013$).

Table 3.3: Multiple regression analysis for aggregate stability and microbial predictor variables for the sandy loam and clay aggregates, using stepwise method.

	Aggregate stability arcsine (Sandy Loam)			**Aggregate stability arcsine (Clay)**		
Predictors	Coefficient estimates	Std. Error	p-value	Coefficient estimates	Std. Error	p-value
Intercept	0.36	0.22	0.1	1.3	0.14	**<0.001**
Respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)	-0.1	0.03	**0.0025**			
Microbial biomass carbon ($\mu g\ g^{-1}$)	0.0004	0.00016	**0.016**			
Gram-negative bacteria (mol%)	0.029	0.0097	**0.0036**	-0.17	0.0065	**0.013**
Observations	81			81		
R^2 / adjusted R^2	0.239 / 0.209			0.076 / 0.064		
p-value	**<0.001**			**0.013**		

3.4 Discussion

The purpose of this experimental laboratory study was to investigate the influence of soil temperature and moisture conditions on soil aggregate stability and whether this was associated with changes in the microbial community at the aggregate scale. Microbial properties were used as proxies for biological stabilisation (Degens *et al.*, 1996; Tang *et al.*, 2011) due to remaining limitations and inconsistencies in the methods for quantifying both EPS adhesion and hyphal enmeshment (Redmile-Gorden *et al.*, 2014; Costa *et al.*, 2018; though see Redmile-Gordon *et al.*, 2020). The main findings were: (1) pre-rainfall temperature and moisture content conditions influenced aggregate stability, dependent on soil texture, (2) soil temperature and moisture content also influenced the soil microbial community, biomass, and respiration and (3) microbial properties were significant predictors of aggregate stability. This is the first empirical investigation of the influence of temperature and moisture content on microbial response and mediation of aggregate stabilisation at the aggregate scale.

3.4.1 Aggregate stability response to pre-rainfall temperature and moisture

In this study, soil temperature and moisture conditions during incubation, prior to air-drying and rainfall simulation, were expected to affect aggregate stability via the influence of climate-induced changes in the soil microbial community and associated biological stabilisation of aggregates. Physical, chemical, and biological stabilising mechanisms act simultaneously so it is important to account for the physical and chemical stabilisation of aggregates and compare the effects of temperature and moisture on aggregate stability to other studies focussed on the physical and chemical stabilising mechanisms, which do not consider microbially-mediated aggregate stabilisation.

The stability of the clay aggregates significantly increased with increasing temperature of the incubation environment, but temperature did not significantly affect aggregate stability of sandy loam aggregates. The observed significant

increase in aggregate stability with increasing incubation temperature for clay aggregates (Figure 3.2) differs slightly from theory and previously published research. Previous studies focussing on the physical properties of aggregates suggest that theoretically aggregate stability would decrease with increasing temperature as interparticle bonds are weakened through several physical and chemical mechanisms. Principally increased temperature causes an increase in the internal energy of particles resulting in the expansion of entrapped air, and double layers in clay, thereby disrupting interparticle bonds and reducing structural stability (Plum & Esrig, 1969; Dexter *et al.*, 2010; Kelishadi *et al.*, 2018). However, testing these relationships has proven difficult with the conflation of numerous clay properties and confounding factors. The approach used to determine aggregate stability can also lead to differences in observations (Amézketa, 1999; Almajmaie *et al.*, 2017). Empirically in a long-term field experiment, Lavee *et al.,* (1996) investigated aggregate stability along a climatological transect using the single drop test and found aggregate stability was highest under temperatures of 15°C and lower for temperatures between 0-10°C and temperatures exceeding 35°C. This unimodal peak in aggregate stability was suggested to be a combination of the physical effects of temperature and biotic activity, though soil microbial properties were not measured. The significant positive relationship between temperature and clay aggregate stability observed here does not follow the expected trends based on theoretical physical mechanisms of internal energy and air expansion. Here aggregates were air-dried post-incubation and under consistent temperatures during the rainfall simulation. As such the response of aggregate stability cannot be explained solely by considering physical mechanisms and so implies that confounding biotic stabilising mechanisms are active.

The stability of the sandy loam aggregates decreased significantly with increasing moisture content of the incubating environment, but soil moisture condition pre-rainfall did not significantly affect aggregate stability of clay aggregates (Figure 3.2). This treatment effect on sandy loam aggregates is independent and unrelated to the more widely reported impact of moisture content at the time of

rainfall (Cousen & Farres, 1984; Ben-Hur & Lado, 2008; Almajmaie *et al.*, 2017). Moisture content was equilibrated for all treatments by air-drying prior to the rainfall event. Thus, these results indicate that moisture content during incubation affects processes internal to the aggregate that affect its stability under air-dry conditions. The physical effects of soil moisture on aggregate stability are influenced by particle interactions and micropore structure. Clay aggregates have a higher proportion of micropores to macropores than sandy loam aggregates, due to smaller particles with a greater proportion of clay minerals and the arrangement of clay layers influencing pore-size distribution. Micropore structures are more stable than macropore structures, as they are less susceptible to fissures (Peng & Horn, 2005; Borken & Matzner, 2009). Increasing moisture content did not alter aggregate stability for the clay aggregates, suggesting that the more cohesive micropores and strong clay-particle interactions were not disrupted (Borken & Matzner, 2009) and all aggregates were air-dried prior to rainfall for consistent testing conditions.

The positive correlation between aggregate stability and temperature reported for the clay aggregates, and negative correlation between aggregate stability and moisture content of the incubating environment observed in the sandy loam aggregates cannot solely be explained by physical and chemical mechanisms and so can be associated with changes in the microbial community. Several microbial properties showed a high level of covariation and strong correlations with temperature and moisture content conditions (Tables 3.1, 3.2). Biological stabilising mechanisms were assumed to be influenced by changes in microbial community composition, biomass, and activity. Microbial biomass carbon and microbial respiration reflect microbial turnover of carbon and biomass synthesis and growth (Degens *et al.*, 1996) and have often been used as indicators of microbially-mediated processes (Schloter *et al.*, 2003; Ritz *et al.*, 2009; Truu *et al.*, 2009), so have been used here as proxies of microbially-mediated aggregation. For example, previous studies have shown increased fungal biomass correlates with higher aggregate stability, with the positive effect of hyphal growth on particle enmeshment (Degens *et al.*, 1996; Lehmann & Rillig,

2015; Lehmann *et al.*, 2020). However, the roles of microbial respiration, biomass carbon, and involvement of bacterial group abundances in the biological stabilisation of aggregates remain largely undocumented. The effects of soil temperature and moisture on soil microbial community are discussed further in Section 3.4.2, and the possible associations with biological stabilisation and aggregate stability explored in Section 3.4.3.

3.4.2 Microbial community response to temperature and moisture content

It was expected that microbial growth and activity would be limited at relatively cold temperatures and stimulated with increasing temperature until exceeding the microbial optimum temperature range. Microbial respiration post-incubation was highest following the coldest temperature treatment for both soil textures (Figures 3.3, 3.4), which may reflect limited microbial activity at cold temperatures during incubation and thus surplus substrate post-incubation (see Section 3.2.4). Microbial respiration response to temperature is often researched within the context of decomposition and soil carbon (Davidson *et al.*, 1998). Studies often agree that temperature has a key role in regulating decomposition, substrate use and enzyme activity via influencing microbial respiration activity. However, the temperature sensitivity of these processes is uncertain and requires further investigation (Koch *et al.*, 2007; Conant *et al.*, 2011; Moyano *et al.*, 2013). The strong relationship between temperature and microbial respiration was expected to regulate energy availability for biological stabilising mechanisms and therefore aggregate stability. However, higher temperatures increase rates of microbial metabolism and lead to faster consumption and depletion of resources, particularly oxygen, when oxygen resupply is slower than consumption (Moyano *et al.*, 2013). The enhanced oxygen consumption and mineralisation with the subsequent depletion of resources such as substrate at higher temperature during incubation may explain the result observed here; respiration was highest at the lowest temperature post-incubation as substrate had not been over-exploited and mineralised.

Alongside microbial respiration, incubation temperature had a concurrent effect on the relative abundances of microbial groups (Figures 3.5, 3.6), which were significant predictors of aggregate stability (Table 3.3), though temperature did not affect microbial biomass carbon significantly (Figures 3.3, 3.4). In the clay aggregates gram-positive bacteria abundance increased with temperature, while gram-negative bacteria abundance decreased (Figure 3.6, Table 3.2). This pattern is consistent with other studies on the effects of warming on the relative abundances of gram-positive and gram-negative bacteria, which suggest that temperature-induced substrate constraints limit gram-negative bacteria, whilst gram-positive bacteria are better adapted to acquire resources at higher temperatures (Biasi *et al.*, 2005; Feng & Simpson, 2009). Further research is necessary to interpret the effects of soil temperature on microbial physiological processes and thermotolerance and resource adaptation. These results indicate that the relative abundances of microbial groups, and microbial respiration, are affected significantly by temperature, either as a catalyst for microbial activity or environmental stress and exhibit interactions with the physical and chemical properties encapsulated in the two soil textures.

Moisture content conditions influence numerous soil properties critical for microbial life, including soil hydration status, oxygen availability, pore connectivity, bacterial motility and thus resource accessibility. Microbial respiration rates were found to decrease with decreasing moisture content in both soil textures (Figures 3.3, 3.4, Tables 3.1 – 3.2). This observed trend has been reported in other research and attributed to the low soil water content creating conditions with reduced pore-water connectivity, subsequently decreasing solute diffusion and therefore limiting substrate supply for microbes (Skopp *et al.*, 1990; Or *et al.*, 2007a; Moyano *et al.*, 2013). Though it has also been reported that under saturated conditions, low oxygen content limits microbial activity and has caused a decrease in decomposition rates (Stres *et al.*, 2008), but this was not observed in this study as the aggregate surface was not oxygen limited. The observed significant increase in microbial biomass with increasing moisture content in clay aggregates (Figure 3.4, Table 3.2) suggests that wet conditions

improved resource accessibility enabling microbial growth as expected, although this was not the case in the sandy loam aggregates (Figure 3.3, Table 3.1). Microbial tolerance to environmental stress caused by moisture content altering physical properties, such as osmotic pressure, pore connectivity and substrate availability, varies between microorganisms and species. Moisture content and associated changes in soil properties were expected to drive compositional changes within the microbial community. Under dry conditions fungi may be more tolerant than bacteria as fungal hyphae can bridge air-filled pores to maintain connection with water-filled micropores and soluble substrates (Jennings, 1987; De Boer *et al.*, 2005), while bacteria rely on a connected aqueous environment for motility, substrate diffusion and resource accessibility (Or *et al.*, 2007a; Lennon, 2012). This is supported in this study, as relative fungal abundance in the sandy loam aggregates significantly increased with decreasing moisture content (Figure 3.5, Table 3.1). Soil moisture content also affects osmotic pressure, with a direct impact on physiological microbial processes. Bacterial groups have varying tolerance to osmotic stress due to differences in osmoregulatory responses and cell wall structures (Csonka, 1989; Wood, 2015). Gram-positive bacteria have a thicker cell wall than gram-negative bacteria and so are expected to have greater resistance to osmotic pressure under dry conditions (Csonka, 1989). However, this was not supported in this study as moisture content during the incubation did not significantly affect the relative abundances of gram-positive or gram-negative bacteria in both soil textures (Figures 3.5, 3.6).

Our study provides evidence that temperature and moisture content can shift the soil microbial community composition and affect microbial biomass carbon and respiration in aggregates, thereby influencing microbially-mediated aggregate mechanisms and aggregate stability overall. Consistently in the sandy loam and clay aggregates, microbial respiration after incubation was negatively correlated with temperature during incubation and was positively correlated with moisture content. However, the response of microbial biomass carbon and composition to temperature and moisture content and their subsequent influence over aggregate

stability was strongly affected by soil texture (Figures 3.3 – 3.6). The environmental pressures associated with changes in moisture content and temperature were expected to depend on soil texture due to variations in physical properties and microstructure, thereby mediating soil-specific microbial response. Alternatively, the differing response between soil textures could be due to variations in the initial microbial community of each soil type, which should be considered in future studies. The variation in microbial responses between the two soil textures is evidence of the continual interactions between microbial and physico-chemical characteristics specific to soil texture.

3.4.3 Microbial community parameters used as proxies for biological stabilising and destabilising aggregate mechanisms

From the review of previous literature, it was expected that temperature and moisture content would drive changes in the microbial community and either stimulate or limit microbial growth and activity, dependent on the microbial optimal range of environmental conditions (Castro *et al.*, 2010; Moyano *et al.*, 2013). It was hypothesised that an increase in microbial activity and growth would enhance biological stabilisation and therefore aggregate stability. Furthermore, shifts in the microbial community were expected to alter the operation and dominance of biological stabilising mechanisms, with fungi stabilising aggregates through hyphal enmeshment and the production of fungal exudates and bacteria through EPS production and particle adhesion (Degens *et al.*, 1996; Ritz & Young, 2004; Blankinship *et al.*, 2016). As such, the response of the microbial community to temperature and moisture treatments was expressed as changes in community composition, biomass, and respiration as indicators of change in biological stabilising mechanisms, such as hyphal enmeshment and EPS particle adhesion.

Aggregate stability was significantly affected by temperature and soil moisture during the incubation phase, though these relationships cannot be solely explained by physico-chemical stabilising and destabilising mechanisms (Section 3.4.1). At the aggregate scale, temperature and moisture content influenced the

microbial community parameters measured, showing the potential for rapid changes in the microbial community (Section 3.4.2). Therefore, the changes observed in aggregate stability with temperature and soil moisture can be associated with changes in microbial properties and related shifts in biological stabilising mechanisms. Aggregate stability in the sandy loam aggregates, which was positively influenced by soil moisture during the incubation phase, was best predicted by microbial respiration, microbial biomass carbon and gram-negative bacteria abundance (Table 3.3). Furthermore, in statistical analyses, gram-negative bacterial abundance was identified as a significant predictor of clay aggregate stability (Table 3.3). This evidence suggests that a rapid shift in microbial community, activity, and growth in response to changing climate conditions has the potential to quickly alter microbially-mediated aggregate stabilisation and overall aggregate stability.

Notably the relationship between the relative abundance of gram-negative bacteria and aggregate stability was positive for sandy loam but negative for clay (Table 3.3). The positive relationship observed for the sandy loam aggregates suggests gram-negative bacteria enhanced the biological stabilisation of aggregates, despite there not being significant responses between this bacterial group and the treatment levels (Figure 3.5). In the clay aggregates, increasing temperature of the incubating environment increased aggregate stability and favoured gram-positive bacteria but reduced the relative abundance of gram-negative bacteria (Figure 3.6), suggesting a shift in the roles of bacterial groups in biological stabilisation. Further investigation is required to better relate microbial characteristics to stabilising functions and better understand how changes in the composition, activity, and growth of the microbial community influence microbial stabilisation of soil aggregates. Therefore, it is recommended that further research on climatic impacts on soil erodibility continue their work at the aggregate scale with further characterisation of the stabilising structures and compounds. Here using microcosms of individual aggregates, this research has begun to assess temperature and moisture effects at a mechanistic scale. This study has shown the complex influence of temperature and moisture content on

microbial community parameters, aggregate stability, and the interaction of soil texture. In order to develop this understanding, the interconnectivity between biological processes and physico-chemical environment must be considered at a scale relevant to microorganisms and aggregate stabilisation.

3.5 Conclusions

In this study, temperature and moisture incubation treatments significantly affected soil aggregate stability, but the effects varied with soil texture. In sandy loam aggregates, increasing moisture content during the incubation phase negatively affected air-dry aggregate stability under rainfall, while temperature of the incubating environment had no effect. Contrastingly, in clay aggregates moisture content during the incubation phase had no effect on aggregate stability, while aggregates were less stable at higher incubating temperatures. Whilst physical or chemical mechanisms might be contributing to overall aggregate stability, the biological evidence suggests that the response of aggregate stability to temperature and moisture pre-treatments was microbially mediated. In both soil textures, temperature and moisture content were drivers for changes in microbial community composition and respiration rates. Shifts in microbial community composition were specific to soil texture, demonstrating an interaction between soil physical properties and the microbial community. Climatic conditions, in terms of soil temperature and moisture content, affected the composition, growth and activity of the soil microbial community. Aggregate stability is a dynamic soil property that varies under climate treatments. This evidence suggests that the climate-induced changes in aggregate stability, and inversely soil erodibility, may be associated with the effects of temperature and soil moisture on the microbial community and potentially the biological stabilising mechanisms. The implications of climate-driven microbial feedbacks and aggregate stability dynamics must be explored in greater detail and at larger spatial scales to consider the implications for soil erodibility and erosion.

3.6 Acknowledgements

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4 The influence of seasonal soil temperature and moisture treatments on soil aggregate stability: an experimental investigation

Abstract

Soil erodibility, the susceptibility of soil to erosion, is an important factor determining erosion rates, but is often considered as a constant value based on static soil properties such as soil texture. However, aggregate stability, the key indicator for erodibility, is a dynamic soil property and has been observed to change seasonally with local climatic conditions. Seasonal dynamics of aggregate stability are not fully understood, and explanations often only consider physico-chemical mechanisms. The microbial community is well known to affect aggregate stability and is also affected by climatic conditions, so climate-induced changes in the microbial community may explain shifts in aggregate stability. The aim of this work is to investigate the effects of seasonal conditions, based on current and future climate scenarios, on aggregate stability, and whether this relationship is provoked by climate-induced changes in the soil microbial community. An experimental investigation using environmental chambers and rainfall simulation examined the effects of pre-rainfall soil temperature and moisture on air-dry aggregate stability under simulated rainfall and the soil microbial community in seasonal treatments (summer followed by winter) and constant seasons (summer-only, and winter-only) based on climate scenarios (current, moderate and high). Treatments were conducted with two soil textures (loamy sand and clay) in single layer aggregate microcosms to investigate the influence of physico-chemical properties and examine the internal aggregate response. Climate scenarios in the seasonal treatments significantly affected microbial properties in clay aggregates but did not affect air-dry aggregate stability significantly, suggesting that the magnitude of change in microbial properties did not meet a critical threshold to induce shifts in aggregate stability. Air-dry aggregate stability was significantly lower in the seasonal treatments than in the constant seasons (summer-only and winter-only). Loamy sand aggregate

stability was approximately 50% in the seasonal treatments, 10% lower than in the constant seasons, while clay aggregate stability was approximately 87% compared to over 90% in the constant seasons. Seasonal treatments and constant seasons significantly affected microbial respiration, biomass, and community composition. Microbial biomass and post-incubation microbial respiration were two to three times higher in the winter-only treatments than the summer-only treatments, and biomass was further stimulated under the seasonal treatments. These changes in the microbial community had a collective influence on biological stabilisation and aggregate stability.

4.1 Introduction

Changes in soil erodibility due to shifts in soil temperature and moisture under seasonal patterns are rarely examined mechanistically, particularly at the aggregate scale. Aggregate stability exhibits a strong inverse relationship with soil erodibility, so is often used to predict soils' susceptibility to erosion (Bryan, 1968; Cousen & Farres, 1984; Cantón *et al.*, 2009). To ensure the future protection of soil and continued provision of essential soil ecosystem services, it is critical to understand how seasonal and climatic changes affect aggregate stability, and thus soil erodibility and erosion rates.

According to climate projections (UKCP09, and more recently UKCP18), climate change will lead to warmer, wetter winters, and hotter, drier summers in the UK (Jenkins *et al.*, 2009; Lowe *et al.*, 2018; Met Office, 2019). These predicted changes in seasonal conditions will directly impact future soil temperatures and moisture contents in the UK, so influencing multiple soil physico-chemical properties (e.g. soil oxygen concentration, saturation of soil pores, and pore-water connectivity) and the microbial communities living within the soil. In turn, these are key factors influencing soil structural development via the microbial community and biotic activity, thereby influencing biogeochemical processes, such as organic matter turnover and nutrient cycling (Bronick & Lal, 2005; Stres *et al.*, 2008; Blankinship *et al.*, 2011; König *et al.*, 2020). Whilst the inherent properties of the soil (e.g. soil texture, mineralogy) generally do not change over the decadal timescales of climate change, the biological community and the stability of soil structure are dynamic and shift in response to climatic conditions over much shorter (e.g. seasonal) timescales (Deviren Saygin *et al.*, 2018).

Aggregate stability and soil erodibility are known to vary seasonally during the year and interannually (Blackman, 1992; Bryan, 2000; Aksakal *et al.*, 2019), but the underlying factors causing those variations are not well understood. While local climatic conditions are often cited as a possible reason for variation in aggregate stability, evidence and mechanistic studies are lacking (Bullock *et al.*, 1988; Sanchis *et al.*, 2008; Dimoyiannis, 2009). An area of uncertainty in

predicting how a changing climate will affect aggregate stability is the response of the microbial community and climate-sensitive biological stabilising mechanisms. Microbes mediate aggregate stability through the enmeshment of particles by fungal hyphae (Bronick & Lal, 2005; Rillig & Mummey, 2006) and the production of stabilising agents such as extracellular polymeric substances (EPS), which increase particle adhesion and can increase soil hydrophobic properties (Ritz & Young, 2004; Lennon, 2012; Guo *et al.*, 2018).

Changes in microbial community properties, such as community composition, respiration, and biomass, can be useful indicators of shifts in microbial aggregation and thus aggregate stability (Chapter 3), and have often been used as indicators of change in microbially-mediated processes (Schloter *et al.*, 2003; Truu *et al.*, 2009). Previous studies have reported a significant relationship between aggregate stability and microbial properties, such as composition, biomass, and respiration (Perfect *et al.*, 1990a; Tang *et al.*, 2011). Soil organisms differ in their functional traits, so changes in community composition and the relative abundance of organisms which regulate a specific process, such as biological stabilisation, will directly affect the rate of the process, i.e. aggregate stabilisation (Griffiths & Philippot, 2013; Classen *et al.*, 2015). Changes in microbial respiration and biomass may also affect microbially mediated aggregate stabilisation by influencing the allocation of resources and energy for hyphal biomass and EPS production (Or *et al.*, 2007b; Moyano *et al.*, 2013). Climate change, by impacting soil temperature and moisture content, will alter soil microbial community composition and relative abundances due to variations in microbes' physiology, temperature sensitivity and growth rates (Castro *et al.*, 2010; Gray *et al.*, 2011; Classen *et al.*, 2015). Microbial metabolism, and thus microbial respiration and biomass, are temperature sensitive (Brown *et al.*, 2004; Clarke, 2006). It is generally expected that elevated temperatures, within an optimal range, will increase microbial metabolic rates and therefore, stimulate microbial activity and growth, providing resources are not limiting (Pietikäinen *et al.*, 2005; Classen *et al.*, 2015). Changes in soil moisture content also affect microbial activity by altering the soil microenvironment and therefore influencing

oxygen availability, osmotic pressure and stress, microbial motility and resource accessibility (Csonka, 1989; Skopp *et al.*, 1990; Lennon, 2012). Therefore, the impact of climatic conditions on aggregate stability may be explained via their effects on the microbial community composition, respiration, and biomass and thus influence on the activity and effectiveness of biological stabilising (and destabilising) mechanisms. It is unclear how seasonal conditions (i.e. summer and winter) influence the microbial community and affect microbially-mediated aggregate stabilisation, and so uncertainty remains on the implications of future changes in seasonal soil temperature and moisture on aggregate stability with climate change.

Observations of aggregate stability have suggested stabilities are lowest during the winter and increase during spring and summer (Bullock *et al.*, 1988; Blackman, 1992; Dimoyiannis, 2009), and have linked this pattern to seasonal changes in climatic conditions, organic amendments, and land management, which induce physico-chemical stabilising and destabilising mechanisms (Bullock *et al.*, 1988; Abiven *et al.*, 2009). Despite evidence of the effects of soil temperature and moisture content in shaping microbial community properties (Evans & Wallenstein, 2012, 2014), the role of the soil microbial community in mediating the seasonal pattern of aggregate stability has generally not been considered. Summer conditions may stimulate microbial activity due to increased soil temperature and thus increase aggregate stability, or summer conditions could repress soil microbes due to low soil moisture content (Stark & Firestone, 1995) and temperature which exceeds the microbial optimal range. The effects of the summer may then influence the impact of the subsequent physical perturbation of rewetting on the soil microbial community during the following winter. Furthermore, studies suggest that previous environmental conditions, such as the frequency and intensity of drying-rewetting cycles, influence the response of the microbial community, in terms of composition, activity, growth and function, to environmental factors (Fierer & Schimel, 2002; Fierer *et al.*, 2003; Evans & Wallenstein, 2012). More information is needed on the relationship between seasonal changes in climatic conditions, the microbial response and

microbially-mediated stabilisation of aggregates at the aggregate scale to better understand the response of aggregate stability, and therefore soil erodibility, to seasonal climatic patterns and climate change.

Empirical evidence on microbial and aggregate response to changing climatic conditions from laboratory and field studies is often inconsistent, due to variations in soil type and texture, site characteristics, initial conditions and applied methodologies (Amézketa, 1999; Strickland & Rousk, 2010). For example, soil texture is closely associated with physico-chemical soil properties (e.g. particle size distribution, clay content, porosity) which affect the soil microbial community and aggregate stability. Previous studies have investigated aggregate stability and microbial response to environmental conditions in short-term laboratory experiments, often measuring soil properties at intervals over 14 to 74 days (Bossuyt *et al.*, 2001; Denef *et al.*, 2001; Fierer *et al.*, 2003; Tang *et al.*, 2011). Relatively short timescales are often used in laboratory experimental studies with the advantage of high control and reproducibility, and are well justified with the naturally rapid generation time of microbes which allows for the observation of multiple generations (Jessup *et al.*, 2004; Hooper *et al.*, 2005). Whilst microbial response to conditions may be rapid, there is uncertainty on the timescale of aggregate formation, stabilisation, and turnover. The influence of the duration of the incubation period is rarely considered, particularly with the activity of microbes at multiple biotic scales. These methodological challenges, coupled with the diversity of soil microorganisms and thus variation in microbial responses, has led to conflicting evidence as to how soil temperature and moisture content directly affect soil microbial activity and aggregate stability (Strickland & Rousk, 2010).

The aim of the study was to determine the effects of seasonal treatments (summer followed by winter conditions, based on predicted emissions scenarios) on aggregate stability and the microbial community. Microbial community composition, respiration, and biomass synthesis have often been used as proxies to monitor changes in microbially-mediated processes (Schloter *et al.*, 2003; Truu *et al.*, 2009), and they are closely related to fungal hyphae biomass and EPS

production, and therefore the microbial stabilisation of aggregates. As such the stimulation or limitation of the microbial community is expected to explain a concomitant increase or decrease in aggregate stability. Using laboratory microcosm experiments, aggregate stability and the microbial community were investigated in soils incubated under summer-winter conditions over different durations. As aggregate stability and the microbial community can be influenced by physico-chemical properties, the effects of climatic conditions are compared for two soil textures. To ascertain possible effects of incubation duration two different timescales will also be studied. The following hypotheses were tested:

1) Aggregate stability will change under seasonal treatments (i.e. summer followed by winter conditions) due to the impacts of soil moisture and temperature on the microbial community, with stability greatest under the moderate seasonal treatment where an increase in temperature and increased winter moisture content will stimulate the microbial community. Under the high seasonal treatment increases in temperature and winter soil moisture will exceed optimal conditions, thereby limiting microbial activity and inducing microbial community composition change.
2) Seasonal treatments (i.e. summer followed by winter) will influence the soil microbial community properties affecting aggregate stability to a greater extent than constant seasons (i.e. summer-only or winter-only).
3) The duration of soil incubation period (two versus four weeks) will not alter the response of the microbial community and therefore aggregate stability as two weeks is sufficient time to observe shifts in microbial processes.

4.2 Materials and methods:

4.2.1 Soil collection and preparation

Surface soil (0–150 mm) was collected from Silsoe Experimental Farm (Bedfordshire, UK, National Grid Reference TL075356/TL075351) in November 2018. The soil was classified as a Cambisol according to the World Reference Base (WRB, 2007). Two soil textures were sampled, a loamy sand from the Bearsted series (3.9% organic matter, 87% sand, 2% silt, 11% clay) and a clay from the Evesham series (7.1% organic matter, 14% sand, 12% silt, 74% clay) to compare the influence of physico-chemical properties on aggregate stability and the microbial community. At the time of sampling fields were in stubble post-harvest and the soil was wet but not saturated. Soil was broken apart by hand and plant material removed. The soil was then air-dried and sieved to 2–5.6 mm to obtain aggregates. Microcosms consisted of 25g of aggregates spread across a 2 mm aperture mesh in a holding hoop. The microcosm structure was enclosed in a container (185 x 130 x 45 mm, l x w x d) with a wetting bed to control moisture content and placed in an environmental chamber to control temperature. A uniform amount of water was added to the wetting bed dependent on the treatment. The aggregates wetted up by capillary action, however actual moisture content varied with soil texture due to differences in soil physical properties.

4.2.2 Seasonal treatment incubation

Microcosms were incubated under three seasonal (summer – winter) treatments, representing current conditions and two future climate scenarios characterized by a moderate and a high increase in temperature in summer and winter and increased winter soil moisture content (Table 4.1). These future seasonal treatments were based on the climate projections under medium and high CO_2 emissions scenarios respectively from UKCP09 (Jenkins *et al.*, 2009), these were superceded by UKCP18 projections which give similar predictions, see Appendix B.1. The current climate treatment was based on the average summer and winter conditions for the UK. The aim was to generate soil moisture contents in line with current conditions and future projections with summer conditions at low moisture

content with limited water available and winter conditions at field capacity for the current climate treatment and approaching saturation in the future seasonal treatments. However, due to intrinsic heterogeneity and variability in the soil and the necessity of methodological maintenance, pilot conditions were tested. Summer moisture conditions were maintained with air-dry aggregates and uniform amounts of water selected to maintain winter moisture content (Table 4.1). Aggregates were saturated during the winter of the high seasonal treatment. All seasonal treatments started incubation under summer conditions (warm and dry) first, followed by winter conditions (cold and wet).

The effects of a constant season (summer-only or winter-only) were compared to two seasons (summer-winter), shown in Figure 4.1, with solid lines showing seasonal summer-winter treatments and dashed lines showing summer-only and winter-only treatments. To allow the investigation of soil temperature and moisture content at the aggregate scale in laboratory conditions, seasonal timescales were accelerated. To test the effects of the duration of incubation period and accelerated timescales, two incubation durations were tested, lasting a total of two weeks (one week summer, followed by one week winter conditions) and four weeks (two weeks summer, followed by two weeks winter conditions). Constant seasons of summer-only or winter-only were also incubated for two weeks and four weeks (Figure 4.1).

Table 4.1: Soil temperature and moisture content treatments for loamy sand and clay aggregates during summer-winter incubations under three different climate scenario treatments.

Climate scenario treatments	Corresponding future climate projection	**Temperature and moisture treatments**			
		Summer temperature and moisture content		**Winter temperature and moisture content**	
		Loamy sand	Clay	Loamy sand	Clay
Current	n/a	15°C, 3%	15°C, 7%	5°C, 18% (+40ml)	5°C, 50% (+40ml)
Moderate	medium emissions from UKCP09	23°C (+8°C), 3%	23°C (+8°C), 7%	8°C (+3°C), 28% (+60ml)	8°C (+3°C), 61% (+60ml)
High	high emissions scenario from UKCP09	30°C (+15°C), 3%	30°C (+15°C), 7%	10°C (+5°C), 39% (+80ml)	10°C (+5°C), 63% (+80ml)

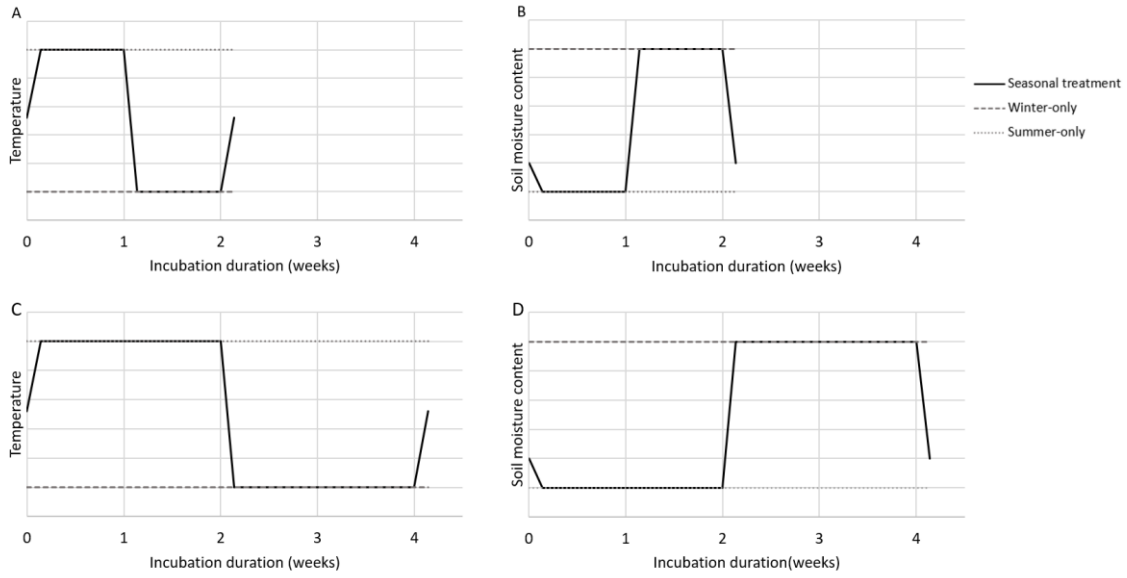

Figure 4.1: Illustrative figure of seasonal treatments (solid line) for summer-winter temperature (left) and soil moisture content (right) conditions over a two-week (top) and four-week incubation (bottom), with constant summer-only and winter-only treatments (dashed lines).

4.2.3 Rainfall simulation and aggregate stability

Aggregate stability was calculated as the proportion of aggregates retained on the microcosm mesh after being subjected to a simulated rainfall event, which was used for all seasonal treatments to hold rainfall erosivity (and its effects on aggregate breakdown) constant. A 9m gravity-fed rainfall simulator was used to generate an event with an intensity of 33 mm hr^{-1}, duration of 5 minutes and median drop size of 2.65 mm. Moisture content at the initiation of the rainstorm will affect aggregate stability, so to test the influence of incubation treatments only (rather than antecedent moisture content), aggregates were air-dried for 48 hours post incubation but pre rainfall to standardise moisture content prior to the rainfall event. Aggregates were then weighed before the rainfall event and air dried again after the rainfall event for 48 hours and reweighed, allowing the calculation of aggregate stability (%).

4.2.4 Microbial biomass carbon

Microbial biomass carbon was extracted by chloroform fumigation based on the method by Vance *et al.,* (1987). Samples were collected post-incubation and pre-rainfall. For each experimental replicate, the wet weight equivalent to 12.5 g air-dried soil was weighed in to a 50 ml Duran bottle and fumigated in a vacuum-sealed desiccator with 25 ml chloroform for 24 hours. Fumigated samples were paired with non-fumigated replicates as a control. Dissolved carbon was extracted from the paired samples by adding 50 ml of 0.5M potassium sulphate and shaking for 30 minutes before filtration with Whatman filter papers. The concentration of dissolved carbon was evaluated on a segmented flow analyser with a calibration curve from known standards. Microbial biomass carbon was estimated as the concentration of the fumigated extract minus non-fumigated extract with a conversion factor of 0.45.

4.2.5 Microbial respiration

Microbial respiration was measured using the rapid automated bacterial impedance technique (RABIT) methodology (Ritz *et al.,* 2006). For each experimental replicate, triplicates of 1g of soil were weighed into glass boats and

sealed in RABIT cells with potassium hydroxide agar. RABIT cells were kept at 25°C and electrical conductance measured between two and four hours enabling the calculation of the rate of respiration. Failed test cells were removed from the dataset and outliers identified as 1.5 times the interquartile range greater or lower than the upper and lower quartiles respectively.

4.2.6 Microbial community composition

Microbial community composition was assessed by phospholipid fatty acid (PLFA) analysis based on the Bligh and Dyer extraction method (Bligh & Dyer, 1959; Frostegård & Bååth, 1994) and measured by gas chromatography (GC-FID). For PLFA extraction, 20 ml of Bligh and Dyer solution was added to 10g of aggregates per sample in clean glass vials with PTFE lined caps. Samples were sonicated in a water bath for 30 minutes and then centrifuged. The upper layer was poured into a clean glass vial with 4 ml each of chloroform and citrate buffer added and centrifuged a second time. A vacuum pump was used to remove the upper layer and the sample was dried under nitrogen. Solid phase extraction was then used to isolate the phospholipids as follows. The SPE column was washed with 4 ml chloroform and was loaded with the sample in 2 ml chloroform. Non-target lipids were eluted with 5 ml of chloroform and 12 ml acetone. Phospholipids were then eluted with methanol and dried under nitrogen. The extracts were then methylated with 1 ml of 1:1 toluene:methanol and 1 ml of potassium hydroxide in methanol at 37°C for 30 minutes. The reaction was stopped by adding 0.25 ml of acetic acid, 5 ml 4:1 hexane:chloroform and 3 ml deionised water, and centrifuged. The organic (upper) phase was filtered through glass wool, dried under nitrogen and resuspended with hexane ready for analysis by GC-FID. Peaks were identified by retention time and calculated as a percentage of the total area of identified peaks. Relative abundances of fungi, gram-negative bacteria and gram-positive bacteria were estimated by combining peak areas into biomarker groups. Fungi were represented by PLFAs 16:1ω5, 18:2ω6, cis18:1ω9 (Frostegård *et al.,* 1993, 1994, 2011), while gram-negative bacteria were represented by trans16:1ω11, cis16:1 ω7, cyc17:0iso, cis17:1ω8, cis17:0, trans17:1ω8, cis19:0, and gram-positive bacteria by i15:0, ai15:0, i16:0, 17:0

i17:0 and ai17:0 (Bardgett *et al.,* 1996; Zelles, 1999; Ruess and Chamberlain, 2010). Other PLFAs measured included 14:0, 15:0, i16:1, 16:0, Me17:0iso, Me17:0iso2, cyc17:0iso. 17:0br, 17:1ω 7, 17:0(12Me), 18:1ω7t, 18:1ω13, 18:0, 19:1ω6, 18:0(Me), 20:4(5,8, 4, 11,14), 20ω5(3), 20:0.

4.2.7 Statistical analyses

Analysis of variance and Tukey's HSD testing was used to assess significant differences in aggregate stability, microbial biomass carbon, microbial respiration and relative abundances of fungi, gram-negative and gram-positive bacteria. Principal component analysis (PCA) of PLFA profiles was used to visualise microbial community composition, while ANOVA analysis was conducted on the relative abundances of PLFA biomarker groups described above.

4.3 Results

There was no significant difference in aggregate stability between the two week and four week incubation periods (data not shown). However, these treatments have been analysed separately as the datasets are methodologically discrete samples. Microbial response varied by incubation duration, although often similar trends were observed. This suggests the processes mediating microbial response and aggregate stability are operating similarly over two and four week incubations.

4.3.1 Seasonal treatments (current versus moderate and high scenarios)

There was no statistically significant difference in aggregate stability between the three seasonal treatments in either soil texture (Figure 4.2). Overall, clay aggregates were significantly more stable than loamy sand aggregates and had a lower variability in their stability results (ANOVA, $p<0.001$, F-test, $p<0.001$).

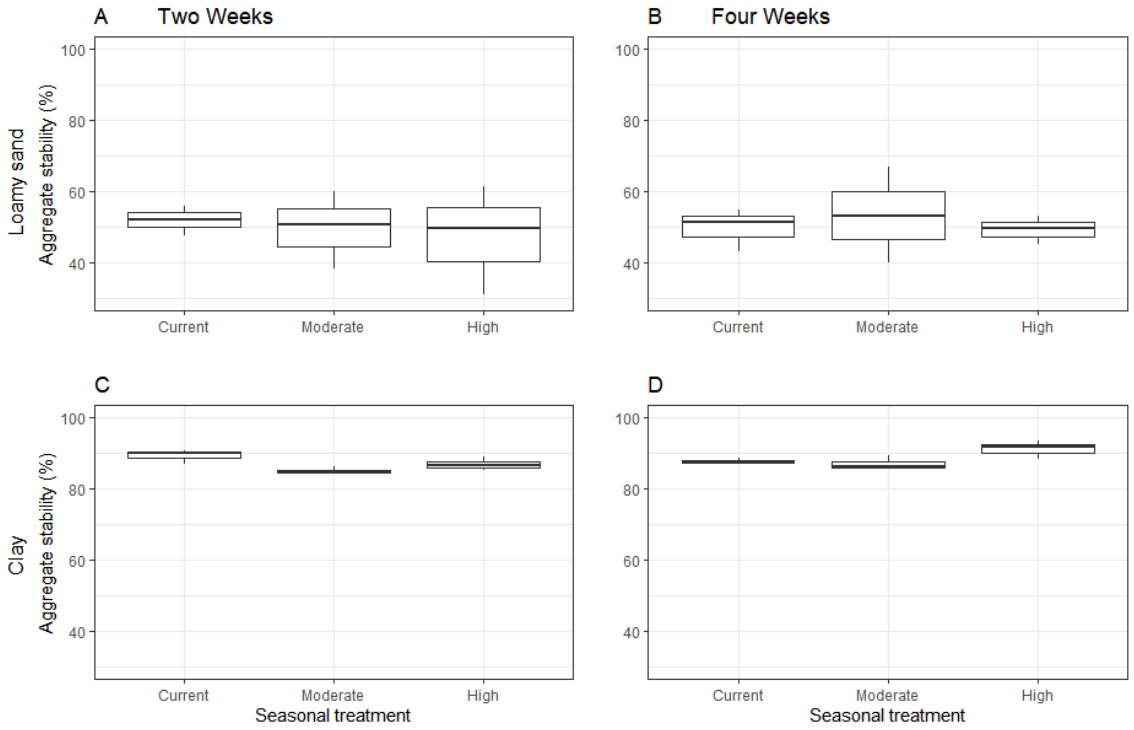

Figure 4.2: Aggregate stability by seasonal treatments representing the current and future climate scenarios for two soil textures: loamy sand (A, B) and clay (C, D), over two incubation periods (left: two weeks; right: four weeks).

The response of microbial respiration and microbial biomass carbon to the seasonal treatments differed by soil texture, with significant differences only observed for clay aggregates (Figure 4.3). In clay aggregates, microbial respiration significantly increased with higher temperature and winter moisture content in the moderate and high seasonal treatments in the two week incubation (Figure 4.3C) with respiration rates two times higher and three times higher than in the current seasonal treatment for the moderate and high seasonal treatments respectively. A similar trend was observed for clay incubated over four weeks, with respiration greatest under the high seasonal treatment (Figure 4.3D). Microbial biomass carbon was also significantly greater under the high seasonal treatment in both incubation periods (Figure 4.3G, H). In loamy sand aggregates microbial respiration and microbial biomass carbon were not significantly different between seasonal treatments (Figure 4.3A, B, E, F).

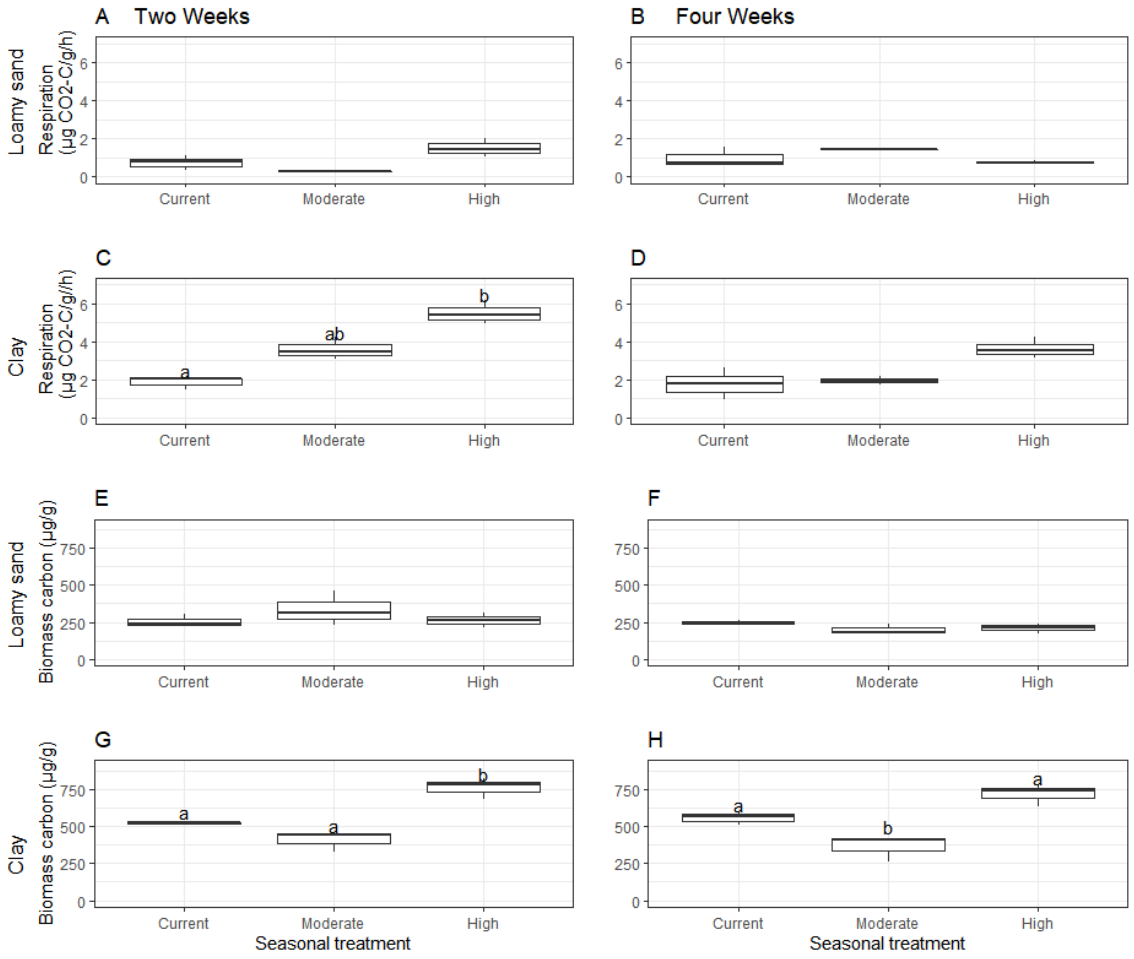

Figure 4.3: Microbial respiration (A-D) and microbial biomass carbon (E-H) under seasonal treatments for loamy sand and clay aggregates incubated for two weeks (left) and four weeks (right).

The effects of seasonal treatments on the microbial community composition differed between soil textures and incubation period, with shifts in the microbial composition observed only in the clay aggregates (Figure 4.4). Here, after two weeks incubation the microbial community was significantly different under the high seasonal treatment compared with the current and moderate seasonal treatments, differentiated by both PC1 and PC2 (Figure 4.4C). After four weeks the microbial community compositions under the moderate and high seasonal treatments were distinct from the current climate treatment and significantly differentiated by PC1 (Figure 4.4D). Therefore for the clay soil, under both

incubation periods, the composition of the microbial community was influenced by seasonal treatment.

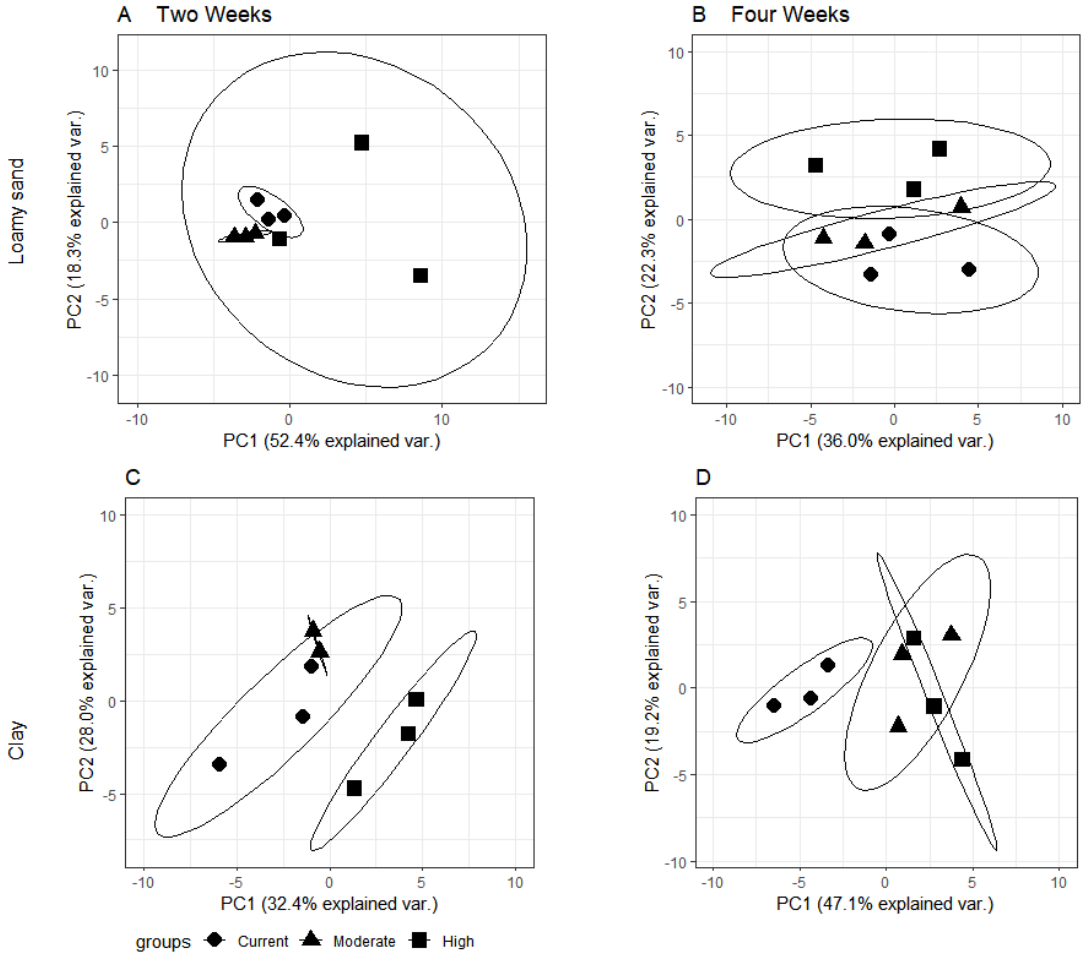

Figure 4.4: First and second principal components of PLFA profiles for loamy sand (A, B) and clay (C, D) after two week (left) and four week (right) incubation period. Groups represent the seasonal treatments; current, moderate, and high.

The relative abundances of the different microbial groups were significantly affected by seasonal treatments, dependent on soil texture and incubation period (Tables 4.2 and 4.3). For the loamy sand, only aggregates incubated over a two week period showed statistically significant results, with abundance of gram-positive bacteria greatest under the moderate seasonal treatment and lowest under the high seasonal treatment (Table 4.2), though the proportional abundance was similar with a small difference of 2% between the moderate and high seasonal treatments. The seasonal treatments did not significantly affect the

relative abundance of gram-negative bacteria or fungi. However, the summer component of the seasonal treatments did have a significant effect on the relative abundance of gram-positive bacteria and fungi. After one week (data not shown, see Appendix B.2) and two weeks in the summer-only treatments, representing the mid-point for the two and four week seasonal treatments, the relative abundance of gram-positive bacteria was significantly higher in the high treatment, whilst fungal abundance was significantly higher in the moderate treatment and lower in the high treatment after one week (Appendix B.2). Therefore, the subsequent winter conditions of the seasonal treatments counteracted the divergent effects of the summer conditions on the microbial community composition.

In clay aggregates, the response of bacterial and fungal abundance varied by incubation period (Table 4.3). Only clay aggregates incubated over a four week incubation period showed statistically significant results. Although there were significant differences the actual values for proportional abundance were similar with shifts of less than 2%. The moderate seasonal treatment had a significantly greater abundance of gram-positive bacteria (16.03%) than under the current (15.36%) and high seasonal treatments (15.44%). Fungal abundance was significantly greater under the current seasonal treatment and significantly lower under the moderate seasonal treatment with a difference of 1.42%. The seasonal treatments did not significantly affect the relative abundance of gram-negative bacteria.

Table 4.2: The response of microbial group relative abundances to seasonal treatments in loamy sand aggregates. Mean and (standard error) where n = 9, ANOVA df = 2, 6. Different letters represent statistically significant difference identified by Tukey's post-hoc testing.

Loamy sand	Two Weeks incubation period				Four Weeks incubation period			
Microbial variables	Current	Moderate	High	F(p-value)	Current	Moderate	High	F(p-value)
Gram-positive bacteria (mol%)	16.65[ab] (0.25)	17.77[a] (0.19)	15.79[b] (0.49)	**8.736 (0.0167)**	17.62 (0.13)	17.47 (0.07)	17.41 (0.25)	0.421 (0.674)
Gram-negative bacteria (mol%)	21.53 (0.14)	21.43 (0.44)	22.89 (1.43)	0.884 (0.461)	21.05 (0.57)	21.33 (0.47)	21.61 (0.42)	0.333 (0.729)
Fungi (mol%)	14.49 (0.52)	14.74 (0.17)	14.32 (1.08)	0.092 (0.913)	14.44 (1.02)	14.35 (0.66)	13.40 (0.14)	0.661 (0.55)

Table 4.3: The response of microbial group relative abundances to seasonal treatments in clay aggregates. Mean and (standard error) where n = 9, ANOVA df = 2, 6. Different letters represent statistically significant difference identified by Tukey's post-hoc testing.

Clay	Two Weeks incubation period				Four Weeks incubation period			
Microbial variables	Current	Moderate	High	F(p-value)	Current	Moderate	High	F(p-value)
Gram-positive bacteria (mol%)	16.15 (0.27)	16.71 (0.05)	15.86 (0.21)	4.705 (0.059)	15.36[a] (0.18)	16.03[b] (0.09)	15.44[a] (0.11)	**7.731 (0.0218)**
Gram-negative bacteria (mol%)	20.58 (0.23)	20.46 (0.14)	20.45 (0.28)	0.103 (0.904)	20.84 (0.01)	20.62 (0.10)	20.74 (0.35)	0.278 (0.767)
Fungi (mol%)	16.17 (0.81)	14.99 (0.17)	15.28 (0.43)	1.293 (0.341)	15.78[a] (0.38)	14.36[b] (0.05)	14.80[ab] (0.14)	**9.301 (0.0145)**

4.3.2 Seasonal treatments and constant seasons

As aggregate stability was not significantly affected by the current and future climate scenarios (current, moderate, and high) in the seasonal treatments, here these scenarios are combined and comparisons made between the summer-only treatments, the winter-only treatments, and the seasonal treatments.

Aggregate stability was generally lower for soil that experienced both summer and winter in the seasonal treatments compared with the constant seasons, with slight differences according to soil texture and incubation period (Figure 4.5). For loamy sand aggregates, aggregate stability was significantly lower in microcosms incubated under the seasonal treatments at 50% compared with the constant seasons (summer-only or winter-only) above 60% over a four-week incubation (Figure 4.5B), and a similar trend was also observed after two weeks (Figure 4.5A). For clay aggregates, aggregate stability was also significantly lower under the seasonal treatments at 87% than the winter-only treatments after two weeks which was above 90% (Figure 4.5C), with a similar trend also observed after four weeks (Figure 4.5D). Aggregate stability was lower under the summer-only treatments than the winter-only treatments by approximately 3%, though this was not statistically significant.

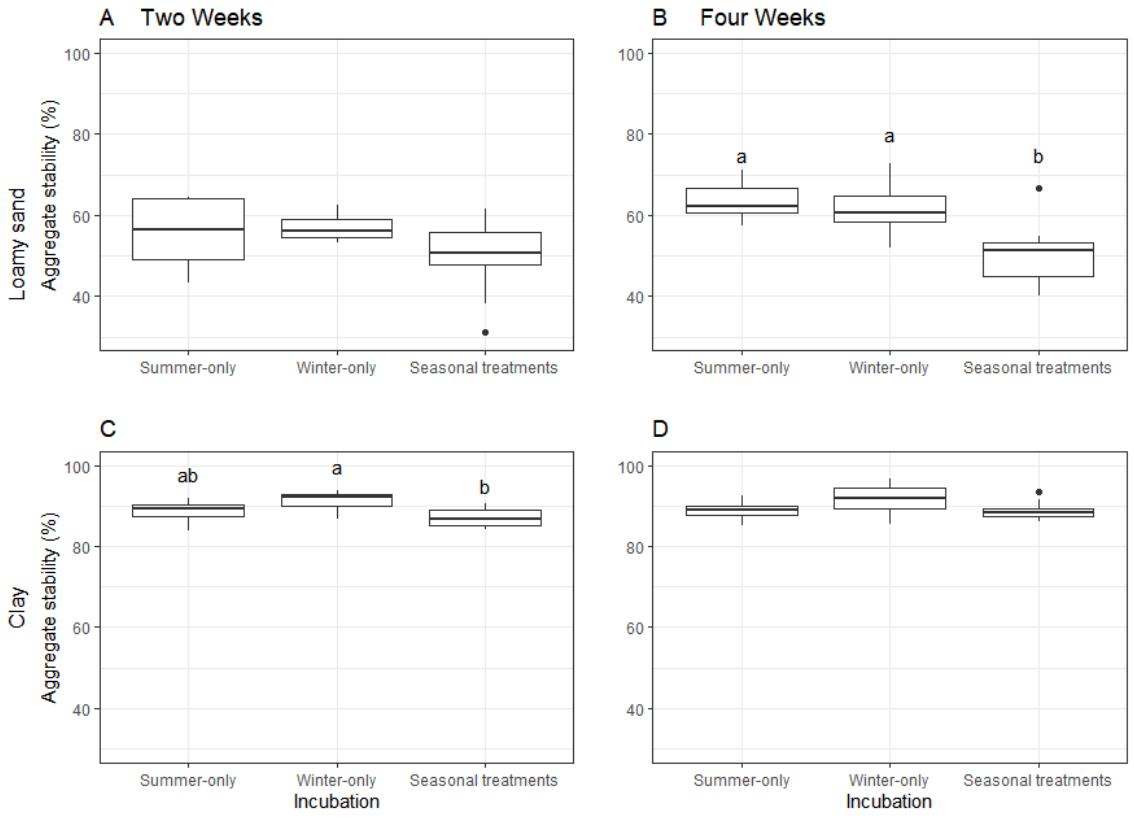

Figure 4.5: Aggregate stability under summer-only, winter-only, and seasonal treatments for loamy sand aggregates (A, B) and clay aggregates (C, D) for two incubation periods (left: two weeks; right: four weeks).

Microbial respiration and microbial biomass carbon were significantly affected by incubation conditions in the constant seasons and seasonal treatments in both loamy sand and clay aggregates over two and four weeks incubation periods (Figure 4.6). Microbial respiration in loamy sand and clay aggregates was higher under the winter-only treatments than the summer-only treatments (Figure 4.6A-D). Though this was more pronounced in clay aggregates where respiration was nearly three times greater under the winter-only than the summer-only, while only two times greater in loamy sand aggregates. For loamy sand aggregates, microbial respiration was also high under the seasonal treatments (Figure 4.6A, B). Whilst microbial respiration in the seasonal treatments in the clay aggregates was intermediate; respiration was significantly lower in the seasonal treatments than the winter-only treatment (Figure 4.6C, D) but significantly higher than the summer-only treatments after two weeks (Figure 4.6C). For both soil textures,

microbial biomass carbon was significantly highest under the seasonal treatments and higher under the winter-only treatments than the summer-only treatments with biomass carbon approximately two times greater in the winter-only than the summer-only treatments.

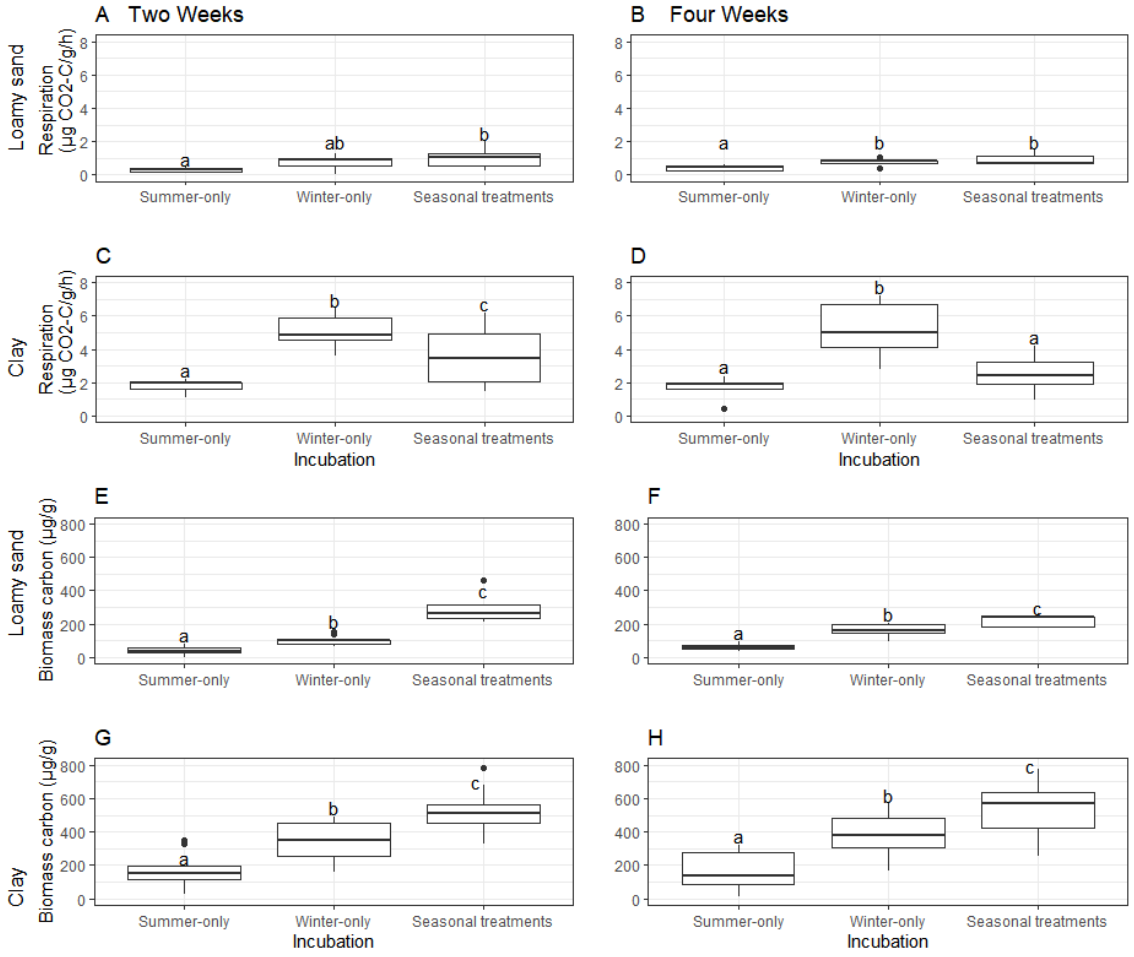

Figure 4.6: Microbial respiration (A-D) and microbial biomass carbon (E-H) for clay and loamy sand aggregates under summer-only, winter-only and seasonal treatments for two weeks (left) and four weeks (right).

Microbial community composition was similar in the constant seasons and seasonal treatments for loamy sand and clay, with no differentiation between communities by PCA of PLFA profiles (Figure 4.7). This suggests that seasonal treatments and constant seasons did not affect the microbial community composition. However, biomarker groups of PLFAs showed differing effects of incubation conditions on the relative abundances of fungi, gram-negative

bacteria, and gram-positive bacteria (Tables 4.4 and 4.5). In loamy sand aggregates, the constant seasons and seasonal treatments did not significantly affect the relative abundances of gram-negative bacteria or fungi. However, the relative abundance of gram-positive bacteria was significantly greatest under the summer-only treatments (at approximately 19.2%), stimulated by the warm and dry conditions, and lowest in the seasonal treatments after two weeks and in the winter-only treatments after four weeks (Table 4.4) with a lower proportional abundance than the summer-only treatments by approximately 2%. In clay aggregates, the relative abundances of microbial groups were significantly affected by the constant seasons and seasonal treatments (Table 4.5). Fungi exhibited the greatest differences between treatments. Relative fungal abundance was stimulated by warm and dry conditions and was greatest under the summer-only treatments at approximately 17% and lowest in the winter-only treatments and seasonal treatments after two and four weeks at approximately 15%. After two weeks, gram-positive and gram-negative bacterial abundance was stimulated by higher moisture content and was significantly greatest under the winter-only treatments and seasonal treatments, and lowest under the summer-only treatments. After four weeks, gram-positive bacterial abundance was higher in the winter-only treatments than both the summer-only treatments and seasonal treatments, whilst gram-negative bacterial abundance was significantly greater under the seasonal treatments and lowest under the constant seasons.

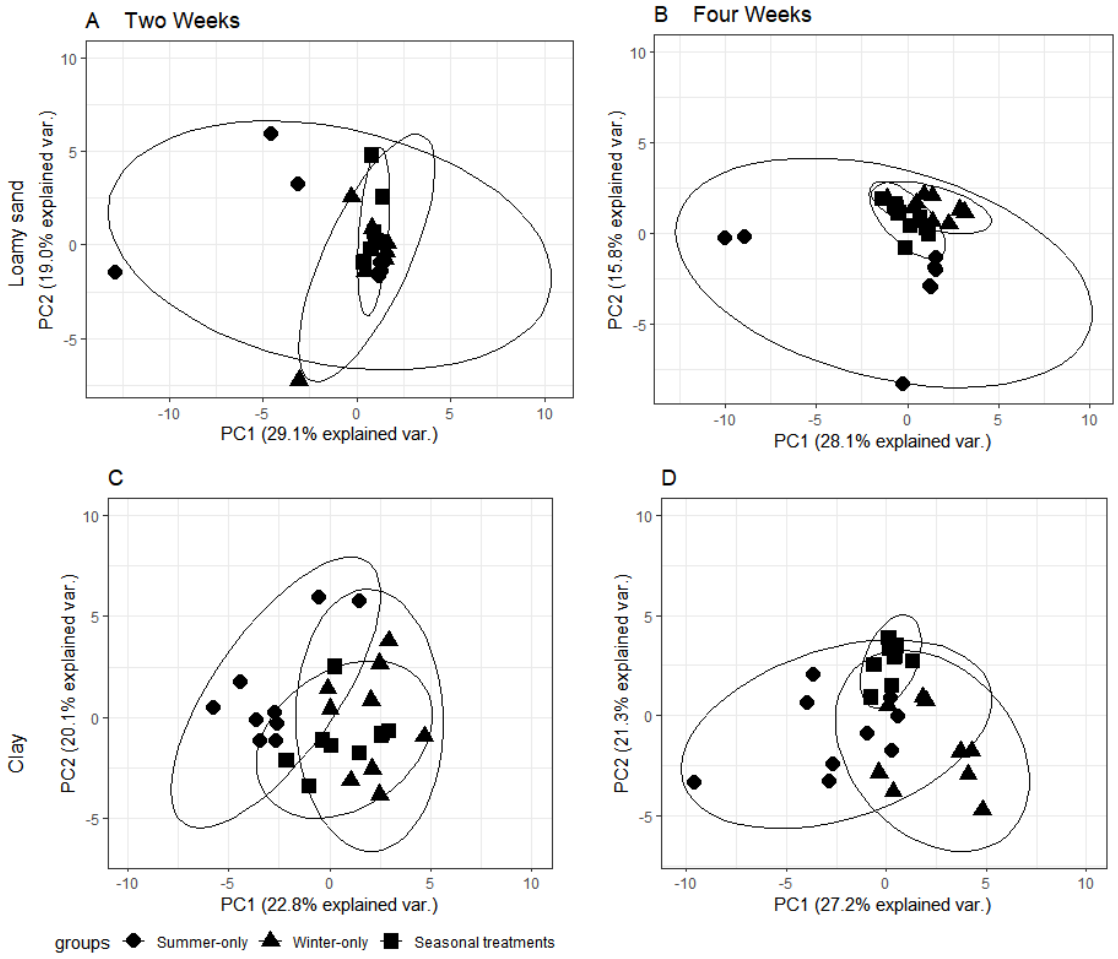

Figure 4.7: First and second principal components of PLFA profiles for loamy sand (A, B) and clay (C, D) after two week (left) and four week (right) incubation period. Groups show summer-only, winter-only and seasonal treatment samples.

Table 4.4: Microbial community composition, represented by PLFA biomarker groups, response to constant seasons (summer-only and winter-only) and seasonal treatments for loamy sand aggregates. Mean and (standard error) where n = 27, ANOVA df = 2, 24. Different letters represent statistically significant difference identified by Tukey's post-hoc testing.

Loamy sand	Two Weeks incubation period				Four Weeks incubation period			
Microbial variables	Summer-only	Winter-only	Seasonal treatments	F(p-value)	Summer-only	Winter-only	Seasonal treatments	F(p-value)
Gram-positive bacteria (mol%)	19.27[a] (0.73)	17.95[ab] (0.28)	16.74[b] (0.33)	**6.652 (0.005)**	19.21[a] (0.79)	17.22[b] (0.26)	17.50[ab] (0.09)	**4.952 (0.0158)**
Gram-negative bacteria (mol%)	20.99 (0.61)	21.35 (0.77)	21.95 (0.49)	0.587 (0.564)	20.38 (0.35)	21.34 (0.31)	21.33 (0.26)	3.171 (0.06)
Fungi (mol%)	14.69 (1.68)	13.87 (1.22)	14.52 (0.35)	0.127 (0.882)	13.67 (0.67)	14.42 (0.39)	14.06 (0.39)	0.553 (0.582)

Table 4.5: Microbial community composition, represented by PLFA biomarker groups, response to constant seasons (summer-only and winter-only) and seasonal treatments for clay aggregates. Mean and (standard error) where n = 27, ANOVA df = 2, 24. Different letters represent statistically significant difference identified by Tukey's post-hoc testing.

Clay	Two Weeks incubation period				Four Weeks incubation period			
Microbial variables	Summer-only	Winter-only	Seasonal treatments	F(p-value)	Summer-only	Winter-only	Seasonal treatments	F(p-value)
Gram-positive bacteria (mol%)	15.17[a] (0.18)	16.22[b] (0.26)	16.24[b] (0.16)	**8.827 (0.00134)**	15.52[a] (0.14)	16.82[b] (0.21)	15.61[a] (0.12)	**19.53 (<0.001)**
Gram-negative bacteria (mol%)	19.77[a] (0.17)	20.43[b] (0.22)	20.50[b] (0.11)	**5.273 (0.0126)**	20.09[a] (0.18)	20.11[a] (0.16)	20.73[b] (0.11)	**5.492 (0.00801)**
Fungi (mol%)	17.51[a] (0.23)	14.96[b] (0.26)	15.48[b] (0.32)	**24.19 (<0.001)**	17.08[a] (0.38)	14.85[b] (0.22)	14.98[b] (0.24)	**18.73 (<0.001)**

4.4 Discussion

4.4.1 Seasonal treatments (current, moderate, and high scenarios)

Aggregate stability was not significantly affected by the three seasonal treatments (Figure 4.2), which does not support the hypothesis that the different soil temperature and moisture content conditions under the seasonal treatments would invoke changes in aggregate stability. The seasonal treatments were expected to affect aggregate stability, due to the impact of soil temperature and moisture conditions on the microbial community (and its stabilising function). However, in the loamy sand aggregates, the differences in temperature and moisture content conditions between seasonal treatments did not affect aggregate stability, microbial respiration, or microbial biomass carbon (Figures 4.2 and 4.3). The relative abundance of gram-positive bacteria was significantly influenced by seasonal treatment over two weeks, but seasonal treatments did not otherwise change microbial community composition (Table 4.2). The abundance of gram-positive bacteria has been observed elsewhere in loamy sand soil, where they responded positively to warmer soil temperatures relative to other microbial groups (Buyer *et al.*, 2010). Evidence in this study suggests that for the loamy sand aggregates the initial summer conditions of the seasonal treatments induced changes in microbial community composition, yet the microbial community compositions converged under the winter conditions. Therefore, the differences in temperature and moisture content conditions between seasonal treatments did not affect the microbial community properties by the end of the incubation period and so did not provoke mechanisms affecting air-dry aggregate stability at the time of the simulated rainfall.

On the other hand, while seasonal treatments did influence microbial respiration, microbial biomass carbon and the relative abundances of gram-positive bacteria and fungi in clay aggregates, there was no corresponding effect on air-dry aggregate stability. These results at first suggest that shifts in the microbial community, and therefore potential shifts in microbial stabilising mechanisms, do not lead to a concomitant change in aggregate stability. However, one

explanation may be the existence of a threshold in the magnitude of microbial change needed to invoke detectable changes in aggregate stability. The relationship between microbial activity and biomass synthesis and aggregate stabilisation has been documented in numerous studies (Six *et al.*, 2004; Cosentino *et al.*, 2006). As previously discussed, fungal and bacterial abundance, as well as microbial activity and biomass synthesis, are closely related to fungal hyphal growth and length and EPS production (Dighton & Kooistra, 1993; Deacon, 1997; Balser *et al.*, 2005; Bittman *et al.*, 2005), which have been shown to increase aggregate stability (Degens *et al.*, 1996; Beare *et al.*, 1997; Lehmann *et al.*, 2020). Therefore, as seen in Chapter 3 it is expected that changes in the microbial community influence biological stabilisation and thus aggregate stability. A further alternative explanation may be that microbial stabilisation was masked by physico-chemical stabilisation associated with the air-drying phase post-incubation and pre-rainfall.

Microorganisms vary in physiological traits which affect temperature sensitivity and osmotic response, and mediates the response of the microbial community to changes in soil temperature and moisture (Csonka, 1989; Wood *et al.*, 2001; Schimel *et al.*, 2007; Wood, 2015). Very little is currently known on how variations in microbes' physiological traits also affect biological stabilising mechanisms and thus aggregate stability. It is possible that the shifts in the abundances of microbial groups observed here (Figure 4.4, Tables 4.2 and 4.3) did not affect overall aggregate stability, as a result of functional redundancy whereby soil microorganisms perform the same function equally, so the reduction of certain microorganisms is functionally substituted with other microorganisms and does not affect overall function (Griffiths & Philippot, 2013). However, recent research has shown how physiological traits, such as fungal hyphal density, can influence the effectiveness of biological stabilisation and aggregate stability (Lehmann & Rillig, 2015; Lehmann *et al.*, 2020), so microorganisms may not have an equal contribution to aggregate stabilisation and so are not functionally redundant. Therefore, the observed changes in microbial community composition are likely to have influenced biological stabilisation of aggregates.

Furthermore, microbial respiration and biomass were significantly affected by seasonal treatment, increasing in the moderate and high seasonal treatments for clay aggregates (Figure 4.3). This supports hypothesis 1 that increasing temperature and winter moisture content would stimulate microbial activity and biomass. However, it appears that in the high seasonal treatment, conditions did not exceed the microbial optimal ranges as expected. Other studies have observed inhibited microbial activity and growth where temperatures above 30°C exceeded the optimal range (Pietikäinen *et al.*, 2005; Bárcenas-Moreno *et al.*, 2009). Extreme soil moisture contents have also been shown to inhibit the microbial community. Aggregates experienced extreme low soil moisture content during summer conditions and high soil moisture content during the winter in the high climate treatment. Low soil moisture content can limit substrate diffusion and lead to osmotic stress and physiological cell damage, whilst high soil moisture content limits oxygen concentration (Csonka, 1989; Stark & Firestone, 1995; Schjønning *et al.*, 2003). This raises further questions on the existence of thresholds or tipping points for the microbial community; and the magnitude of change in the microbial community that will instigate changes in aggregate stability.

4.4.2 Seasonal treatments and constant seasons

The observed decrease in aggregate stability under the three seasonal treatments compared with the constant seasons supports hypothesis 2 that changing conditions in the summer-winter cycle of the seasonal treatments reduced aggregate stability in comparison to constant conditions. Studies have previously shown that the number of drying-wetting cycles influences aggregate stability, attributing the variation in aggregate response to the effects of the perturbation on the microbial community (Denef *et al.*, 2001; Cosentino *et al.*, 2006). Evidence suggests that preceding climatic conditions (e.g. preceding season) have a prolonged effect on aggregate stability and determine the influence of current and future climatic conditions on aggregate stability. Therefore, understanding the influence of seasonal conditions on aggregate

stability and the underlying mechanisms would improve our ability to predict future aggregate stability and soil erodibility.

The summer-winter pattern of the seasonal treatments appeared to stimulate microbial biomass carbon in both soil textures and stimulated respiration in the loamy sand aggregates (Figure 4.6). For both soil textures, microbial biomass carbon and respiration were significantly higher under the winter-only treatments than the summer-only treatments and for loamy sand aggregates microbial biomass was highest under the seasonal treatments. Soil moisture content was very low during the summer-only treatments, and the higher moisture content during the winter-only treatments and seasonal treatments would have increased microbial motility and substrate diffusivity, thereby increasing resource accessibility (Skopp *et al.*, 1990; Or *et al.*, 2007b; Lennon, 2012). This suggests the higher soil moisture content in the winter-only treatments and increase in soil moisture during the seasonal treatments is likely to have stimulated microbial biomass carbon and respiration, and low moisture content during the summer-only treatments suppressed microbial biomass synthesis and respiration (Meisner *et al.*, 2013, 2015, 2017). However, microbial respiration is theoretically expected to increase with increasing temperatures based on metabolic relationships (Kirschbaum, 2006; Classen *et al.*, 2015), so respiration was expected to be higher in the warmer summer-only treatments than the winter-only treatments. Contrastingly, microbial respiration was found to be higher in the winter-only treatments than the summer-only treatments in both the loamy sand and clay aggregates. Though it has been suggested that the energy required to maintain microbial biomass is greater with increased soil temperature, the allocation of energy for maintenance rather than growth may constrain microbial activity (Li *et al.*, 2019). This negative relationship between temperature and respiration may be a result of interactions with soil moisture content as suggested earlier, rather than temperature alone. Furthermore, microbial respiration responded to changes in climatic conditions differently between the two soil textures (Figure 4.6). Microbial respiration in clay aggregates was stimulated during the seasonal treatments in comparison to the summer-only treatments, but

unlike loamy sand aggregates, respiration was not as high as levels under the winter-only treatments. This finding may demonstrate the role of soil texture, which would have affected physical properties such as particle size and porosity, in mediating microbial response. Additionally, microbial respiration and biomass are interconnected with microbial community composition (Zogg *et al.*, 1997; Karhu *et al.*, 2014; Auffret *et al.*, 2016), so simultaneous changes in composition, as well as soil texture, may influence respiration and biomass response. Studies have reported conflicting responses in shifts in microbial properties as a result of changes in temperature, moisture and drying-wetting (Denef *et al.*, 2001; Bárcenas-Moreno *et al.*, 2009; Strickland & Rousk, 2010), so there are currently no consistent patterns or general systematic explanations for microbial response to climatic conditions. Despite the generally stimulating influence of seasonal treatments on microbial biomass and respiration, aggregate stability was not increased under seasonal treatments and was less stable under the seasonal treatments than under the constant season's treatments.

The constant seasons and seasonal treatments did have a differing effect on the relative abundances of biomarker groups (Tables 4.4 and 4.5). The observed shifts in microbial community composition suggested that the summer-only conditions favoured fungi, whilst bacterial abundance was highest in the winter-only treatments and seasonal treatments. This finding corresponds to other studies which report that fungi are more tolerant to dry conditions than bacteria, as hyphae are able to bridge pore spaces to reach water and nutrients, and hyphal walls are more resistant to osmotic stress than bacterial cells (Schimel *et al.*, 2007; Preece *et al.*, 2019). Seasonal fluctuations in soil temperature and moisture have been observed to correlate with the abundance and biomass of gram-positive and gram-negative bacteria and fungi (Smit *et al.*, 2001; Bell *et al.*, 2009; Castaño *et al.*, 2017). It was expected that climatic conditions, in terms of soil temperature and moisture, would induce shifts in the microbial community composition due to variations in the resistance of different microbial groups to changing conditions as a result of differences in physiological traits. For example, soil moisture content influences substrate diffusivity, bacterial motility, osmotic

pressure, oxygen concentration, and microbial response to these changing conditions is dependent on physiological traits which define cell wall characteristics, osmoregulation, resource allocation and mobility (Or *et al.*, 2007b; Schimel *et al.*, 2007; Lennon, 2012). As mentioned in Section 4.4.1 the observed shifts in fungal and bacterial abundances (Tables 4.4 and 4.5) would influence the relative contribution of the biological stabilising mechanisms. The relative contribution of each of these biological stabilising mechanisms to aggregate stability has not yet been resolved, though fungal stabilisation is generally more associated with macroaggregates (Tisdall & Oades, 1982; Lehmann *et al.*, 2017). Aggregate stability was lowest under the seasonal treatments, despite stimulated microbial biomass and respiration, yet this may be explained by the influence of microbial composition interacting with biomass and respiration collectively to mediate the effectiveness of the biological stabilising mechanisms. However, little is known how shifts in microbial composition, such as those observed here, in turn influence biological stabilisation. Furthermore, as suggested earlier, the magnitude of microbial change may not have been significant enough to meet the critical threshold to invoke changes in aggregate stabilisation.

As stated previously the implications of shifts in the relative abundances of microbial groups for aggregate stabilisation are largely unknown. Previous work has shown that favourable conditions for fungi are likely to promote aggregate stabilisation with hyphal growth enhancing hyphal enmeshment (Degens *et al.*, 1996). However, hyphal morphology varies within fungal populations, influencing hyphal growth, branching and tensile strength with potential consequences for hyphal enmeshment and aggregate stability (Rillig & Mummey, 2006; Lehmann & Rillig, 2015). Furthermore, fungi are also thought to influence soil aggregation through the production of exudates and EPS, such as glomalin, which increases particle adhesion and binding (Tisdall & Oades, 1982; Wright & Upadhyaya, 1998; Caesar-Tonthat, 2002), as well as influencing soil hydrophobicity and fungal desiccation resistance (Hallett *et al.*, 2001; Wösten, 2001; Rillig, 2005). With the complexity of these multiple fungal mechanisms, it remains challenging

to relate shifts in fungal abundance directly to aggregate stability as seen here. Bacterial production of EPS also serves many functions; as EPS provides a protective environment and desiccation resistance, enhances the accumulation of nutrients and enzymes within the EPS matrix and enables cell-cell communication, as well as aggregate stabilisation (Roberson & Firestone, 1992; Sutherland, 2001b; Flemming & Wingender, 2010). Relatively little is known about the production, nature and function of soil microbial EPS (Chenu, 1995), with no standardised extraction procedure and issues in extraction efficiency, contaminants and alterations of the original composition (Liu & Fang, 2003; Redmile-Gorden *et al.*, 2014). Therefore, there is little specific evidence of the relationship between microbial abundances and aggregate stabilising mechanisms. Such evidence is often correlative with aggregate stability, and so further research is necessary to analyse the underlying mechanisms (Rillig *et al.*, 2002; Rillig & Mummey, 2006). In order to elucidate the mechanistic relationship between the microbial community and aggregate stability, further research on the shaping of the microbial community through sequential seasonal conditions must be expanded to consider the magnitude and temporal pattern of microbial change and influence over microbially-mediated aggregation.

4.5 Conclusions

These results show that different soil temperature and moisture content conditions under three seasonal treatments did not affect aggregate stability in either soil texture. The microbial community was not significantly affected by the climate scenarios in the seasonal treatments in the loamy sand, thus no microbial-mediated changes in aggregate stability were expected. However, in clay aggregates microbial respiration was stimulated by increasing temperature and winter moisture content in the seasonal treatments and microbial biomass was stimulated under the high seasonal treatment, yet these microbial changes did not result in greater aggregate stability. It is suggested that the magnitude of microbial change did not meet a critical threshold to invoke changes in aggregate stability, or biological effects were masked by physico-chemical stabilisation associated with drying.

This study has revealed the importance of seasonal conditions and constant seasons on aggregate stability and in shaping the microbial community. Aggregate stability was significantly lower when microcosms experienced the transition from summer to winter conditions in the seasonal treatments, compared to the constant seasons. Meanwhile the seasonal treatments stimulated microbial respiration and biomass in the loamy sand, and microbial biomass in the clay, reaching higher levels than in the constant season controls. It is therefore unclear why the shift between two different seasons in the seasonal treatments caused a decrease in aggregate stability, despite the stimulation of the microbial community and shifts in community composition. Investigation on the stabilising functionality of the microbial community would ascertain the underlying mechanisms that associate changes in the microbial community to shifts in aggregate stability. There is continual interplay between microbial community composition, respiration, and biomass, that will have complex implications for aggregate stabilising mechanisms. This research provides evidence that climatic conditions influence the microbial community, but whether these climate-induced microbial changes are of a sufficient magnitude to provoke changes in aggregate stability depends on many factors that need exploring further.

5 Soil temperature and moisture content effects on microbial stabilisation affects soil erodibility and erosion

Abstract

Whilst studies have predicted increased rainfall erosivity associated with climate change will result in further accelerated rates of erosion, a complete evaluation of future changes to soil erosion rates must consider soil erodibility, i.e. its susceptibility to erosion. Aggregate stability, a well-known indicator of soil erodibility, is influenced by soil temperature and moisture and related to climate-induced shifts in the soil microbial community. Therefore, to understand how the effects of climatic conditions on soil erodibility, particularly with future climate change, will affect soil erosion rates, it is important to investigate the impact of changing climatic conditions on aggregate stability, and the role of the soil microbial community. This study investigates how climatic conditions, in terms of soil temperature and moisture, affect soil erodibility and hydrological processes, through the influence of climatic conditions on the soil microbial community and microbial-induced aggregate stability. An experimental approach used multi-layered aggregate packed soil trays investigating two soil textures (loamy sand and clay), environmental chambers, and a rainfall simulator to examine the effects of temperature (5°C, and 30°C) and soil moisture (representing dry, and wet conditions) in a 2x2 factorial incubation. Climate treatments did not significantly affect runoff or infiltrate volumes but did significantly affect sediment load, suggesting changes in aggregate stability altered soil erodibility but not hydrological processes. Increasing pre-rainfall incubation temperature in the loamy sand, and increasing soil moisture in both soils, significantly reduced aggregate stability and increased sediment load. Wetter soil conditions caused a significant (approximately five-fold) increase in sediment load in the runoff for the loamy sand ($p=0.002$) and approximately a two-fold increase from 0.00025g to 0.0005g in the infiltrate for the clay ($p=0.015$). Incubation temperature significantly affected microbial properties in the loamy sand, which may explain

the observed shifts in aggregate stability, but had no significant effects in the clay. Soil moisture did not significantly affect the soil microbial community, therefore changes in clay aggregate stability may not be associated with microbially-mediated stabilisation. Alternatively, the measured soil microbial properties as proxies of biological stabilisation may not have been detectably affected by soil moisture, but soil moisture may have influenced the effectiveness of biological stabilising mechanisms directly.

5.1 Introduction

Soil is a natural resource that provides numerous ecosystem services including biomass production, biogeochemical cycling, and water storage and regulation (Blum, 2005; Haygarth & Ritz, 2009; Davies, 2017). The degradation and removal of soil through soil erosion processes places pressure on soils and threatens the provision of essential environmental functions (Lal, 2003; Pimentel & Burgess, 2013). Soil erosion also has the potential to cause environmental damage through the sedimentation and pollution of water bodies, damage to aquatic life and increased flooding (Pimentel *et al.*, 1995; Pagiola, 1999; Gorlach *et al.*, 2004). Climate change will place even greater pressure on soil systems and impact soil erosion rates through changes in rainfall erosivity and the frequency of extreme events (Nearing, 2001; Nearing *et al.*, 2004). However, a critical gap in our knowledge is how climate change could influence soil erodibility, the susceptibility of soil to erosion processes.

Soil erodibility is influenced by multiple factors including vegetation and land use and soil properties, such as soil structure, soil texture, organic matter content, and aggregate stability (Deviren Saygin *et al.*, 2018). Several studies have documented the strong relationship between aggregate stability, soil erodibility, and erosion (Barthès & Roose, 2002; Cantón *et al.*, 2009; Dimoyiannis, 2009). Aggregate stability is a critical component of soil structure, affecting porosity and thus hydrology (infiltration rate, water retention, runoff generation). The breakdown of aggregates releases primary particles and finer fragments affecting the redistribution of soil particles, preferential transportation by runoff and

raindrops, and subsequent erosion (Le Bissonnais, 1996, 2016; Nciizah & Wakindiki, 2015). As such, aggregate stability is often considered the most important factor influencing soil erodibility, and is repeatedly used as an indicator of soil erodibility (Bryan, 1968; Le Bissonnais, 1996; Cantón *et al.*, 2009). Soil erodibility and aggregate stability have been observed to vary with climatic conditions, including temperature and soil moisture (Blackman, 1992; Sanchis *et al.*, 2008; Dimoyiannis, 2009), yet the mechanistic causes for these variations are not well understood. Soil temperature and moisture content are suggested as critical factors driving changes in aggregate stability and soil erodibility (Bullock *et al.*, 1988; Dimoyiannis, 2009). Previous studies have found a strong negative relationship between antecedent moisture content and aggregate stability related to slaking (Perfect *et al.*, 1990a; Aksakal *et al.*, 2019), though other studies on drying-wetting cycles suggest there are observed contradictions, with inconsistencies in the parameters measured and methodologies used (Denef *et al.*, 2001; Cosentino *et al.*, 2006). This variation in results may also be dependent on the influence of numerous soil properties affecting soil aggregation, including soil particle size distribution, clay content, organic matter content, and microbial community properties (Amézketa, 1999; Aksakal *et al.*, 2019). Therefore, the effects of temperature and moisture content on aggregate stability and soil erodibility remain uncertain and are difficult to generalise.

A number of studies have documented the importance of the microbial community in stabilising soil aggregates through biological aggregating agents such as fungal hyphae and microbial extracellular polymeric substances (EPS) (Oades, 1993; Beare *et al.*, 1997; Ritz & Young, 2004; Tang *et al.*, 2011). Fungal hyphae promote aggregate stabilisation through the enmeshment of soil particles and are closely related to fungal community and biomass (Tisdall, 1991; Dighton & Kooistra, 1993; Miller & Jastrow, 2000; Barbosa *et al.*, 2019). EPS acts as an adhesive agent promoting soil aggregation and has a close relationship to bacterial responses to temperature and moisture conditions (Costa *et al.*, 2018; Guo *et al.*, 2018). These biological stabilising mechanisms are closely related to the microbial community properties. As such microbial community properties,

including composition, biomass, and respiration, have shown a significant relationship with aggregate stability in previous studies (Perfect *et al.*, 1990a; Degens, 1997; Bossuyt *et al.*, 2001; Tang *et al.*, 2011) and are used as indicators of biological stabilisation of aggregates (Chapters 3 and 4). Microbial community composition affects EPS production and composition and the abundance of fungi and fungal biomass influences the effectiveness of hyphal enmeshment (Degens *et al.*, 1996; Lehmann *et al.*, 2017; Costa *et al.*, 2018). Furthermore, hyphal growth and EPS production require a high level of energy expenditure, mediated by biomass synthesis and respiration (Ritz & Young, 2004; Costa *et al.*, 2018). The soil microbial community itself is influenced by changes in temperature and soil moisture content. Temperature and soil moisture influence microbial metabolic activity, osmotic pressure, pore connectivity, microbial motility and resource accessibility (Clarke, 2006). Climatic conditions therefore affect the microbial community directly metabolically and indirectly through changes in the microbial physical habitat. Thus, soil temperature and moisture content have been shown previously to influence microbial community composition, respiration and biomass carbon (Chapters 3 and 4; Fierer *et al.*, 2006; Moyano, Manzoni and Chenu, 2013; Karhu *et al.*, 2014; Supramaniam *et al.*, 2016). It is generally expected that with available resources an increase in soil temperature and moisture will stimulate microbial respiration and biomass carbon, until a threshold exceeding optimal conditions and limiting oxygen availability (Conant *et al.*, 2011). In this study, it is hypothesised that the impact of climatic conditions on the microbial community invokes climate-associated changes in aggregate stability and soil erodibility through the influence on biological stabilising mechanisms.

There are complex feedbacks where microbes influence soil structure but are simultaneously influenced themselves by the influence of soil structure on the microhabitat. It is therefore necessary to investigate the microbial community at a scale relevant to microbial processes. Whilst aggregate stability has been shown to be strongly correlated with erodibility and erosion rates (Bryan, 1968, 2000; Barthès & Roose, 2002; Nciizah & Wakindiki, 2015), there remains a critical

challenge in the upscaling of soil microbial responses to biotic functions and understanding microbial processes at a larger scale (Vereecken *et al.*, 2016; Juyal *et al.*, 2019). At the erosion scale (with run-off generation), aggregate stability can influence soil erosion by affecting aggregate breakdown and the mobilisation of particles, and therefore soil erodibility. Yet changes in aggregate stability and therefore soil structure also influence soil hydraulic properties and hydrological processes (Barthès & Roose, 2002; Cantón *et al.*, 2009). Aggregation of soils influences hydraulic conductivity, infiltration rate, and pore-size distribution, and therefore affects surface runoff and infiltrate (Amézketa, 1999). Therefore, differences in aggregate stability as a result of climatic conditions and microbially-mediated stabilisation could impact runoff generation, and infiltration, and thus surface erosion rates. It is expected that increasing soil temperature and moisture stimulates microbial activity and growth (respiration and biomass, and biological stabilisation), enhancing aggregate stability and thereby reducing aggregate breakdown and soil erodibility and thus limiting sediment load in the runoff and infiltrate. In colder and drier treatments, the microbial community is expected to be limited, inhibiting biological stabilisation, and leading to less stable aggregates that are more likely to breakdown resulting in higher soil erodibility with higher sediment load in runoff and infiltrate.

This study aims to investigate the effects of temperature and moisture content on soil erodibility (sediment load), hydrological processes (runoff and infiltrate generation), and the soil microbial community. It is hypothesised that soil temperature and moisture content of the incubation phase will influence sediment load due to changes in soil erodibility, as a result of changes in aggregate stability and breakdown via the climate-driven shifts in microbial community composition, microbial biomass and respiration and thus biological stabilisation of aggregates. It is also expected that microbial changes and associated shifts in aggregate stability induced by temperature and moisture of the incubation environment will affect hydrological properties of soil, as measured by runoff and infiltrate volume, due to the role of aggregates in determining soil structure.

5.2 Methods

5.2.1 Soil collection and preparation

Two surface soils (0-150 mm depth), both classified as Cambisols (WRB, 2007), were collected from two fields at the Silsoe Experimental Farm (Bedfordshire, England, National Grid Reference TL075356/TL075351) in August 2019. The two contrasting soils were from the Bearstead and Evesham series; a loamy sand (4% organic matter, 86% sand, 5% silt, 9% clay) and a clay (6.7% organic matter, 13% sand, 21% silt, 66% clay) respectively. The soils were gently broken apart by hand and air-dried for 48 hours. Once dry the soil was sieved to obtain aggregates between 2-5.6 mm. Aggregates were packed in to 60 x 110 x 200 mm (d, w, l) foil trays with an internal volume of 600 cm^3 to a bulk density of 1.5 g cm^3. The foil trays were fitted with a 2 mm aperture mesh (Wondermesh) at the base to allow for infiltration. The packed soil trays were placed into containers (185 x 130 x 45 mm, l x w x d) with a cotton wool wetting bed to control moisture content and were incubated in temperature controlled environmental chambers. For wet treatments, 300 ml of water was initially added to the wetting bed and 100 ml added every three days in order to maintain soil moisture content based on pilot observations. There was variation in moisture content between soil textures due to physical soil characteristics; the loamy sand wetted up to 26% and the clay to 30%.

5.2.2 Microcosm treatments and rainfall simulation

Four treatments were applied multi-factorially to the soil trays: two air temperatures representing relatively cold and hot conditions (5°C and 30°C) and two moisture contents (air-dried and wet, 2-7% and 26-30% respectively). Each treatment was replicated six times, with paired soil trays in order to allow three replicates for the destructive analysis of microbial properties prior to the rainfall event and three replicates for the rainfall simulation and hydrological and erosion analyses. After the climate treatment incubation all soil trays were wetted to 26-30% moisture content over 24 hours via wetting beds, as initial soil moisture content is known to affect aggregate stability by rainfall simulation. Half the soil

trays were analysed directly after incubation to assess pre-rainfall microbial conditions, whilst the other half were subjected to a rainfall event generated by a 9m gravity-fed hypodermic needle rainfall simulator. The soil trays were placed in purpose-built containers with a funnel system to collect runoff and infiltrate and a raised bed of 4 mm gravel to allow for infiltration (Figure 5.1). The soil trays were placed at a 12° incline for the rainfall event with an intensity of 66 mm hr^{-1} for 45 minutes, representing a rainfall event with an estimated return period of 1 in 100 years (NERC, 1975). The runoff and infiltrate volumes were recorded after the rainfall event and stored at 4°C for sediment load analysis. Sediment load and concentration was calculated using 200ml aliquots of infiltrate and total runoff volume.

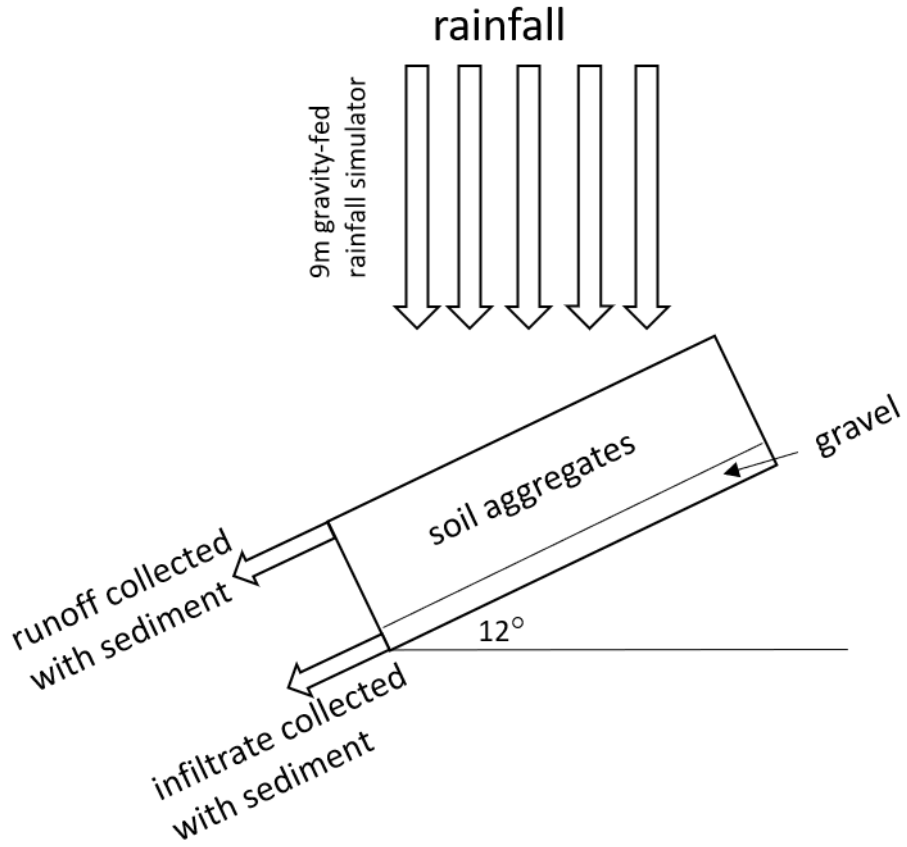

Figure 5.1: Schematic diagram of rainfall testing with 9m gravity-fed rainfall simulator and inclined soil trays in a funnel system to collect runoff and infiltrate with sediment. Not drawn to size.

5.2.3 Soil tray sampling

The surface 10 mm of each soil tray was removed using a palette knife and sub-sampled for microbial respiration, microbial biomass, and microbial community composition analysis. These microbial properties are used as proxies of changes in biological stabilising mechanisms and have often been used to indicate changes in microbially-mediated processes (Schloter *et al.*, 2003; Truu *et al.*, 2009).

5.2.4 Phospholipid Fatty Acid (PLFA) analysis

The composition of the microbial community was characterised by phospholipid fatty acid (PLFA) analysis based on the procedures developed by Frostegård et al.,(1993; 1994), Bardgett et al., (1996) and Bligh and Dyer (1959). Soil tray subsamples were frozen at -20°C and freeze-dried for 24 hours. PLFAs were extracted from 10g of soil, analysed by gas chromatography with flame ionisation detector (GC-FID), and identified by retention time. PLFAs were assessed as the relative abundance of the total area of identified peaks and grouped to represent the relative abundances of fungi, gram-negative bacteria and gram-positive bacteria (Frostegård *et al.*, 1993, 2011; Frostegård & Bååth, 1994; Bardgett *et al.*, 1996; Zelles, 1999; Ruess & Chamberlain, 2010). Gram-positive bacteria were represented by i15:0, ai15:0, i16:0, 17:0 i17:0 and ai17:0, gram-negative bacteria were represented by trans16:1ω11, cis16:1 ω7, cyc17:0iso, cis17:1ω8, cis17:0, trans17:1ω8, cis19:0 and fungi were represented by 16:1ω5, 18:2ω6, cis18:1ω9. Other identified PLFAs included 14:0, 15:0, i16:1, 16:0, Me17:0iso, Me17:0iso2, cyc17:0iso. 17:0br, 17:1ω 7, 17:0(12Me), 18:1ω7t, 18:1ω13, 18:0, 19:1ω6, 18:0(Me), 20:4(5,8, 4, 11,14), 20ω5(3), 20:0.

5.2.5 Microbial respiration rate

The rate of microbial respiration was measured using rapid automated bacterial impedance technique (RABIT) developed by Ritz et al., (2006). Microbial respiration rate was measured post-incubation and pre-rainfall, which reflected the response of the microbial community to the consistent temperature during RABIT measurements influenced by thermal stress and resource consumption in

the previous incubation. For each sample, three replicates of 1g of soil were sealed inside RABIT cells with electrodes in 1ml of potassium hydroxide agar. The rate of microbial respiration was measured between 2 and 4 hours with a decrease in conductance as microbially produced CO_2 was absorbed by the agar. Faulty cells were terminated and outliers, specified as 1.5 times the interquartile range outside the upper and lower quartiles, were removed from the dataset.

5.2.6 Microbial biomass carbon

Microbial biomass carbon was extracted using chloroform fumigation following the procedure by Jenkinson (1976) and Vance *et al.,* (1987), and compared to non-fumigated paired samples to analyse microbial biomass carbon flush. The air-dry equivalent of 12.5g soil from soil tray subsamples were weighed into Duran bottles and paired, with one subsample fumigated for 24 hours and the other non-fumigated as a control. Both subsamples were mixed with 50 ml of 0.5M K_2SO_4 for 30 minutes and filtered through Whatman 2V filter papers to extract dissolved organic carbon (DOC). DOC concentration was analysed using a segmented flow analyser and estimated with a calibration curve constructed from known concentrations of carbon standards. Non-fumigated DOC concentration was subtracted from fumigated DOC concentration to identify microbial biomass carbon and was estimated with a conversion factor of 0.45 (British Standards Institution, 1997).

5.2.7 Statistical analysis

Statistical analysis was completed using R3.2.5 (R Core Team, 2018), and results determined significant at $p \leq 0.05$. Mann-Whitney U tests and Kruskal-Wallis tests were used to assess differences in runoff and infiltrate volume, sediment load, microbial biomass carbon, microbial respiration and microbial group relative abundances due to non-normal distribution and differences in variances (measured by F-tests and the Levene's test). Post-hoc testing was completed using Dunn's test. Principal component analysis (PCA) was conducted for PLFA profiles to investigate all identified PLFA peaks.

5.3 Results

5.3.1 Hydrology, soil erodibility and soil erosion

Climate-induced changes in aggregate stability were expected to affect soil porosity and hydrological processes. However, there were no significant differences in runoff or infiltrate volumes between climate treatments for the loamy sand or clay soils (Figure 5.2). The volume of runoff for both loamy sand and clay appeared to have a higher variability under the hot-wet climate treatment (Figure 5.2A, C), though this was not significant (Levene's test, p >0.05).

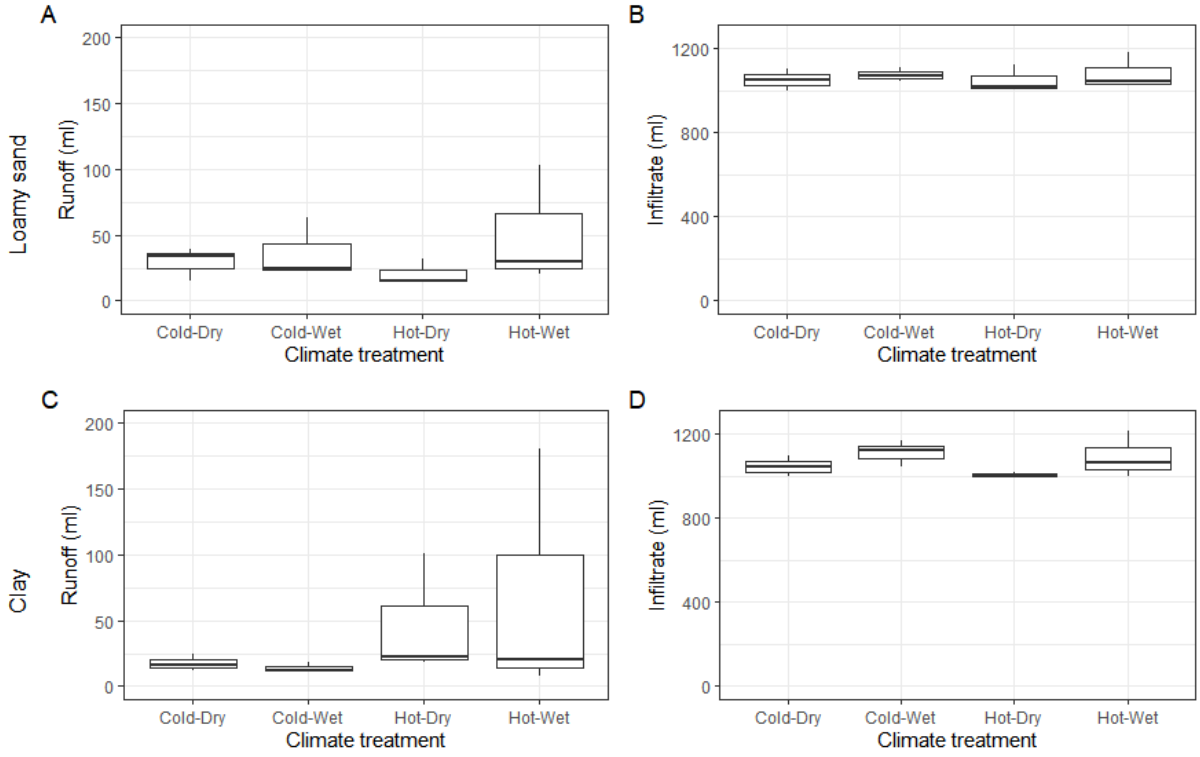

Figure 5.2: Runoff and infiltrate volumes (ml) for loamy sand (A, B) and clay (C, D) analysed by Kruskal-Wallis and Dunn's post-hoc test.

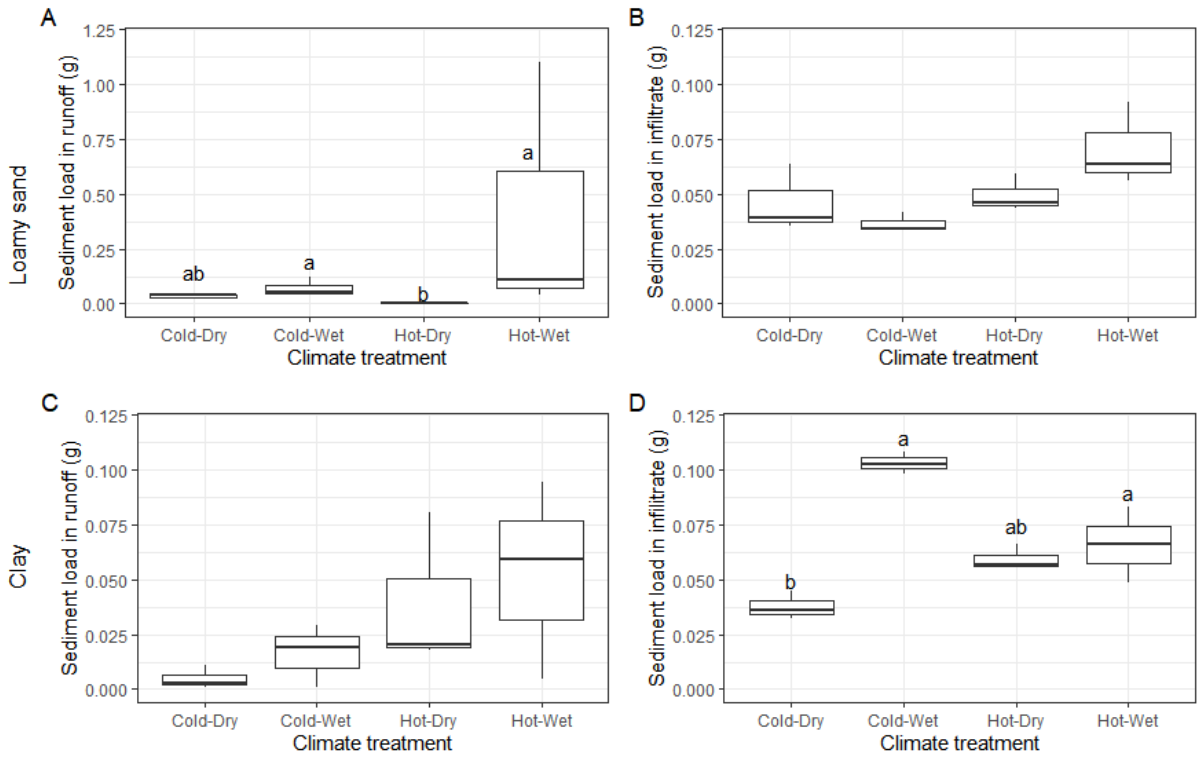

Figure 5.3: Sediment load (g) in infiltrate and runoff for loamy sand (A, B) and clay (C, D) analysed by Kruskal-Wallis and Dunn's tests

Sediment load in the runoff for the loamy sand soil was significantly different between climate treatments (Kruskal-Wallis, p=0.02, Figure 5.3A). Soil moisture during the incubation was found to have a significant positive impact on sediment load (Mann-Whitney, p=0.002), irrespective of temperature. For the infiltrate of the loamy sand soil, comparison of all the climate treatments did not detect significant differences in the sediment load (Kruskal-Wallis, p>0.05, Figure 5.3B), however temperature did have a significant positive effect on sediment load (Mann-Whitney, p=0.04). For the clay soil climate treatment did not significantly affect sediment load in the runoff (Kruskal-Wallis, p>0.05, Figure 5.3C). However, climate treatment did significantly affect sediment load in the infiltrate (Kruskal-Wallis, p=0.04, Figure 5.3D) with a significant positive effect of moisture content (Mann-Whitney, p=0.015), which was particularly pronounced in the cold treatments. Variability of sediment load was tested with Levene's tests but were not significant.

5.3.2 Soil microbial community

The composition of the microbial community based on the PCA analysis of all identified PLFA peaks showed a high level of similarity between climate treatments in the loamy sand (Figure 5.4A). For the clay soil, the microbial community composition in the climate treatments can be differentiated by the second principal component; suggesting distinct microbial communities for the hot-wet and cold treatments, whilst the hot-dry treatment showed some similarity with the hot-wet and cold-dry treatments (Figure 5.4B). Whilst there were no detectable significant differences in microbial group abundances between climate treatments in both soil textures (Kruskal-Wallis, $p > 0.05$), overall, temperature was found to have mixed significant effects. In the loamy sand, soil temperature had an overall significant positive effect on the relative abundance of gram-positive bacteria and significant negative effect on fungi (Mann-Whitney, $p=0.015$, $p=0.04$ respectively, Figure 5.4C). Gram-positive bacterial abundance was higher at 30°C, whilst fungal abundance was highest at 5°C. In the clay soil, temperature had a significant negative effect on the abundance of gram-negative bacteria (Mann-Whitney, $p=0.04$, Figure 5.4D).

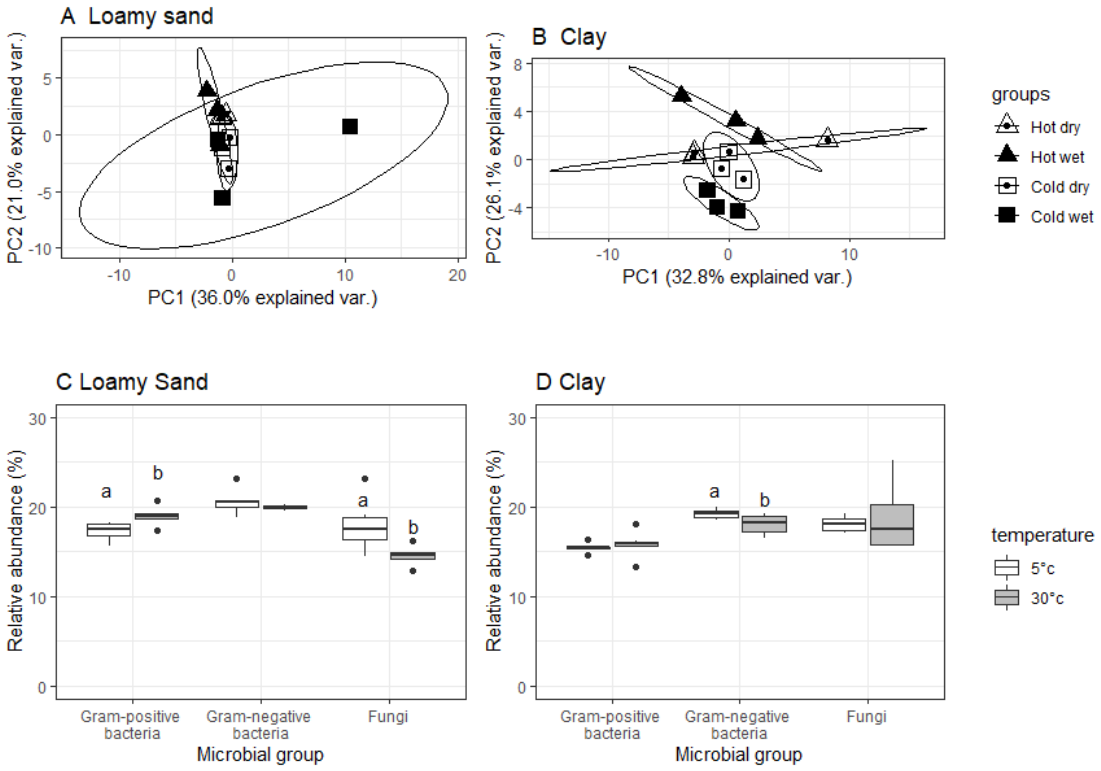

Figure 5.4: Principal component analysis of PLFA peaks for loamy sand (A) and clay (B) by climate treatments. The relative abundance of gram-positive bacteria, gram-negative bacteria, and fungi in loamy sand (C) and clay (D) separated by temperature treatments; hot (30°C) and cold (5°C).

Microbial respiration and biomass carbon were much higher in the clay than the loamy sand (Figure 5.5). Microbial respiration was significantly affected by climate treatments in the loamy sand (Kruskal-Wallis, p=0.03, Figure 5.5A), as temperature had a significant positive effect on microbial respiration (Mann-Whitney, p=0.04). Microbial respiration was not significantly affected by the climate treatments in the clay soil (Figure 5.5C). Microbial biomass carbon was not significantly affected by climate treatment in either soil texture (Kruskal-Wallis, p>0.05, Figure 5.5B, D).

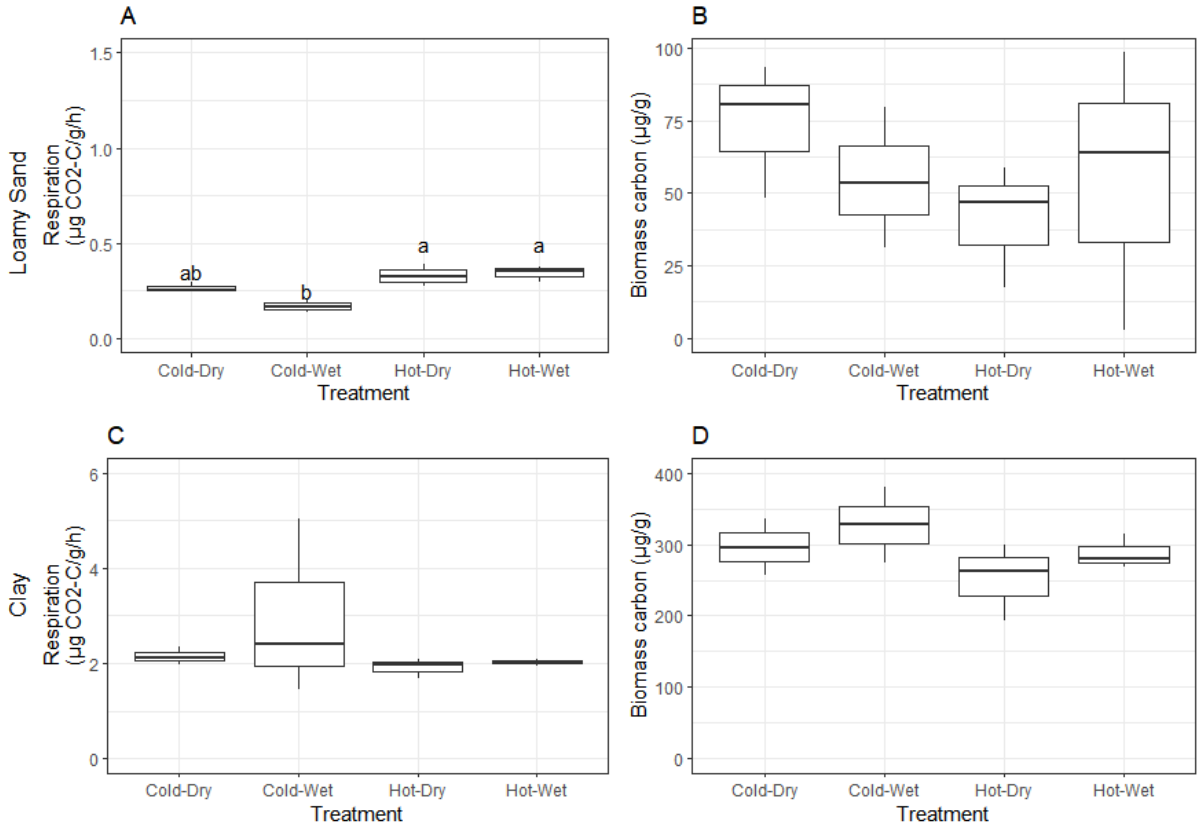

Figure 5.5: Microbial respiration and biomass carbon for loamy sand (A, B) and clay (C, D) soils. Significant differences identified by Kruskal-Wallis and Dunn's post-hoc test and shown with different letters. Axes scales are different for the two soil textures, due to much higher values in the clay soil.

5.3.3 Correlation and regression analysis between climatic conditions, microbial properties, and soil erodibility

Pearson correlation analysis for the loamy sand soil showed significant positive correlation between sediment load in the runoff and infiltrate (Table 5.1). The correlation analysis also showed a significant positive correlation between sediment load in the infiltrate and microbial respiration (Table 5.1). Sediment loads in the infiltrate and runoff were positively correlated with temperature and moisture content, though not significantly at $p = 0.05$. Temperature was significantly positively correlated with microbial respiration and the relative abundance of gram-positive bacteria and was significantly negatively correlated with fungal abundance. Microbial respiration was strongly positively correlated with gram-positive abundance and negatively correlated with fungal abundance, whilst fungal abundance and gram-negative abundance were also negatively correlated.

For the clay soil, sediment load in the runoff and infiltrate were positively correlated, though this was not significant (Table 5.2). Sediment load in the infiltrate was significantly positively correlated with temperature, whilst sediment load in the runoff was significantly positively correlated with soil moisture (Table 5.2). Temperature also showed a significant negative correlation with the relative abundance of gram-negative bacteria. Fungal abundance was negatively correlated with gram-positive and gram-negative bacterial abundance.

Multiple regression analysis identified significant microbial properties as significant predictors of sediment load for loamy sand, for both the infiltrate and runoff (Table 5.3). Microbial respiration was the sole significant predictor for sediment load in the infiltrate, whilst sediment load in the runoff was best predicted by microbial biomass carbon and gram-positive abundance. No significant regression model was found for clay soil.

Table 5.1: Pearson correlation analysis of sediment load, climatic variables and microbial properties in loamy sand soil

Loamy sand	Sediment load in infiltrate	Sediment load in runoff	Temperature	Moisture content	Microbial respiration (µg CO_2-C g^{-1} h^{-1})	Microbial biomass carbon (µg g^{-1})	Gram-positive bacteria (mol%)	Gram-negative bacteria (mol%)
Sediment load in runoff	**0.750****							
Temperature	0.574	0.266						
Moisture content	0.173	0.381	-					
Microbial respiration (µg CO_2-C g^{-1} h^{-1})	**0.677***	0.372	**0.770****	-0.270				
Microbial biomass carbon (µg g^{-1})	0.141	0.424	-0.293	-0.048	-0.011			
Gram-positive bacteria (mol%)	0.383	0.573	**0.669***	0.066	**0.577***	-0.046		
Gram-negative bacteria (mol%)	-0.166	-0.068	-0.325	0.310	-0.337	-0.290	-0.215	
Fungi (mol%)	-0.539	-0.379	**-0.638***	0.146	**-0.764****	-0.050	-0.573	**0.629***

Table 5.2: Pearson correlation analysis of sediment load, climatic variables and microbial properties in clay soil

Clay	Sediment load in infiltrate	Sediment load in runoff	Temperature	Moisture content	Microbial respiration (µg CO_2-C g^{-1} h^{-1})	Microbial biomass carbon (µg g^{-1})	Gram-positive bacteria (mol%)	Gram-negative bacteria (mol%)
Sediment load in runoff	0.092							
Temperature	0.067	**0.578***						
Moisture content	**0.756****	0.200	-					
Microbial respiration (µg CO_2-C g^{-1} h^{-1})	0.285	-0.107	-0.343	0.265				
Microbial biomass carbon (µg g^{-1})	0.300	-0.311	-0.467	0.374	-0.151			
Gram-positive bacteria (mol%)	0.133	-0.065	0.178	0.327	0.017	-0.154		
Gram-negative bacteria (mol%)	0.173	-0.353	**-0.608***	0.098	0.338	0.152	0.349	
Fungi (mol%)	-0.109	0.111	0.136	-0.144	-0.107	0.154	**-0.799****	**-0.781****

Table 5.3: Multiple regression analysis for sediment load in the runoff and infiltrate with microbial properties as predictor variables for loamy sand soil using backwards stepwise regression

Loamy sand	**Sediment load in the infiltrate**			**Sediment load in the runoff**		
Predictors	Coefficient estimates	Std. Error	p-value	Coefficient estimates	Std. Error	p-value
Intercept	0.011	0.014	0.46	-2.6	0.98	**0.025**
Respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)	0.14	0.05	**0.016**			
Microbial biomass carbon ($\mu g\ g^{-1}$)				0.0048	0.0024	0.079
Gram-positive bacteria (mol%)				0.14	0.05	**0.029**
Observations	24			24		
R^2 / adjusted R^2	0.459 / 0.405			0.532/ 0.428		
p-value	**0.016**			**0.033**		

5.4 Discussion

5.4.1 Climate effects on hydrology and soil erosion

It was expected that the influence of climate treatments (as represented by soil moisture content and temperature) on aggregate stability would affect soil erosion, measured as sediment load, two-fold; first by influencing soil erodibility and secondly by influencing soil structural properties and thus hydrological processes of runoff generation and infiltration. Whilst the climate treatments did not significantly affect the volumes of runoff or infiltrate for either soil texture (Figure 5.2), they did affect sediment load, with significant increases in sediment load with increasing temperature and moisture during the incubation phase dependent on soil texture (Figure 5.3). Therefore, the effects of the climate treatments on soil erosion are principally due to changes in aggregate stability and thus soil erodibility, rather than hydrology. However, the hydraulic conductivity of the aggregate-packed soil trays would have been high due to large inter-aggregate pores, and so this may have limited the detectability of changes in hydrological processes.

Other studies have reported that aggregate stability, soil structure, and soil hydraulic properties are interrelated (Jirků *et al.*, 2013; Alletto *et al.*, 2015), due to the influence of aggregate stability and aggregate size distribution on porosity and therefore hydrological partitioning between runoff and infiltrate. Stable soil aggregates that do not breakdown retain soil-pore structure and infiltrability. Lower aggregate stability can reduce soil infiltrability with the sedimentation and blocking of pores by soil particles, leading to surface sealing, increased surface ponding and greater runoff generation. Though it has been reported that lower aggregate stability may not necessarily lead to reduced infiltrability if drainage pores are not fully blocked (Jakab *et al.*, 2019). The observed change in soil erodibility, but not hydrological partitioning, suggests that any reduction in aggregate stability did not lead to changes in soil infiltrability.

5.4.2 Effects of climate on soil erosion, the microbial community, and biological mediation of soil erodibility

Sediment load was significantly influenced by climate treatments in both soil textures (Figure 5.3), via the influence of temperature and moisture on aggregate stability and soil erodibility. Soil aggregate stability and erodibility have been observed to change with soil temperature and moisture (Dimoyiannis, 2009; Aksakal *et al.*, 2019). Previous studies have shown that seasonal variations in aggregate stability are strongly associated with soil moisture content and suggest that aggregates are less stable under seasonally high soil moisture content (Perfect *et al.*, 1990a; Chan *et al.*, 1994). Mechanistic explanations for changes in aggregate stability focus on antecedent soil moisture at the time of the rainfall and physico-chemical mechanisms and seldom consider shifts in the microbial community and biological stabilising mechanisms. This study showed that temperature and moisture content induced changes in sediment load associated with aggregate stability and soil erodibility and influenced the soil microbial community (Figures 5.3-5.5, Tables 5.1 and 5.2). Furthermore, microbial properties, measured as proxies for biological stabilisation, were found to be significant predictors of sediment load for loamy sand (Table 5.3), and so provide evidence that the changes in aggregate stability and soil erodibility may be provoked by climate-induced shifts in the microbial community.

5.4.2.1 Temperature

There was significant variation in the effects of temperature on soil erodibility and the microbial community between soil textures. Temperature was a key factor for changes in microbial community and respiration in both soil textures (Figures 5.4 and 5.5), but it is unclear how these changes in microbial properties affect soil structural stability.

For the loamy sand soil, warmer temperature caused an increase in sediment load in the infiltrate (Figure 5.3B, Table 5.1). Sediment load, and soil erodibility, increased significantly with increasing temperature. Soil temperature was expected to stimulate the microbial community and enhance aggregate stability;

however, the opposite was observed with reduced aggregate stability at higher temperature. Temperature did have a significant positive impact on microbial respiration and stimulated gram-positive bacterial abundance, which may have enhanced biological stabilisation by EPS. Warming has previously been observed to stimulate gram-positive bacterial abundance, and drive a shift in the balance between gram-positive and gram-negative favouring gram-positive bacteria in a sandy loam (Pisani *et al.*, 2015). In this study, loamy sand aggregates incubated at higher temperature treatment had a lower fungal relative abundance (Figure 5.4C). The negative effect on fungal abundance could have reduced biological stabilisation of aggregates through decreased hyphal enmeshment and fungal exudate production. In a previous study, the experimental suppression of fungal biomass and activity with the use of fungicides was reported to reduce aggregate stability, emphasising the important role of fungi in biological stabilisation (Tang *et al.*, 2011). Therefore, the stabilising effects of the stimulation of respiration and gram-positive bacteria may have been outweighed by the reduced fungal abundance and thus resulted in a corresponding decrease in aggregate stability and increase in soil erodibility and sediment load. Therefore, future work investigating microbially-mediated aggregate stability should examine the balance between different biological stabilising mechanisms with concurrent changes in microbial composition and respiration.

For the clay soil, temperature did not have a significant effect on sediment load, suggesting temperature did not significantly affect soil erodibility (Figure 5.3). Temperature did not significantly affect microbial respiration, biomass or the relative abundances of fungi and gram-positive bacteria (Figure 5.5). However, the microbial profiles under the hot-wet and cold-wet treatments were distinct, revealing the influence of temperature on community composition (Figure 5.4B). Temperature was observed to have a strong negative influence on the relative abundance of gram-negative bacteria (Table 5.2). Despite the effects of temperature on microbial community composition, and therefore potential effect on biological stabilising mechanisms, corresponding changes in aggregate

stability, soil erodibility and sediment load were not observed. Possible explanations for this are i) there was no effect of microbial composition change on soil structural stability, or ii) changes in microbial composition were not large enough to result in changes to aggregate stability / erodibility. Firstly, changes in microbial composition may affect the activity of biological stabilising mechanisms, as shifts in fungal:bacterial dominance alters EPS:hyphal stabilisation, yet may not affect overall biological stabilisation if these mechanisms perform the same function equally. Therefore, changes in microbial community composition may not pertain to changes in aggregate stability due to functional redundancy; whereby fungi and bacteria contribute equally to biological stabilisation and so perform equivalent roles. Functional redundancy of fungi and bacteria has been observed for other microbial processes, such as respiration and carbon mineralisation, as a result of overlaps in microbial genes, traits and functional performance (Allison & Martiny, 2008; Rousk *et al.*, 2008, 2009; Martiny *et al.*, 2015). Secondly, shifts in the microbial community, and thus biological stabilisation by proxy, may not have been large enough to meet a critical threshold at which changes in microbial properties relate to a measurable change in aggregate stability. The potential existence of a critical threshold for shifts in the microbial community and biological stabilising mechanisms to alter aggregate stability has important implications for the amendment and inoculation of soil with EPS-producers (Vardharajula & Ali, 2015; Costa *et al.*, 2018). The experimental inoculation of soil with *Bacillus* strains found that aggregation effect was influenced by bacterial population size (Vardharajula & Ali, 2014), though it remains uncertain the minimum required inoculant volume to alter aggregate stability.

A third possibility may be that changes in aggregate stability were not effectively detected by sediment load measurements due to particle recapture in the multi-layered aggregate matrix following aggregate breakdown.

5.4.2.2 Soil moisture

Wetter soil caused a significant increase in sediment load in both soil textures (Figure 5.3). For loamy sand, sediment load in the runoff was significantly affected by climate treatments, primarily driven by an increase in sediment load with increasing moisture content. For the clay, climate treatment did not significantly affect sediment load in the runoff but did significantly affect sediment load in the infiltrate with a significant positive effect of moisture content. Therefore, a higher moisture content during incubation was positively correlated with a greater sediment load (Figure 5.3), but without a significant effect on the hydrology (Figure 5.2), suggesting higher soil moisture during incubation had a destabilising effect on aggregates for both soil textures.

Whilst soil moisture was related to aggregate stability, soil erodibility and thus sediment load, soil moisture did not drive significant changes in microbial properties. This suggests that the shift in aggregate stability due to soil moisture was not influenced by the microbial community properties measured and so could be attributed to the influence of physico-chemical stabilising mechanisms, or the biological stabilising mechanisms mediating aggregate stability were not detectably captured by the microbial properties measured as proxies. Whilst it is possible that soil moisture may still have influenced interparticle bonds through physico-chemical dispersion, thus reducing aggregate stability (Le Bissonnais, 1996), methodologically soil wetting was very slow to avoid slaking and aggregate breakdown during incubation.

Whilst there were no moisture-driven changes in the microbial community, the regression analysis for the sandy loam showed that microbial biomass and gram-positive abundance were significant predictors of sediment load in the runoff, alongside the influence of soil moisture (Table 5.3). The regression analysis therefore suggests that these microbial properties were still closely related to aggregate stability, though do not explain the observed change in aggregate stability with soil moisture. Microbial properties, including respiration, biomass, and community composition, were measured as proxies of biological stabilising

mechanisms. These microbial properties have often been measured as indicators of change for microbial processes (Bending *et al.*, 2004; Ritz *et al.*, 2009; Truu *et al.*, 2009). It is possible that the microbial properties measured may not have fully characterized the operation of the biological stabilising mechanisms at the inter-aggregate scale. Changes in microbial properties were expected to indicate changes in EPS stabilisation through shifts in EPS production and composition, however a number of EPS properties also may affect aggregate stabilisation. Adjustments in the composition of EPS in response to soil moisture changes may alter biological stabilisation and EPS fractions, such as EPS-protein and EPS-polysaccharides, have been shown to have a varying contribution to the stability of aggregates (Redmile-Gordon *et al.*, 2020). Therefore, a subtle adjustment in EPS composition may affect the efficacy of aggregate stabilisation. Furthermore, soil moisture content may also interact directly with the biological stabilising mechanisms to affect the efficacy of biological stabilisation. EPS is well known to interact with soil moisture and affect soil water retention by adjusting EPS hydration, reducing soil evaporation rates and increasing soil hydrophobicity (Hallett *et al.*, 2001; Ritz & Young, 2004; Or *et al.*, 2007a; Deng *et al.*, 2015). Additionally, EPS promotes particle adhesion through ionic bonding and hydrogen bonds, which may be influenced by soil moisture and solution properties. It is relatively unexamined how changes in EPS and soil hydration influence particle interactions and the efficacy of EPS-mediated aggregate stabilisation. In this study, it may be possible that shifts in the efficacy of biological stabilising mechanisms with soil moisture mediated aggregate stability but were not uncovered here through measurement of microbial properties as proxies. Currently, studies extracting and characterising EPS, and linking EPS properties to soil aggregate stability are lacking, however recent methodological developments provide an opportunity to advance knowledge in this area (Redmile-Gorden *et al.*, 2014; Costa *et al.*, 2018; Wang *et al.*, 2019c).

5.5 Conclusions

Increasing soil temperature and moisture significantly increased soil erodibility and soil erosion, dependent on soil texture. Runoff and infiltrate volumes were not affected by soil temperature and moisture, therefore the impact of climate treatments on sediment load are attributed to changes in aggregate stability which affected soil erodibility, but not hydrology. Temperature had a significant positive effect on sediment load and soil erodibility in the loamy sand soil, with increased soil erodibility inversely related to decreased aggregate stability. Temperature simultaneously influenced multiple microbial properties in the loamy sand soil, therefore changes in aggregate stability with temperature may be a result of the shifting balance between biological stabilising mechanisms. Temperature did not affect aggregate stability and sediment load in the clay soil but did significantly affect microbial composition. This suggests that microbial compositional changes did not result in a corresponding shift in aggregate stability in this study, or the magnitude of microbial change was not large enough to meet a critical threshold and measurably influence overall aggregate stability. Soil moisture had a significant positive relationship with sediment load in both soil textures. It is suggested that increasing moisture content, decreased aggregate stability and increased soil erodibility which resulted in an increase in sediment load. However, soil moisture did not drive significant changes in the microbial properties, suggesting physical and chemical stabilising mechanisms may have been influenced by soil moisture and responsible for the changes in aggregate stability. In addition to the activity of physical and chemical mechanisms during wetting-drying (such as shrinking, swelling and physico-chemical dispersion), it is possible that soil moisture did not significantly affect the microbial properties measured as proxies of biological stabilisation but may have affected the efficacy of the biological stabilising mechanisms directly.

6 The effects of rainfall on the soil microbial community

Abstract

Few studies have characterised the effects of rainfall on in-situ soil microbial properties, which may have important consequences for subsequent microbial biomass and diversity and thus microbial functioning (such as biologically mediated structural recovery) following rainfall. The simulation of a rainfall event is expected to affect the soil microbial community via changes in soil moisture, the redistribution of microbes and resources upon aggregate breakdown, and the selective mobilisation of a component of the microbial community in the sediment and liquid phases in runoff and infiltrate. Furthermore, the effects of pre-rainfall climatic conditions on the soil microbial community could alter the response of microbes to rainfall. This study investigated the effects of simulated rainfall preceding climatic treatments on the soil microbial community. The first objective was to examine the effects rainfall on the in-situ soil microbial properties, and the influence of preceding climatic treatments on the response of microbes. The second objective was to investigate the selective mobilisation of soil microbes in the runoff and infiltrate and examine the influence of preceding climatic treatments. Aggregate-packed soil trays were incubated under four climatic treatments, and half of samples subjected to rainfall simulation (the eroded soil). The results indicate that the effects of rainfall on microbial respiration and biomass carbon were dependent on soil texture and the preceding climatic treatment. In the clay microbial biomass was significantly lower in the eroded soil (approximately 100 µg/g) compared to the control soil (approximately 300 µg/g), though biomass was not significantly affected in the loamy sand. In both soils, simulated rainfall generally stimulated the relative abundance of gram-negative bacteria by approximately 2% and limited fungal relative abundance in the eroded soil by approximately 4%, whilst the effect on the relative abundance of gram-positive bacteria was dependent on soil texture and climatic treatment. The rainfall event selectively mobilised a component of the microbial community, dependent on climatic treatment, which is thought to be a result of climatic

conditions altering microbial distribution, adhesion to soil particles, and aggregate stability.

6.1 Introduction

Soil microbes are fundamental drivers of multiple soil processes and have a critical function in mediating soil properties such as aggregate stability (Tisdall & Oades, 1982; Six *et al.*, 2004). Rainfall events can affect the soil microbial community via changes in soil moisture, aggregate breakdown and the associated redistribution of nutrients and microbes within the soil mass (Cosentino *et al.*, 2006; Manzoni *et al.*, 2011), and the mobilisation of microbes in the runoff and infiltrate (Allton *et al.*, 2007). Despite dedicated studies on the effects of soil moisture and rewetting on microbial respiration, the effects of rainfall on the soil microbial community composition, activity, and distribution, are not fully understood. Furthermore, soil temperature and moisture have been shown to affect the soil microbial community (Manzoni *et al.*, 2011; Lenoir *et al.*, 2016) and may thus alter the response of microbes to rainfall by influencing microbial abundance and distribution, as well as affecting microbially-mediated aggregate stability (Chapters 3 and 4) and susceptibility to mobilisation. Therefore, the potential for a rainfall event to drive differences in the soil microbial community, influenced by preceding climatic conditions, has critical implications for the microbial functioning of in-situ eroded soil and effects of subsequent rainfall events in terms of microbial diversity, biomass, and microbial functioning (Baxter *et al.*, 2013; Le *et al.*, 2020), such as biologically mediated structural recovery.

Rainfall events have a direct influence on soil moisture conditions and drying-wetting cycles, therefore affecting the soil microbial community (Cosentino *et al.*, 2006; Manzoni *et al.*, 2011), aggregate stability (Le Bissonnais, 1996), and the mobilisation of microbes (Allton *et al.*, 2007). First, the rewetting of soil after a dry period is well known to stimulate microbial respiration and activity, known as the Birch effect, due to the increased availability of substrate (Birch, 1958; Borken & Matzner, 2009; Moyano *et al.*, 2013). Second, rainfall events cause the

breakdown of soil aggregates through rain-splash erosion, weakening of interparticle bonds, and slaking (Le Bissonnais, 1996, 2016; Nimmo, 2004a; Hu *et al.*, 2018). Aggregate breakdown by rainfall alters the microhabitat structure and releases soil particles, associated nutrients, and previously occluded organic matter. Aggregate breakdown may also affect the redistribution of microbial communities, previously embedded and protected internally in aggregates or in EPS biofilms. Finally, rainfall will also have an impact on the soil microbial community through the detachment, entrainment, and transportation of microorganisms in runoff and infiltrate, as well as the restructuring of the microhabitat and microbial redistribution in the in-situ eroded soil with the breakdown of aggregates. Previous research has reported that a rainfall event mobilised a component of the microbial community in the runoff and infiltrate (Allton *et al.*, 2007). This finding implies that rainfall events affect the microbial abundance and relative community composition of in-situ eroded soil. In studies on the effects of rainfall and overland flow on microorganisms, primarily the transportation of pathogens and pollution of water bodies, microorganisms are often assumed to act like soil particles physically (Tyrrel & Quinton, 2003). However, there is little empirical evidence of the erosion processes acting on microorganisms, and the mechanisms for the mobilisation of microorganisms and effects on subsequent microbial properties have received relatively little focus (Tyrrel & Quinton, 2003; Baxter *et al.*, 2013; Le *et al.*, 2020).

The effects of rainfall on the soil microbial community has significant implications for the subsequent microbial properties and therefore functioning of soil (Le *et al.*, 2020) such as biologically mediated structural recovery. Furthermore, preceding temperature and soil moisture conditions may influence the response of soil microorganisms to rainfall and also affect the mobilised component of the microbial community. Soil temperature and moisture content have been shown to influence microbial community composition, biomass, and respiration, with changes in microbial response to rewetting, the processing of previously occluded organic matter, and indicating an impact on biological stabilisation and aggregate stability (Chapter 3). Temperature has a strong influence on microbial

metabolic rates (Brown *et al.*, 2004; Clarke, 2006), and increasing temperature is expected to stimulate microbial respiration and biomass synthesis (Moyano *et al.*, 2013). Soil moisture influences osmotic pressure, oxygen availability and pore-water connectivity, thereby affecting the microbial community (Keift *et al.*, 1987; Or *et al.*, 2007b). Soil microbes are known to respond to changes in soil moisture through osmoregulation, though physiological differences affect the optimal range of conditions for microbes and their response to soil moisture conditions (Csonka, 1989; Wood *et al.*, 2001). For example, gram-positive bacteria have thicker cell walls than gram-negative bacteria, which increases bacterial tolerance to osmotic pressure (Csonka, 1989). Therefore, soil temperature and moisture content affect multiple soil microbial properties. It has been suggested that the mobilisation and transportation of the microbial community may be affected by the abundance of microorganisms, the protection of microorganisms within aggregates or biological structures and the strength of microbial adherence to soil particles (Tyrrel & Quinton, 2003). Soil temperature and moisture content can influence the soil microbial abundance, biomass, and respiration (Chapters 3, 4, and 5), and thus alter how microorganisms respond to rainfall events through the effects on microbial motility and aggregate stability. The influence of temperature and moisture content on microbial properties could affect biological stabilising mechanisms, thereby mediating the strength of microbial adherence to soil particles and the susceptibility of aggregates to breakdown. The influence of temperature and moisture on the soil microbial community, and the potential interaction with the effects of rainfall event on the mobilisation of the microbial community, remains unclear.

The aim of this study was to investigate how the response of the soil microbial community to climatic incubations affects their inherent mobility and selectivity of rainfall disturbance events. The objectives of this study were to i) investigate the effects of preceding climatic treatments, with differing temperature and soil moisture, and rainfall on soil microbial community in soil and ii) investigate the effects of simulated rainfall on microbial displacement and mobilisation in combined liquid and sediment samples (runoff and infiltrate). It was hypothesised

that the rainfall event would alter microbial community properties, by stimulating microbial respiration due to the release of nutrients by aggregate breakdown, but reduce microbial biomass and adjust community composition as a result of microbial mobilisation and transportation. It was also expected that the effects of the rainfall event on the microbial community would be dependent on the preceding climatic incubations, as temperature and soil moisture conditions influence the soil microbial properties and susceptibility to mobilisation, and thus affect the response of soil microbes to rainfall.

6.2 Methods

6.2.1 Soil collection, preparation, and climatic treatment

The experiment was carried out as part of the same experiment in Chapter 5. Two surface soils (0-150 mm depth) were collected from two fields at the Silsoe Experimental Farm (Bedfordshire, England, National Grid Reference TL075356/TL075351) in August 2019, with a loamy sand from the Bearsted series (4% organic matter, 86% sand, 5% silt, and 9% clay) and a clay from the Evesham series (6.7% organic matter, 13% sand, 21% silt, and 66% clay). The loamy sand and clay were both classified as Cambisols according the World Reference Base (WRB, 2007). The soil was air-dried for 48 hours and sieved for aggregates between 2-5.6 mm. Two soil textures were compared to account for variations in physico-chemical properties (e.g. particle size distribution, clay content, porosity), which are known to affect aggregate stability and the microbial habitat (Amézketa, 1999; Ranjard & Richaume, 2001). Aggregates were packed into foil trays (60 x 110 x 200 mm, d, w, l) with an internal volume of 600 cm^3 to a bulk density of 1.5 g cm^3, with a 2 mm aperture mesh fitted to the base for infiltration. The packed aggregates in the foil trays were then placed into containers (185 x 130 x 45 mm, l x w x d) with a cotton wool wetting bed to determine soil moisture through slow capillary rise. Soil environmental conditions were represented by two factors, temperature and soil moisture, each with two treatment levels. The trays of aggregates were incubated at either a cold (5°C) or hot (30°C) temperature, and with or without a wetting bed to represent dry or

wet conditions. In total, four different climate treatments were tested: cold-dry, cold-wet, hot-dry, and hot-wet. To maintain soil moisture in the wet treatments, 300 ml of water was added to the wetting bed initially with 100 ml added every three days to maintain moisture content. Average soil moisture during the wet climatic treatment for the loamy sand was 26%, and 30% for the clay. Each climatic treatment was replicated six times, with three replicates analysed pre-rainfall after the climatic incubation as the control soil and three replicates subjected to the rainfall event, referred to as the eroded soil meaning the in-situ soil remaining in the soil tray, in order to assess the effects of the rainfall event.

6.2.2 Rainfall simulation, sediment load, and hydrology

Three replicates of each climatic treatment were analysed directly after incubation as the control soil, whilst another three were subjected to a rainfall event generated by a 9m gravity-fed hypodermic needle rainfall simulator with reverse osmosis water. As moisture content at the time of the rainfall is known to affect aggregate stability, the soil trays for all treatments were slowly wetted to 26-30% moisture content (loamy sand-clay respectively) over 24 hours via capillary rise with wetting beds following incubation and prior to the rainfall event. The three control soil tray replicates were then air-dried for 72 hours ready for the microbial analyses. The remaining three soil tray replicates per treatment were placed in a container with a raised bed of 4 mm gravel to enable infiltration and a purpose-built funnel system to collect runoff and infiltrate separately. Soil trays were positioned at a 12° incline for the simulated rainfall event with an intensity of 66 mm hr^{-1} for 45 minutes (Allton, 2007), approximating a 1 in 100 year rainfall event (NERC, 1975). The rainfall simulation used reverse-osmosis water at room temperature to limit confounding effects due to ionic interactions or raindrop temperature (Sachs & Sarah, 2017a; b). The runoff and infiltrate were stored at 4°C and aliquoted into 200ml samples for sediment and PLFA analysis. The aliquots were filtered and weighed to analyse sediment load and for PLFA analysis of the sediment and aqueous community. Sediment load and volumes of runoff and infiltrate are presented in Chapter 5 in Section 5.3.1. The soil trays were then air-dried for 72 hours before sampling. The upper 10 mm of each soil

tray was then removed using a palette knife and sub-sampled for analysis of the microbial properties. Microbial respiration was measured by rapid automated bacterial impedance technique (RABIT), microbial biomass by chloroform fumigation extraction and microbial community composition by phospholipid fatty acid (PLFA) analysis. The eroded and control soil trays were sampled to analyse the effects of rainfall on the soil microbial community and provide further information on the potential impact on future microbial response.

6.2.3 Microbial community composition

Microbial community composition was characterised using phospholipid fatty acid (PLFA) analysis following standard operating procedures based on the techniques developed by Frostegård et al., (1993; 1994), Bardgett et al., (1996), and Bligh and Dyer (1959). Soil samples were frozen at -20°C and freeze-dried for 24 hours. For each sample, PLFAs were extracted from 10g of soil and analysed using gas chromatography with flame ionisation detector (GC-FID). PLFA peaks were identified by retention time and expressed as a percentage of the total area of the identified peaks. PLFA peaks represented microbial groups of gram-negative bacteria, gram-positive bacteria, and fungi, and so PLFAs were expressed as the relative abundance of the total area of grouped peaks. Gram-negative bacteria was represented by trans16:1ω11, cis16:1 ω7, cyc17:0iso, cis17:1ω8, cis17:0, trans17:1ω8, cis19:0 (Zelles, 1999; Ruess & Chamberlain, 2010). Gram-positive bacteria was represented by i15:0, ai15:0, i16:0, 17:0 i17:0 and ai17:0 (Bardgett *et al.*, 1996; Zelles, 1999; Ruess & Chamberlain, 2010). Finally, 16:1ω5, 18:2ω6, cis18:1ω9 represented fungi (Frostegård *et al.*, 1993, 2011; Frostegård & Bååth, 1994). Other PLFAs identified included 14:0, 15:0, i16:1, 16:0, Me17:0iso, Me17:0iso2, cyc17:0iso. 17:0br, 17:1ω 7, 17:0(12Me), 18:1ω7t, 18:1ω13, 18:0, 19:1ω6, 18:0(Me), 20:4(5,8, 4, 11,14), 20ω5(3), 20:0. Microbial community composition for the infiltrate and runoff was analysed using 200ml aliquots. The aliquots of infiltrate and runoff were filtered using pre-freeze-dried and weighed quartz filter paper and vacuum pump. The filters and retained sediment were then freeze-dried for 24 hours, re-weighed, and preserved for PLFA analysis. PLFAs were extracted and identified in the same manner as the

soil tray samples. The soil microbial community in the runoff and infiltrate samples include microorganisms in the liquid phase and microbes attached to the sediment phase. The intention was to monitor these phases separately, however low sediment load and methodological limitations prevented the partitioning of these phases.

6.2.4 Microbial respiration rate

Microbial respiration rate was estimated by rapid automated bacterial impedance technique (RABIT, Don Whitley Neil, UK), according to (Ritz *et al.*, 2006; Butler *et al.*, 2011, 2012). RABIT cells were created with electrodes immersed in 1 ml of potassium hydroxide agar. For each sample, triplicates of 1g of soil were weighed into glass boats and sealed inside RABIT cells. The microbial respiration rate was calculated by the decrease in conductance of alkaline agar with the absorption of microbially produced CO_2 between two and four hours incubation at 25°C. Outliers were identified as one and a half times the interquartile range outside of the upper and lower quartiles.

6.2.5 Microbial biomass carbon

Microbial biomass carbon was estimated by comparing the microbial biomass carbon flush from fumigated soil to non-fumigated replicate. Microbial biomass carbon was extracted using the chloroform fumigation extraction technique developed by Jenkinson (1976) and Vance *et al.,* (1987). For each subsample from the soil trays, two paired samples of 12.5g air-dried soil were weighed into glass Duran bottles. One of these bottles was fumigated with chloroform for 24 hours and the other non-fumigated as a control. Following fumigation, 50ml of 0.5M K_2SO_4 was added to both samples and shaken for 30 minutes, and then filtered through Whatman 2V filter papers to extract dissolved organic carbon (DOC). A segmented flow analyser was used to estimate DOC concentration by comparison to a calibration curve using carbon standards of potassium hydrogen phthalate mixed with potassium sulphate sodium polyphosphate. Microbial biomass carbon was identified by subtracting non-fumigated DOC concentration

from the fumigated sample and estimated with a conversion factor of 0.45 (British Standards Institution, 1997).

6.2.6 Statistical analysis

As a result of non-normal distributions and differences in variance (tested by F-tests and Levene's tests), Kruskal-Wallis and Mann-Whitney U tests were used to test significant differences in microbial respiration, microbial biomass carbon, and the relative abundances of microbial groups. PLFA profiles were visualised and assessed by principal component analysis (PCA) to investigate all identified PLFA peaks. Statistical analysis was conducted with R3.2.5 (R Core Team, 2018).

6.3 Results

6.3.1 Effects of rainfall on the soil microbial community

The rainfall event did have a significant effect on microbial respiration in the cold-wet treatment for loamy sand, where the event of rainfall stimulated respiration in the eroded soil (Kruskal-Wallis, p=0.05, Figure 6.1A). The rainfall event significantly reduced microbial respiration following the hot treatments in the eroded clay soil (Kruskal-Wallis, p=0.05, Figure 6.1C). The application of rainfall also significantly decreased microbial biomass in both the cold treatments and the hot-wet treatment for the eroded clay soil (Kruskal-Wallis, p=0.05, Figure 6.1D). The combination of the climatic treatment and application of rainfall had a significant effect on microbial respiration and microbial biomass between treatments for the eroded clay soil. After rainfall, microbial respiration was significantly decreased with increasing temperature (Kruskal-Walls, p=0.03, Figure 6.1C), whilst microbial biomass was significantly higher in the hot treatment (Kruskal-Walls, p=0.03, Figure 6.1D).

Climatic treatment had a significant effect on microbial respiration in the control soil in the loamy sand (Kruskal-Wallis, p=0.03, Figure 6.1A), primarily driven by a positive relationship with temperature (Mann-Whitney, p=0.04). Climatic

treatment did not otherwise affect microbial respiration or biomass for the control soil (Kruskal-Wallis, p>0.05).

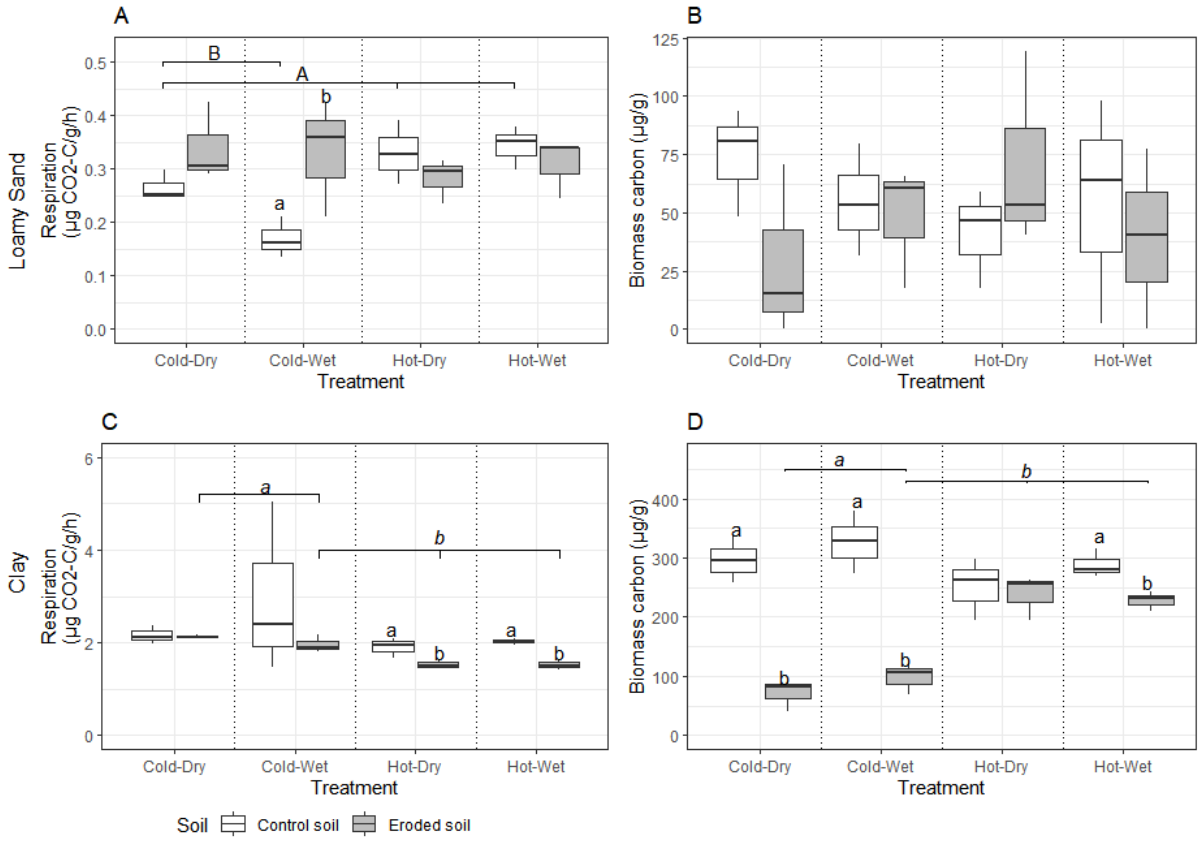

Figure 6.1: Microbial respiration and biomass carbon for control and eroded soil, separated by climatic treatment and soil texture. Two temperatures were applied; hot (30°C) and cold (5°C), and two moisture contents; dry (air-dry) and wet (28-30%). Different lowercase letters represent significant differences identified by Kruskal-Wallis tests between control and eroded soil within the climatic treatments: capital letters represent significant differences in microbial respiration in the control soil (A), and italic letters in represent significant differences in the eroded soil (C and D).

The rainfall event had a significant effect on microbial relative abundances for both soil textures, generally stimulating gram-negative bacterial abundance, but reducing fungal abundance (Figure 6.2). The effect on gram-positive bacteria was mixed. For loamy sand, the relative abundance of gram-positive was significantly lower in the eroded soil than in the control soil for the hot-dry treatment (Figure 6.2A). Meanwhile the relative abundance of gram-positive bacteria was significantly higher in the eroded soil than the control soil in the cold-dry and both the hot treatments (Figure 6.2B). Fungal abundance was significantly lower following the rainfall event in the eroded soil than the control soil (Figure 6.2C). For the clay, the relative abundance of gram-positive bacteria was significantly higher in the cold-dry eroded soil (Figure 6.2D), whilst gram-negative bacterial abundance was significantly higher in the cold-dry and hot-wet eroded soil (Figure 6.2E). In the clay eroded soil, fungal abundance was significantly lower than the control soil for all climatic treatments (Figure 6.2F). However, for eroded soil relative abundances of microbial groups were generally not significantly different dependent on climatic treatment, with the exception of fungal abundance in the clay eroded soil (Kruskal-Wallis $p=0.03$), where fungal abundance decreased with increasing temperature.

Climatic treatment did not have an overall significant influence of the relative abundances of microbial groups in the control soil for both soil textures (Kruskal-Wallis, $p > 0.05$, Figure 6.2A-F). Though in the control soil, temperature did have a significant impact on microbial abundances irrespective of moisture, as warming caused an increase in gram-positive bacterial abundance and decrease in fungal abundance in the loamy sand, as well as a decrease in gram-negative bacterial abundance in the clay at warmer temperature (Mann-Whitney tests, $p<0.05$).

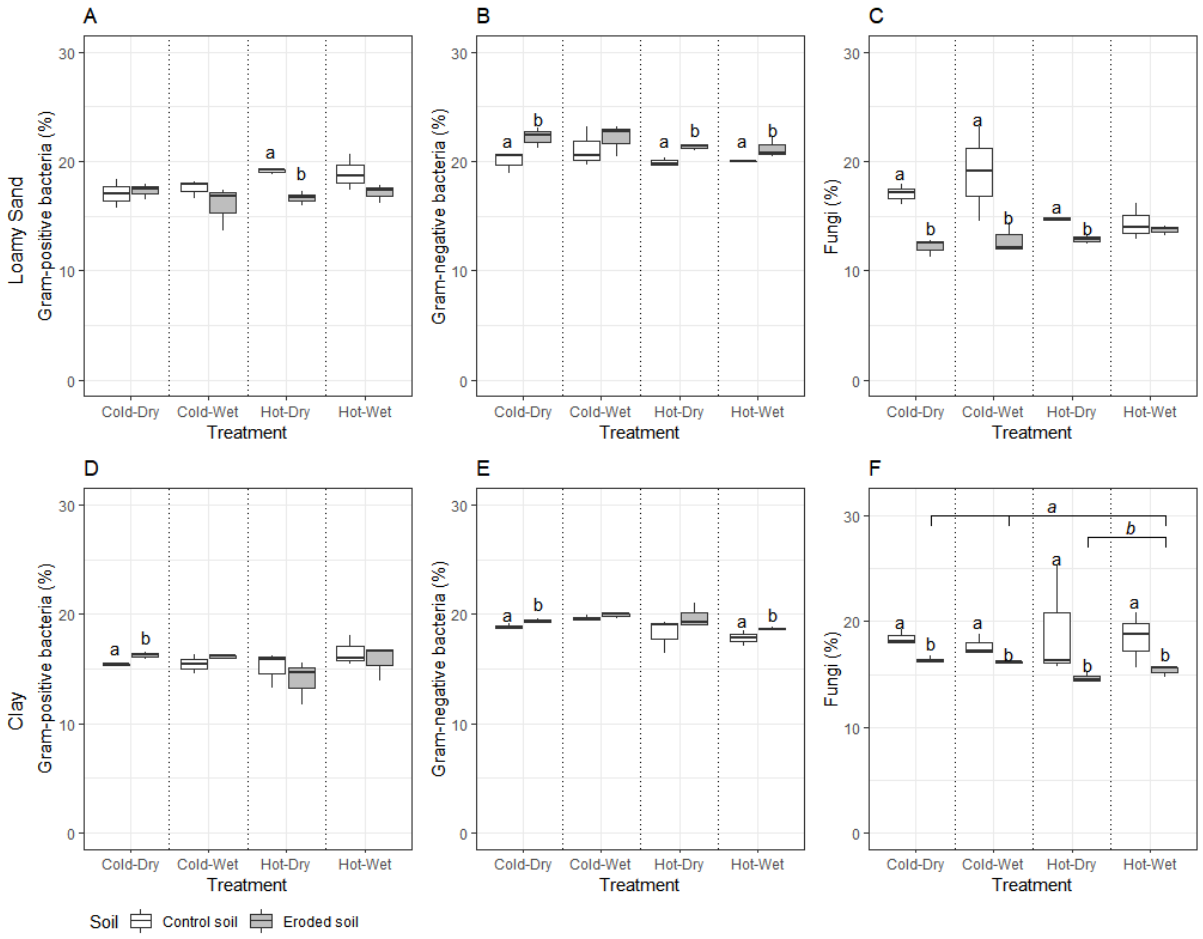

Figure 6.2: Relative abundances of gram-positive bacteria, gram-negative bacteria, and fungi in loamy sand (A – C) and clay (D – F) control and eroded soil. Different letters represent significant differences between the control and eroded soil per climatic treatment, italic letters are used in panel F to represent significant differences between climatic treatments in the eroded soil.

6.3.2 Mobilisation of microbial community

The soil microbial community in the runoff and infiltrate samples include free microorganisms in the water, as well as microbes attached to the sediment. Whilst for several temperature:moisture treatments, microbial group abundances were significantly different in the control and eroded soils (Figure 6.2), there was a much greater difference in the microbial profiles between the soil (control and eroded) and the runoff and infiltrate (Figure 6.3). For both soil textures the microbial communities in the runoff and infiltrate, were distinct from the microbial

communities in both the control and eroded soil, assessed by PCA of all PLFA peaks (Figure 6.3). In addition, the microbial profiles for the runoff and infiltrate samples show a greater spread suggesting higher variation between microbial communities.

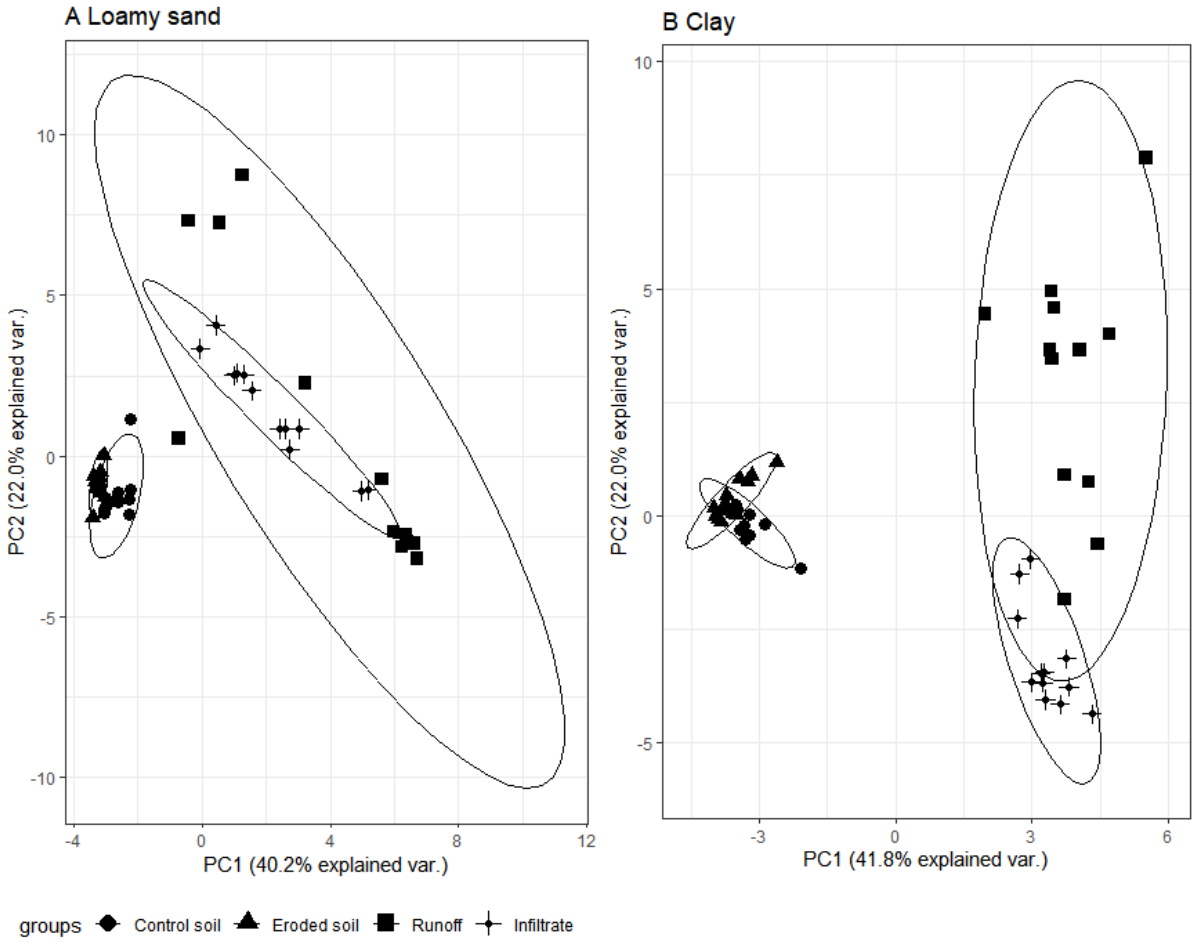

Figure 6.3: PCA analysis for control and eroded soils, runoff, and infiltrate for loamy sand (A), and clay (B).

The large spread in the microbial profiles for runoff and infiltrate (Figure 6.3) may be a result of the divergent effects of climatic treatments on the microbial community. For the loamy sand, the microbial community in the hot-dry treatment was distinct from other climatic treatments in the runoff (Figure 6.4A). In the infiltrate for loamy sand (Figure 6.4B), there were distinct microbial profiles for the hot and cold treatments across both moisture treatments, and microbial community composition also differed with moisture content.

In the runoff for the clay (Figure 6.4C), temperature led to distinct microbial profiles in the dry treatments. In the infiltrate for the clay, microbial community composition was similar in the cold treatments and there was larger variability in the hot-dry treatment (Figure 6.4D).

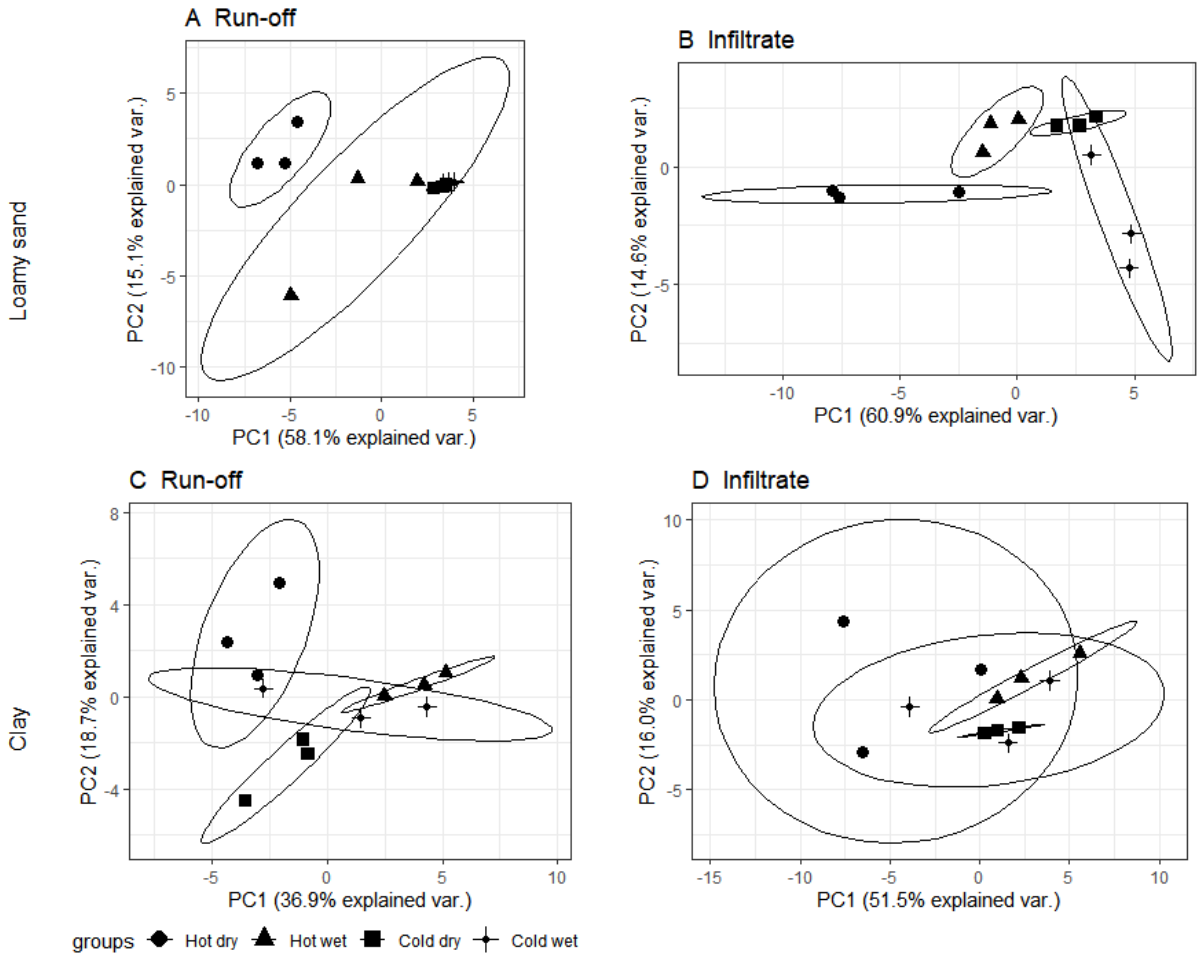

Figure 6.4: PCA analysis by climatic treatments for runoff and infiltrate from the loamy sand (A, B), and clay (C, D).

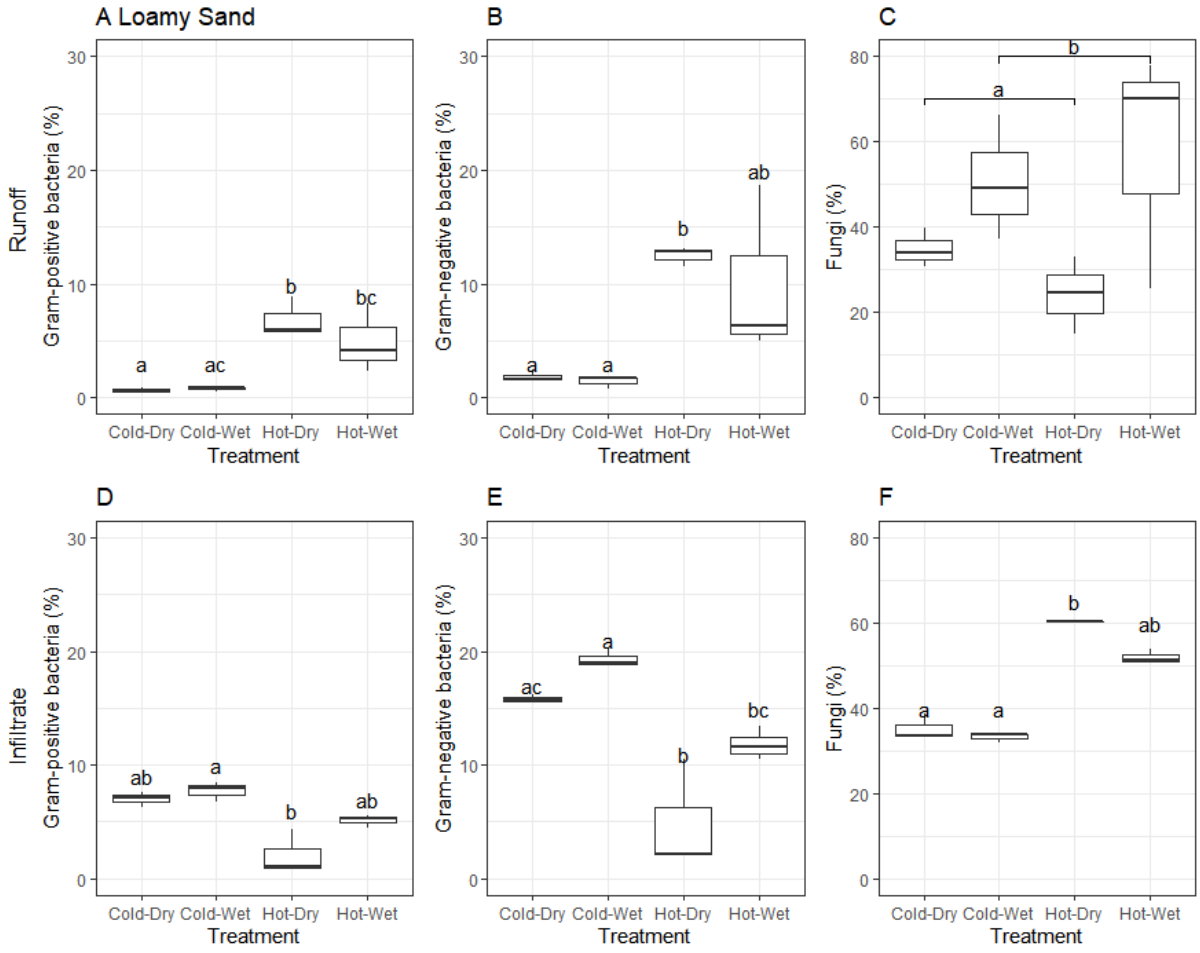

Figure 6.5: Relative abundances of gram-positive bacteria, gram-negative bacteria, and fungi in runoff (A-C) and infiltrate (D-F) from the loamy sand soil. Different letters represent significant differences between climatic treatments. Brackets in panel C show significant differences by moisture treatment. Axes scales are different for fungi (C, F) due to the much higher relative abundances.

In the runoff and infiltrate for the loamy sand, the relative abundance of fungi was consistently greater than gram-positive and gram-negative bacteria (Figure 6.5). Climatic treatment had a significant effect on the relative abundance of gram-positive and gram-negative bacteria in the runoff from the loamy sand soil (Figure 6.5A, B), with higher relative abundance associated with higher temperature. Fungal abundance was greater in the wet treatment than the dry (Figure 6.5C), but greater in the infiltrate in the hot treatment, compared to the cold (Figure 6.5F). In the infiltrate, temperature had a significant positive effect on the relative

abundance of fungi (Figure 6.5F), but a significant negative effect on the relative abundance of gram-positive (Figure 6.5D) and gram-negative bacteria (Figure 6.5E).

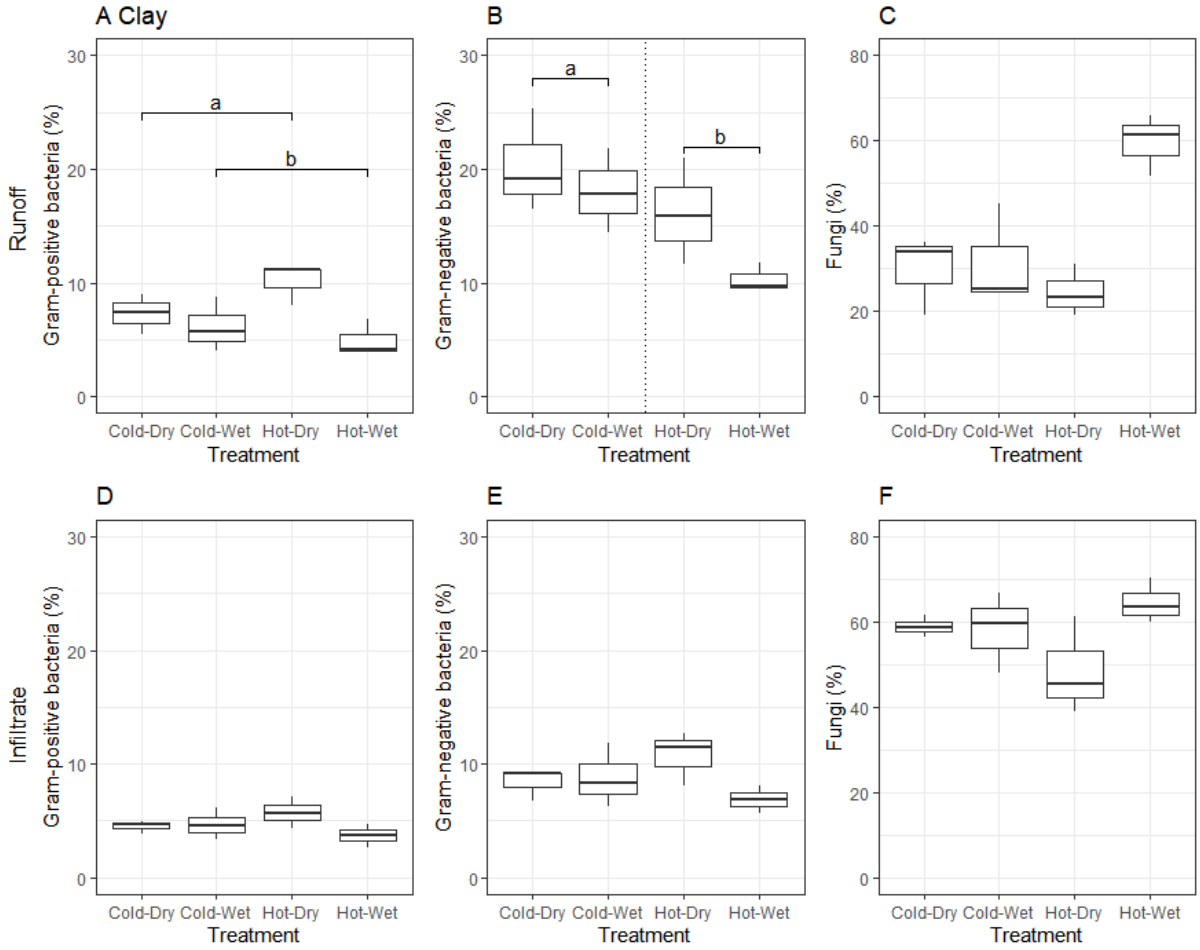

Figure 6.6: Relative abundances of gram-positive bacteria, gram-negative bacteria, and fungi in runoff (A-C) and infiltrate (D-F) from the clay soil. Brackets show differences between moisture (A) and temperature (B) treatments. Axes scales are different for fungi (C, F) due to the much higher relative abundances.

The relative abundances of fungi in the runoff and infiltrate from the clay soil was greater than gram-positive and gram-negative bacteria (Figure 6.6). Soil moisture during the climatic treatment had a significant negative effect on the relative abundance of gram-positive bacteria with significantly lower abundance in the runoff from wet treatments (Figure 6.6A). In the runoff, temperature had a significant negative effect on the relative abundance of gram-negative bacteria,

which significantly decreased with increasing temperature, especially under wet conditions (Figure 6.6B). Climatic treatment did not significantly affect the relative abundances of gram-positive bacteria, gram-negative bacteria, and fungi in the infiltrate.

6.4 Discussion

The effects of rainfall on microbial respiration, biomass, and composition in the eroded soil, in comparison to the control soil are discussed followed by the potential interaction of the effects of rainfall with climatic treatment. The rainfall event was also expected to mobilise a component of the microbial community, as influenced by soil temperature and moisture conditions in the climatic treatment due to climate-driven alterations in microbial properties and aggregate stability, which is discussed in Section 6.4.2. Rainfall was hypothesised to affect soil microbial properties in these laboratory experiments through the stimulation of microbial respiration and the selective mobilisation and transportation of the microbial community, thus reducing microbial biomass and altering microbial community composition, which would be affected by the preceding climatic treatments.

6.4.1 The effects of rainfall on the soil microbial community (control versus eroded soils)

The simulation of a rainfall event was expected to affect microbial respiration, biomass carbon, and community composition. In this study, the effects of rainfall on the soil microbial community varied by soil texture. In the loamy sand, the rainfall event did not significantly affect microbial respiration, except for the eroded soil pre-incubated in the cold-wet treatment (Figure 6.1A), and for eroded clay soil the rainfall event reduced microbial respiration dependent on the preceding climatic treatment (Figure 6.1C). Therefore, the hypothesis that rainfall would stimulate microbial respiration cannot be accepted. Aggregate breakdown during the rainfall event can alter microhabitats and the distribution of nutrients with the release of previously-occluded organic matter (Navarro-García *et al.*, 2012), which was expected to stimulate microbial respiration in the eroded soil.

However, there is limited empirical data on the relationship between microbial activity and aggregate breakdown, and interpretation of this relationship is further complicated by the confounding effects of temperature and soil moisture. Soil moisture has been reported to limit microbial respiration when oxygen availability becomes limited (Conant *et al.*, 2011; Manzoni *et al.*, 2011; Moyano *et al.*, 2013). There may be additional processes operating which explain the reduced respiration in the eroded soil, such as the lysing of microbial cells with changes in osmotic pressure, and mobilisation of microbes. However, cell lysis with changes in osmotic pressure has been observed to stimulate the surviving microbes (Borken & Matzner, 2009; Blazewicz *et al.*, 2014). Therefore, it is more likely that the reduced microbial respiration was a result of the redistribution and loss of microbes following rainfall with aggregate breakdown and mobilisation (discussed in Section 6.4.2).

The clay soil exposed to rainfall had significantly lower microbial biomass carbon, particularly in soil previously incubated at cold treatments (Figure 6.1D), which supports the study hypothesis. There were no significant effects of rainfall on microbial biomass in the loamy sand (Figure 6.1B). The rainfall event was expected to mobilise a component of the microbial community, thereby reducing microbial biomass, and altering microbial community composition in the eroded soil. Soil texture has an important influence on the effects of simulated rainfall, which is likely related to differences in soil properties including porosity, aggregate stability, organic matter, and interactions between clay particles and water (Amézketa, 1999; Nimmo, 2004b). Previous studies have observed decreased microbial biomass and organic carbon in eroded soil and enriched sediment and soil at depositional sites (Nie *et al.*, 2014; Li *et al.*, 2015). It is suggested that this is caused by the changes in soil physico-chemical properties associated with soil erosion, including the selective transportation of soil particles, carbon, and other nutrients, and the resultant creation of different microhabitats at erosional and depositional sites (Li *et al.*, 2013a, 2015; Huang *et al.*, 2014; Nie *et al.*, 2014).

The patterns observed in the composition of the eroded soil community differ to the control community revealing the impact of the rainfall event itself. In these laboratory studies, rainfall stimulated gram-negative bacteria with significantly higher relative abundance observed in the eroded soil compared to the control soil (Figure 6.2B, E), suggesting that the gram-negative bacteria were able to survive the rainfall event and exploit post-rainfall conditions such as the release of previously occluded organic matter. In experimental investigations of the effects of rewetting, bacterial growth and abundance have been reported to increase after rewetting with increased resource availability, particularly gram-negative bacteria which is strongly influenced by carbon availability (Iovieno & Bååth, 2008; Huang *et al.*, 2014). In contrast, soil fungi were susceptible to disruption and mobilisation by rainfall, with a generally negative effect observed on fungal abundance (Figure 6.2C, F). The differences in the responses of microbial groups to the rainfall event can be partly explained by variations in physiological traits, which would have altered survival and resistance to disruption, as well as microbial adhesion to soil aggregates, thereby also influencing the selective mobilisation of microbes in water flow (discussed further in Section 6.4.2). Fungal abundance has been observed to decrease with rewetting, with the proposed explanation that soil fungi are more vulnerable to disruption as a result of their predominant location in large pores and on the surface of aggregates (Gordon *et al.*, 2008; Huang *et al.*, 2014). The results show that the effects of rainfall on the different abundance of gram-positive bacteria by soil texture and climatic treatment show that climatic treatment and soil physico-chemical properties had a strong influence on the survival and resistance of gram-positive bacteria. For loamy sand, gram-positive bacterial abundance was significantly lower in the eroded soil than the control soil in the hot-dry treatment (Figure 6.2A). For clay, the abundance of gram-positive bacteria was significantly higher in the eroded soil than the control soil in the cold-dry treatment (Figure 6.2D).

Aggregate stability has been previously observed to change as a result of climatic treatments, and it has been suggested this change was mediated by shifts in the

microbial community and biological stabilisation (Chapters 3, 4, and 5). Here, the pre-rainfall climatic treatments did affect soil microbial properties and so could have altered aggregate stability, which subsequently would have affected aggregate breakdown and the redistribution of microbes in the eroded soil and mobilised in the runoff and infiltrate.

6.4.2 Hydrological mobilisation and partitioning of the microbial community

The microbial community composition in soil (control and eroded) was distinct from the microbial communities in the runoff and infiltrate samples (Figure 6.3). Allton *et al.* (2007) also found evidence of distinct microbial community separation in runoff and infiltrate, suggesting the presence of a water-mobile fraction of the community. The runoff and infiltrate may mobilise microbes from three states: microorganisms that are freely motile in pore-spaces, free microorganisms that have been released through aggregate breakdown, and microorganisms attached to soil particles that have been detached and mobilised, similarly to the three states of microorganisms in soil-slurry mixture (Tyrrel & Quinton, 2003). The runoff and infiltrate samples (representing combined sediment and liquid phases) had a very high relative abundance of fungi for both loamy sand and clay (Figures 6.5, 6.6).

The microbial community composition and the relative abundances of microbial groups observed in the runoff and infiltrate in this study were dependent on the climatic treatments (Figures 6.4 – 6.6). This suggests that temperature and soil moisture in the climatic treatment pre-rainfall determined the soil microbes mobilised by the runoff and infiltrate. The effects of climatic treatment on microbial mobilisation may be explained through climate-induced shifts in the susceptibility of the microbial community to displacement and mobilisation, related to changes in the abundance of soil microbes and the distribution and protection of soil microbes.

In this study, the relative abundance of microbial groups in the control soil varied with temperature, though were not affected by soil moisture (Section 6.3.1).

Increased abundance of a microbial group would likely increase susceptibility to mobilisation, but climatic conditions did not significantly affect microbial biomass in the control soil (Figure 6.1) and only slightly affected the relative abundances of microbial groups, despite the significant effects of temperature (Figure 6.2). It is therefore proposed that differences in the selective mobilisation of soil microbes could have been driven by variations in the location and protection of microbes, rather than abundance of soil microbes. Climatic conditions may alter the susceptibility of the microbial community to displacement and mobilisation by influencing the distribution and protection of microorganisms within soil-pore water, aggregates, and biological structures of EPS (biofilms). First, soil moisture greatly affects the connectivity of soil pore-water and therefore the aqueous microhabitat (Or *et al.*, 2007b). As such soil moisture has been suggested to affect bacterial density gradients and hotspots by influencing motility as well as resource accessibility (Bundt *et al.*, 2001; Nunan *et al.*, 2002, 2003). Therefore, it is suggested that increasing soil moisture encourages a higher bacterial density in soil water and, therefore, an increased number of freely motile microorganisms susceptible to mobilisation and transportation. Second, soil texture and aggregate stability are key determinants of porosity and thus microbial distribution and density (Rabbi *et al.*, 2016). Porosity has a great influence on bacterial density by controlling gas and water diffusion and protection from predation (Ranjard & Richaume, 2001; Schjønning *et al.*, 2003), with previous studies reporting a greater abundance of bacteria within aggregates than in larger inter-aggregate pores (Ranjard *et al.*, 2000). Therefore, aggregates can provide a favourable microhabitat and protection for microorganisms and so aggregate breakdown, here dominated by raindrop impact, would have reduced microbial protection and resulted in the release and redistribution of microbes during rainfall simulation. Upon aggregate breakdown detached soil particles are susceptible to entrainment and redistribution as sediment, also transporting soil microbes associated with the detached soil particles (Tyrrel & Quinton, 2003; Almajmaie *et al.*, 2017; Rabot *et al.*, 2018). Microbial adhesion to detached soil particles and stable aggregates therefore affects the selective entrainment of soil microbes.

Although here aggregate breakdown was dominated by raindrop impact, which may not have necessarily been represented as soil loss through sediment load if particles were retained within the multi-layered aggregate structure, though aggregate breakdown still had an ecological effect by impacting the microbial community. Finally, microorganisms also alter their own environment with the production of EPS for protection, which enhances biological adhesion to soil particles and aggregate stability. In a previous study, sediment load was influenced by climatic conditions, possibly as a result of changes in biological stabilisation and aggregate stability (Chapter 5). Therefore, climatic conditions may have affected the location of bacteria directly through the influence of soil moisture on pore-water connectivity and indirectly by affecting aggregate stability, porosity, and biological stabilisation.

6.5 Conclusions

In this laboratory study, the effects of rainfall on microbial respiration and biomass varied by soil texture and climatic treatment. The rainfall event significantly reduced respiration rates and biomass carbon in the clay eroded soil, but generally had no significant effects in the loamy sand. The rainfall event generally stimulated the relative abundance of gram-negative bacteria and limited fungal abundance in both soils. Pre-rainfall climatic treatments altered the response of the soil microbial community to rainfall. Therefore, future studies should consider the effect of rainfall itself on the microbial community, as well as the interaction of preceding climatic conditions and soil texture and feedbacks with aggregate stability. The influence of climatic treatments and rainfall events on the selective mobilisation of microbes has important implications for future studies on soil erosion. Firstly, the microbial biomass and composition was significantly different in eroded soil compared to control soil, which may alter soil functioning post-rainfall (such as biologically mediated structural recovery) and could alter the effects of subsequent rainfall events. Secondly, the selective mobilisation of the microbial community in runoff and infiltrate dependent on climatic treatment affects the dispersal of microbes throughout the soil system and transportation into the hydrological system, with possible consequences for water quality.

7 Synthesis

7.1 Introduction

Soil erodibility is a critical variable influencing erosion rates and is typically represented as a constant value in erosion models, based on physico-chemical properties that are relatively static over decadal timescales. However, significant variations in soil erodibility have been observed and may be caused by the dynamics of aggregate stability, a well-accepted indicator of soil erodibility (Bryan, 2000; Barthès & Roose, 2002; Nciizah & Wakindiki, 2015). Furthermore, the soil microbial community has a principal role in altering aggregate stability and is responsive to shifts in climatic conditions, that directly affect soil temperature and moisture (Six *et al.*, 2004; Cosentino *et al.*, 2006). The response of the microbial community to temperature and soil moisture, and influence on biological stabilisation, is one possible explanation for the differences in aggregate stability and soil erodibility observed with climatic conditions but these relationships are infrequently studied.

This research sought to investigate the influence of climatic conditions on aggregate stability and soil erodibility, and is the first of its kind to measure the effects of climatic conditions on both aggregate stability and the soil microbial community. A review of the literature in Chapter 2 identified critical gaps in knowledge on the effects of climatic conditions on aggregate stability and the underlying stabilising and destabilising mechanisms, particularly microbially-mediated aggregation and the rapid response of the microbial community. The experimental research analysed the effects of constant soil temperature and moisture content treatments on aggregate stability and the microbial community at the aggregate-scale (objective 1, Chapter 3). It then investigated the influence of seasonal treatments (summer-winter), representing future climatic scenarios, on aggregate stability and the microbial community (objective 2, Chapter 4). The research then examined the effects of soil temperature and moisture content on soil erodibility, with aggregates packed in soil trays at the erosion scale (objective 3, Chapter 5). Finally, the research considered the effects of rainfall, and the

influence of pre-rainfall climatic incubations, on the soil microbial community post-rainfall and the mobilisation of the microbial community in the sediment, runoff, and infiltrate (objective 4, Chapter 6).

In this chapter, cross-study insights on the effects of soil texture, soil temperature, moisture content, and scale will be discussed in Section 7.2. The upscaling of aggregate processes to soil erodibility and erosion, as well as the associated challenges of current methods and the novel application of aggregate microcosms in this research, are discussed in Section 7.3. Finally, directions for future research are suggested in Section 7.4 within the proposed mechanistic framework of interactions between climate, soil properties, aggregate stability, and soil erodibility as described in Chapter 2 (Figure 2.1). This mechanistic framework identifies the need to a) examine further the effects of climatic conditions on soil microbes and aggregate stability (Section 7.4.1); b) link the microbial community to stabilising functions better (Section 7.4.2); c) quantify biological stabilising mechanisms (Section 7.4.3); and d) identify the multi-way interactions between soil architecture, chemical environment, and inhabitants (Section 7.4.4).

7.2 Cross-study insights on the effects of soil texture, temperature, moisture, and scale

Previous synthesis of aggregate-scale mechanisms and responses to climatic conditions have been hindered by the intrinsic heterogeneity of soil and inconsistency in the methodologies applied and variables measured. In this research, the use of aggregates, aggregate-packed soil trays, and consistent application of methodologies to control climatic conditions and measure soil properties allow much greater collation of trends between experimental findings. This section will present the cross-study insights on the effects of soil texture, temperature, moisture, and scale on aggregate stability and the microbial community.

Soil texture was a variable in each of the experiments and the results indicate that it strongly influenced the effects of soil temperature and moisture on the

microbial community, aggregate stability, and soil erodibility. Two soil textures (high percentage clay versus high percentage sand content) were tested in each chapter to represent differences in physico-chemical soil properties. The difference in particle size distribution due to the proportion of sand and clay particles would have influenced porosity, with a higher proportion of larger pores with higher sand content and influenced clay particle interactions due to the electrostatic nature of clay particles (Lal & Shukla, 2004). These physico-chemical properties may have directly affected aggregation as discussed in Chapter 2, and indirectly affected the microbial community and mediated the influence of climatic conditions, through the effects of soil texture and structure on microhabitat, particularly influencing porosity, tortuosity and pore-water connectivity (Nimmo, 2004b; Ebrahimi & Or, 2016; Rabot *et al.*, 2018). Soil texture varied slightly between experiments and this could have influenced the results (Appendix C). Each experiment presented in Chapters 3 and 4 was designed to inform the expected response of aggregate stability and thus soil erodibility to climate conditions in subsequent experiments. Broadly the baseline soil physico-chemical properties were expected to be similar in the clay soils and sandy soils, and therefore aggregate stability and the soil microbial community were expected to respond similarly and consistently across the experimental chapters.

The effects of temperature on aggregate stability, microbial community composition and respiration differed between soil textures. Clay aggregate stability increased significantly with increasing temperature (Chapter 3). For soil textures with high sand content (sandy loam in Chapter 3, loamy sand in Chapters 4 and 5), temperature did not significantly affect aggregate stability when aggregates were arranged in a single layer (Chapters 3, and 4), though did influence aggregate stability when aggregates were packed together to represent the soil matrix (Chapter 5). Temperature was observed to impact the relative abundances of the microbial groups and microbial respiration. Temperature had a significant negative impact on fungal abundance in the loamy sand soil trays (Chapter 5), though this relationship was not observed in individual sandy loam

aggregates (Chapter 3). Temperature did not significantly affect the fungal abundance in clay aggregates (Chapters 3, 4, 5). Generally, the relative abundance of gram-positive bacteria was observed to increase with increasing temperature in both soil textures (loamy sand aggregates: Chapters 4 and 5, clay aggregates: Chapter 3), whilst the relative abundance of gram-negative bacteria significantly decreased with increasing temperature in clay aggregates (Chapters 3 and 5). Temperature had a significant negative impact on microbial respiration in individual aggregates for both soil textures (Chapter 3). Contrastingly, microbial respiration increased significantly with temperature in loamy sand soil trays, though microbial respiration was also affected simultaneously by moisture content in the cold temperature treatment (Chapter 5). Generally, microbial respiration is expected to increase with increasing temperature and a positive relationship between respiration and temperature has been observed in several short term studies (Kirschbaum, 2006; Auffret *et al.*, 2016). Though the longer term effect of temperature on respiration remains uncertain with observations complicated by the confounding effects of substrate availability and compositional change of the microbial community adjusting microbial sensitivity to temperature (Bradford *et al.*, 2008; Auffret *et al.*, 2016). Aggregate stability was expected to increase with stimulated microbial respiration. However in clay aggregates, aggregate stability increased with increasing temperature despite a negative correlation between temperature and microbial respiration. The relationship between aggregate stability and temperature may therefore be a result of the effects of temperature on the ratio of gram-positive and gram-negative bacterial abundances.

Aggregate stability decreased with increasing moisture content in sandy loam aggregates (Chapter 3). Consistently soil erodibility was found to increase with increasing moisture content relating to a decrease in aggregate stability and resulting in a higher sediment load in the runoff for loamy sand soil (Chapter 5). Additionally for clay aggregates, increasing moisture content increased significantly sediment load in the infiltrate, which was related to a decrease in aggregate stability (Chapter 5). Soil moisture also affected microbial community

composition in individual aggregate populations in both soil textures, constraining fungal abundance in sandy loam aggregates (Chapter 3) and interacting with temperature to adjust fungal abundance in clay aggregates (Chapter 4). However, soil moisture did not cause significant differences in the relative abundances of microbial groups in aggregate packed soil trays (Chapter 5). Alongside changes in microbial community composition, increasing soil moisture content significantly stimulated microbial respiration in both soil textures (Chapter 3) and stimulated microbial biomass in clay aggregates (Chapters 3 and 4). However, the stimulation of microbial respiration and biomass carbon with increasing soil moisture content did not translate to a concurrent increase in aggregate stability as expected. Therefore, it was suggested that the simultaneous changes in microbial community composition, respiration and biomass carbon may have a complex composite influence via different biological stabilising mechanisms. Furthermore, the results may also suggest that there is a threshold in the magnitude of microbial change required to influence biological stabilisation and drive measurable change in aggregate stability.

Climatic conditions significantly affected aggregate stability, soil erodibility, and the soil microbial community at the scales studied in this research, though certain relationships were affected by the scale of study. Two experiments were conducted at the aggregate scale, with individual aggregates in a single layer (Chapters 3 and 4). This allowed the investigation of changes in stability within aggregates but made the local environment around the aggregate very artificial. Further experimental work examined a matrix of aggregates packed in soil trays in multiple layers (Chapters 5 and 6), which enabled the study of both intra- and inter-aggregate processes. The single layer and multi-layered construction of aggregates is a significant distinction between Chapters 3 and 4, and Chapters 5 and 6, which aided the upscaling of mechanisms and showcases contrasts due to inter-aggregate processes, which is discussed in Chapter 7, Section 7.3. Climatic conditions significantly affected aggregate stability and soil erodibility at the scales considered. Aggregate stability was significantly affected by climatic conditions when aggregates were structured in a single layer (Chapter 3),

Additionally, climatic conditions affected soil erodibility, measured by changes in sediment load, in aggregate-packed soil trays (Chapter 5). Soil moisture tended to exhibit a negative relationship with aggregate stability, or no significant effect, for both soil textures at the single-layer and multi-layered scale (Chapters 3 and 5). Meanwhile, the relationship between temperature and aggregate stability differed between single layers and multiple layers of aggregates. For sandy aggregates in a single layer, temperature did not affect aggregate stability significantly (Chapters 3 and 4) but did have a significant negative effect on aggregate stability in a multi-layered structure (Chapter 5). For clay aggregates in a single layer, temperature had a significant positive effect on aggregate stability (Chapter 3) but did not affect sediment load, suggesting no effect on aggregate stability when aggregates are packed in multiple layers (Chapter 5). Generally, the relationships between temperature and the relative abundance of microbial groups were consistent across structural scales for both soil textures. Although for sandy soils, the negative effect of temperature on fungal abundance was only observed when aggregates were structured in multiple layers (Chapter 5) and not in a single layer (Chapter 3). Additionally, the relationships observed between soil moisture and microbial properties were generally consistent across scales for both soil textures. The response of microbial respiration to temperature varied slightly dependent on the structuring of aggregates in a single layer or multiple layers. Temperature and microbial respiration exhibited a negative relationship in a single layer of aggregates in both soil textures (Chapters 3 and 4), yet a positive relationship was observed in the loamy sand and no effect in the clay with multiple layers of aggregates (Chapter 5). The multi-layered structure of aggregates and therefore operation of inter-aggregate processes thus have significant implications for the effects of temperature on microbial respiration and aggregate stability, potentially due to alterations in pore connectivity and resource distribution and accessibility.

7.3 Upscaling aggregate-scale mechanisms to soil erosion-scale processes

This study sought to explore how changing climatic conditions affect aggregate stability and soil erodibility and thus soil erosion, with other erosive factors held constant. The underpinning hypothesis was that climatic conditions affect aggregate stability and thus soil erodibility, due to the influence of climatic conditions on the soil microbial community and effects on biological stabilising mechanisms. In order to address the effects of climatic change on soil, it is critical to understand the nature and extent to which microbial processes operate at both the aggregate-scale and upscale to the runoff scale, so affecting the response of the soil system (König *et al.*, 2020). The spatial heterogeneity of soils is very high, creating a great challenge to generalise findings, and produce accurate models of soil processes at the macroscale. Our interpretation of processes at macroscales must therefore reflect the heterogeneity of soil at the microscale (Baveye *et al.*, 2018). Microbial processes must be investigated at relevant scales at which microorganisms operate, accounting for microenvironmental variations (Baveye, 2015; Baveye *et al.*, 2018).

The research presented in this thesis focussed on two scales, a single layer of aggregates and a multi-layered matrix of aggregates, because these scales have a significant influence on the mechanisms mediating aggregate stability and the soil microbial community (Or *et al.*, 2007b; Ebrahimi & Or, 2016). The investigation of single layer aggregates (Chapters 3 and 4) identified the effects of moisture and temperature on the soil microbial community and its role on aggregate stability without confounding factors caused by inter-aggregate microhabitats. Scaling up from single layer aggregates to a multi-layered aggregate matrix would have influenced soil architecture and created microhabitats between aggregates. The soil microbial community has been shown to be strongly related to aggregate stability (Chapter 3) and is greatly influenced by soil architecture, as discussed in Section 3.4. As such, a key reason for the investigation of single layer and multi-layered aggregate structures was the two-way interaction between soil architecture and the microbial community.

The structuring of aggregates in single-layer microcosms (Chapters 3 and 4) enabled the identification of individual aggregate changes and microbial response at the intra-aggregate scale without confounding influences of soil physico-chemical properties from inter-aggregate structures. Multilayer packed soil trays (Chapters 5 and 6) were used to inform understanding of aggregate changes and microbial response with interactions of soil architecture. Therefore, the response of the microbial community was collectively due to climatic conditions and soil architecture. The structuring of aggregates in single layers and multi-layers altered important soil physical properties including structural porosity and connectivity, which in turn is known to influence microbial distribution (Juyal *et al.*, 2019). The structural role of aggregates on microhabitats also mediates the effects of climatic conditions such as soil moisture on pore-water connectivity and resource accessibility (Or *et al.*, 2007b).

Where aggregates were structured in single layer microcosms (Chapters 3 and 4), aggregate breakdown resulted in the loss of particles through the mesh base if the particles were less than 2 mm. Comparatively, where aggregates were packed into soil trays forming an aggregate matrix (Chapter 5), released particles and aggregate fragments from the breakdown of aggregates may have been retained within the aggregate matrix, or transported via the infiltrate as porosity was high. Additionally, aggregates within a multilayer matrix were more protected from external disruptive forces, such as raindrop impact, than aggregates in a single layer. In single-layer aggregate populations, approximately 20-40% of aggregates were lost through breakdown (Chapters 3 and 4). Without taking into account particle retention and aggregate protection in the multi-layered aggregate matrix, simply upscaling from the single layer aggregate scale to the multilayer soil tray would predict that 20-40% of the aggregates within the soil tray would be lost, yet in multi-layer soil trays this level of sediment loss was not observed (Chapter 5). Multiple methodologies for aggregate stability measuring the breakdown of individual aggregates or populations of aggregates do not encapsulate all mechanisms of aggregate breakdown or accurately represent natural breakdown processes and overlook critical scale-dependent processes

including the interaction between aggregates in a matrix (Amézketa, 1999; Almajmaie *et al.*, 2017). Investigation and comparison of aggregate stabilisation and breakdown in aggregates structured in a single layer and in multiple layers can therefore overcome previous shortcomings that have oversimplified inter-aggregate processes. However, further upscaling to in-situ soil would need to consider the differences between a packed tray of aggregates and undisturbed soil.

Previous studies have documented a repeatedly strong relationship between aggregate stability and soil erodibility (Bryan, 1968; Cantón *et al.*, 2009; Nciizah & Wakindiki, 2015), as aggregate stability determines the intrinsic susceptibility and degree of aggregate breakdown (Bryan, 2000). The suitability of aggregate stability as an indicator for soil erodibility has been revisited on numerous occasions. Studies conclude that aggregate stability is a good indicator of soil erodibility despite variations in methods of measurement (Cantón *et al.*, 2009; Ahmadi *et al.*, 2011; Nciizah & Wakindiki, 2015; Ding & Zhang, 2016). Research has often used methods based on the wet-sieving approach (Kemper & Koch, 1966) and the unified framework with three treatments of wetting (Le Bissonnais, 1996) to evaluate aggregate stability and indicate soil erodibility, as previously discussed in Chapter 2 Section 2.1.1.1. While these methodologies enable the separation of breakdown mechanisms, they require the application of mechanical stresses that are not necessarily realistic of field conditions. The approach used here of testing aggregate stability by rainfall simulation has been recognised as the most realistic method of measuring aggregate stability (Almajmaie *et al.*, 2017), as aggregate stability and breakdown is tested through realistic processes, predominantly raindrop impact. The concept and use of aggregate-based research to investigate the soil system has advanced scientific understanding of soil processes. The use of aggregates in laboratory microcosms and soil trays enables the investigation of fundamental soil mechanisms and functional processes at scales relevant to microbes. Aggregates extracted from soil and studied in controlled laboratory experiments increase the replication and repeatability of experiments and constrain the inherent spatial heterogeneity

abundant in soil, offering a starting point for research on complex microbial processes (Hallett *et al.*, 2013). However, it is recognised that there are limitations on the concept of aggregates with some contention surrounding the natural existence of aggregates and usefulness of upscaling results from research conducted on aggregates to the field scale to understand soil system processes (Kravchenko *et al.*, 2019; Wang *et al.*, 2019a; b; Yudina & Kuzyakov, 2019). A common criticism of research completed with aggregates is the breakdown of soil into its constituents, which causes the loss of the soil's inherent internal structure and results in the retraction from realistic field conditions. Individual aggregates have defined boundary conditions that are not analogous to in-situ soil systems (Kravchenko *et al.*, 2019). There is also the issue of size indeterminacy, whereby the operational conditions under which aggregates are extracted has great influence on aggregate size distribution (Díaz-Zorita *et al.*, 2002; Hallett *et al.*, 2013). As such several authors have argued that the repacking of aggregates in soil cores cannot recreate the undisturbed soil (Baveye *et al.*, 2018; Kravchenko *et al.*, 2019). Thus, caution is required in the extrapolation of aggregate-scale responses to processes at larger scales because soil is not the sum of individual components (Kravchenko *et al.*, 2019). Upscaling aggregate dynamics to large-scale processes risks the oversimplification of aggregate and soil processes, omitting the occurrence of synergies and feedbacks within the mechanisms operating at different scales. However, the investigation of aggregates and use of repacked aggregates in soil trays allows the direct examination of fundamental mechanisms that influence aggregate stability (the most important parameter that determines soil erodibility) without multiple confounding effects obscuring key relationships. Without a detailed systematic knowledge of aggregate-scale mechanisms, understanding of the interactions and feedbacks between climatic conditions, aggregate stability and the microbial community cannot be fundamentally developed.

7.4 Wider implications and future research

The research conducted in this thesis raises further research questions (Figure 7.1) on the effects of climatic patterns on aggregate stability and the microbial community (Section 7.4.1), the connection between the microbial community and biological stabilising mechanisms (Section 7.4.2), the quantification of biological stabilising mechanisms (Section 7.4.3) and the multi-way interaction between microbes and the soil physico-chemical environment (Section 7.4.4).

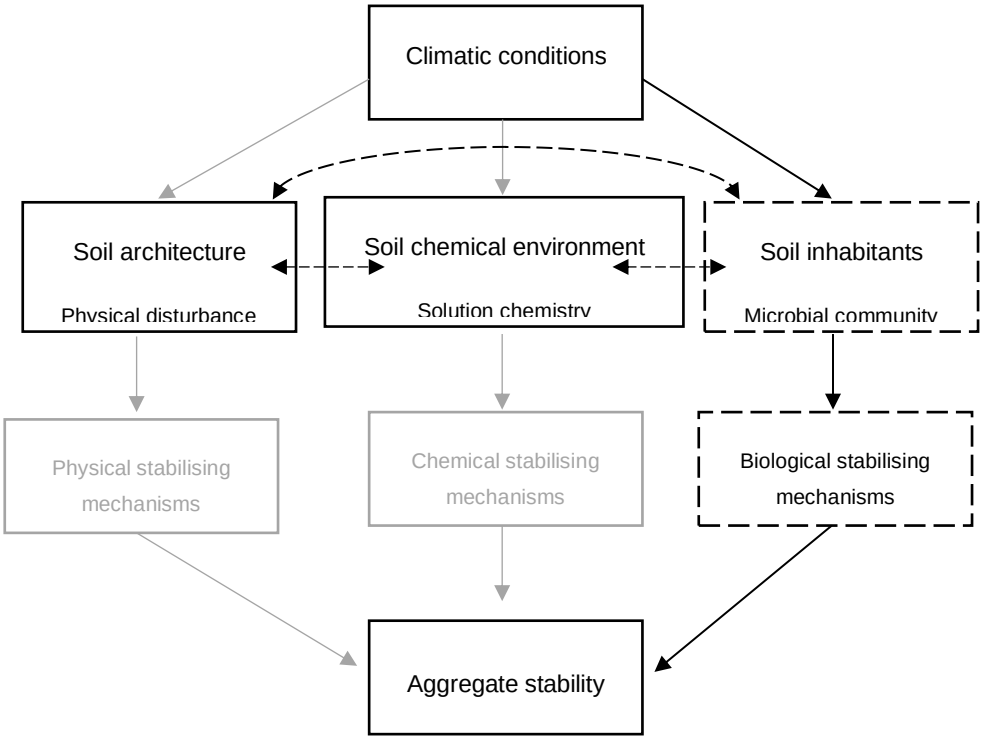

Figure 7.1: Framework of mechanistic interactions between climate, soil physical, chemical, and biological properties and aggregate stability. Black continuous lines show the links where this thesis has contributed to knowledge and raised further research questions on the effects of climatic conditions discussed in Section 7.4.1. Black dashed boxes identify the biological properties and stabilising mechanisms for further research discussed in Sections 7.4.2 and 7.4.3. Black dashed lines show the multi-way interactions between soil properties discussed in Section 7.4.4.

7.4.1 The importance of climatic patterns and the magnitude of microbial response

In this research, changes in aggregate stability and soil erodibility were examined in response to changing climatic conditions of soil temperature and moisture, with other erosive factors held constant. Rain splash erosion was the dominant erosion process considered at the aggregate scale, though other water erosion processes including sheet erosion, rill erosion and gully erosion, as well as wind erosion processes (Bryan, 2000; Toy *et al.*, 2002; Song, 2005) are expected to be affected by climate change (Li & Fang, 2016). This research focussed on climatic changes driving soil temperature and moisture, though climate change will affect other climatic conditions and influence soil erosion rates through the impact on crop biomass and land management practices (Nearing *et al.*, 2004; Zhang & Liu, 2005; Zhang & Nearing, 2005). Climatic changes in temperature and precipitation will also affect rainfall patterns, intensity, and frequency, and thus impact soil erosion processes by influencing climatic erosivity (Pruski & Nearing, 2002; Segura *et al.*, 2014). Furthermore, atmospheric CO_2 concentrations are predicted to change, alongside precipitation and temperature, and future climatic conditions may also be affected by fluctuations in solar radiation levels (Jenkins *et al.*, 2009; Murphy *et al.*, 2018). Whilst the effects of elevated atmospheric CO_2 concentration on soil microbial community composition has experimentally been reported to be relatively small (Castro *et al.*, 2010), the potential for interactive effects between multiple climatic factors is an important consideration.

The potential changing pattern of climatic conditions could cause significant changes in the soil microbial community and aggregate stability. In this research, changing climatic conditions from relatively hot and dry (with summer temperature ranging between 15-30°C dependent on scenario) to cold and wet (with winter temperature ranging between 5-10°C dependent on scenario) in the summer-winter conditions lowered aggregate stability in comparison to constant conditions (Chapter 4, Figure 4.5). Further work should be completed on the influence of sequential climatic conditions, the transition between them, and the

effects of prolonged climatic periods (e.g. droughts) on the microbial community, biological stabilisation and thus soil aggregate stability / erodibility. For example, prolonged drought periods have been observed to significantly impact microbial activity, growth, and microbial community physiology and structure (Hueso *et al.*, 2012; Barnard *et al.*, 2015). The impact of climatic conditions on the microbial community is well recognised, but this has rarely been extended to the effects on biological stabilisation and aggregate stability. Aggregate stability has been shown to be closely related to microbial properties (Chapter 3) with the conclusion that microbial change indicated associated changes in biological stabilising mechanisms and thus aggregate stability. However, seasonal treatments (summer-winter) were observed to significantly influence microbial properties, though did not affect aggregate stability (Chapter 4). Subsequently, it was proposed that the magnitude of microbial change may not have met a threshold to collectively alter aggregate stability. Therefore, the impact of prolonged and sequential climatic conditions on the microbial community should be further examined in the context of biological stabilisation and thresholds of aggregate stability.

Further research should be completed on the impacts of rainfall on the soil microbial community post-rainfall and investigate the subsequent impact on microbial functioning (such as biologically mediated structural recovery). In this research, the rainfall event significantly affected microbial community composition, biomass, and respiration in post-rainfall soil, in comparison to pre-rainfall microbial properties and instigated the selective mobilisation of a component of the microbial community in runoff and infiltrate (Chapter 6). This research also reported the influence of pre-rainfall climatic conditions on the effects of rainfall and suggested that climatic conditions mediated the effects of rainfall on the microbial community by inducing changes in pre-rainfall microbial properties, and thus potentially influencing microbial distribution and susceptibility to mobilisation directly, as well as indirectly by influencing microbially-mediated aggregate stability and biological stabilising mechanisms (Chapter 6). Previous studies have mainly focussed on the export of faecal bacteria following a rainfall

event and the consequences for water quality, but very few studies have investigated the implications of selective mobilisation and transportation of soil microbes for microbial diversity and functioning in post-rainfall soil (Le *et al.*, 2020). Further research should also investigate the effects of multiple rainfall events and rainfall regimes on the soil microbial community and aggregate stability, as well as biologically mediated structural recovery and how this could be accelerated. Climate change will affect rainfall regimes and alter the erosivity and frequency of erosion events as well as affecting soil moisture conditions. Rainfall characteristics, such as intensity, kinetic energy, duration and frequency, and rainfall patterns, have been reported to influence infiltration rates, runoff generation, surface roughness and soil erosion (Ran *et al.*, 2012; He *et al.*, 2018). Several studies have reported effects of altered precipitation patterns on the microbial community (Fierer *et al.*, 2003; Evans *et al.*, 2014; Barnard *et al.*, 2015). Therefore, knowledge should be expanded on the impacts of rainfall events and patterns on the distribution, activity and composition of microbes, and the feedbacks between soil structure and microbial changes.

7.4.2 Connecting the microbial community to functional microbial aggregation mechanisms

In this research soil microbial properties, including community composition, biomass, and respiration, have been used as proxies for microbial stabilising mechanisms (Chapters 3, 4, 5). These soil microbial properties have often been used as indicators for microbially mediated processes, due to the ease and higher accuracy of measurement and sensitivity to land-use and environmental changes (Ritz *et al.*, 2009; Truu *et al.*, 2009). Microbial community composition, biomass and respiration are closely associated to the operation of microbially mediated aggregation through their influence on the dominance of biological aggregating mechanisms (fungal vs bacterial), and nutrient and energy availability and therefore resource allocation to stabilising growth and products.

Whilst microbial community properties are closely related to biological stabilising mechanisms (Lehmann *et al.*, 2017, 2020), further examination of the influence

of changing microbial communities on the delivery of soil system functions should aim to integrate soil microbial community taxonomy and activity with microbial functioning (Zogg *et al.*, 1997; Ranjard & Richaume, 2001; Frostegård *et al.*, 2011). As detailed in Chapter 2, the microbial community mediates soil aggregate formation, stabilisation and turnover in a number of ways. Biological stabilising mechanisms include particle enmeshment by fungal hyphae, and particle reorientation and adhesion by fungal and bacterial exudates, such as glomalin and EPS (Lehmann *et al.*, 2017). However, directly and accurately measuring these mechanisms is methodologically challenging (see Section 7.4.3).

Future examination of microbially-mediated aggregation should further elucidate the relationship between microbial community composition and biological stabilising mechanisms at a higher taxonomic and functional resolution. Physiological traits of fungi and bacteria have been reported to have a significant influence on biological stabilisation, and affects microbial responses to changing climatic conditions (Lehmann & Rillig, 2015; Wood, 2015). Fungal hyphal traits such as hyphal branching and movement have been theorised to influence the effectiveness of biological stabilising mechanisms (Rillig & Mummey, 2006; Lehmann & Rillig, 2015) and empirical evidence reports fungal hyphal density and enzyme activity to be significant traits related to aggregation (Lehmann *et al.*, 2020). Additionally, microbial composition influences multiple properties of exudates and EPS, including production rate, chemical composition, and distribution and residual time in the soil environment (Sutherland, 2001b; a; Sheng *et al.*, 2010), which in turn may influence the effectiveness of EPS for aggregation. For example, characteristics of the chemical composition of EPS, such as chain conformation and internal particle activity, are suggested to alter the adhesiveness of EPS (Berne *et al.*, 2015; Costa *et al.*, 2018). Therefore, climate-driven shifts in microbial community composition within bacterial and fungal populations may also influence physiological traits, thereby affecting the efficacy of biological stabilisation and thus aggregate stability.

7.4.3 Quantifying microbially mediated aggregation

The influence of the microbial community on biological stabilising mechanisms is well recognised, yet the examination and quantification of these mechanism remains a challenge (Figure 7.1). To progress the scientific understanding of biological stabilising mechanisms more precise techniques are required to characterise and quantify EPS, measure in-situ stabilising processes and monitor microbial activity affecting biological stabilisation.

Currently few studies attempt to directly quantify microbial stabilising mechanisms. Methodologies for the direct identification and quantification of EPS are limited due to the broad range of EPS components, numerous extraction procedures and lack of a standardised approach, and the vulnerability of EPS extraction to contaminants (Flemming & Wingender, 2010; Tang *et al.*, 2011; Redmile-Gorden *et al.*, 2014). Furthermore, the extraction method selected affects the quantity and chemical structure of the extracted EPS, so shifts in EPS production and their influence on aggregate stability are difficult to capture. Therefore, measuring the potential changing characteristics of EPS with shifting microbial composition or response remains nearly impossible.

The development and advancement of observational non-destructive techniques, such as high-resolution x-ray computed tomography (CT) scanning, and molecular methods offers the opportunity to enhance our understanding of soil-microbial interactions. CT-scanning can be used for in-situ measurements and could provide further insight on the complex two-way interaction between microbes and soil architecture (see Section 7.4.4), though spatio-temporal measurements are limited with a trade-off between sample size and resolution and currently there are limitations in the differentiation of liquid components (Garbout *et al.*, 2013; Lafond *et al.*, 2015). Therefore, despite great advances in 3D-scanning, structural visualisation and numerical modelling (Mueller *et al.*, 2013; Stuckey *et al.*, 2017), technical and analytical limitations mean it remains challenging to characterise physico-chemical and biological processes at the sub-micron and microaggregate scale (Baveye *et al.*, 2018).

Molecular methods allow very high resolution, taxonomic identification and are not based on our ability to culture organisms, however PCR amplification is a highly sensitive process, which can be significantly influenced by soil physico-chemical properties (such as organic matter, pH), contaminants and the extraction method and PCR programme (Anderson & Cairney, 2004; Carrigg *et al.*, 2007). The application of molecular methods in future research to characterise and monitor the microbial community composition, activity and function could help resolve the challenges of connecting the microbial community to functionality discussed in Section 7.4.2. For example, recent methodological research has designed PCR primers for the amplification of a glomalin-associated gene (Magurno *et al.*, 2019), though further validation is necessary before the application to experimental studies.

7.4.4 Interpreting feedback mechanisms with the two-way interaction between soil architecture and microbes

Aggregate stability is a complex product of physico-chemical characteristics and biological processes and their interactions (Figure 7.1). Our mechanistic understanding of soil aggregate stability and soil erodibility is dependent upon an integrated knowledge of the dynamic physical, chemical, and biological processes operating at the aggregate scale. However, the two-way nature of interactions between soil structure and the soil microbial community leads to complex feedback mechanisms. The soil microbial community is known to influence soil structure, through the hyphal enmeshment of particles and the production of extracellular compounds, causing the mechanical movement of particles and formation and stabilisation of aggregates. In turn, aggregate stability and soil structure influence the microbial community by defining the microhabitat through physical properties, such as particle surface area, pore-water connectivity and resource availability and accessibility, and chemical properties including pH, available cations and pore-water solutes. Soil texture in this research encapsulates the collective effects of physico-chemical properties on the microbial community and aggregate stability and has highlighted the

significance of texture in determining the influence of climatic conditions on the microbial community.

The links between the soil microbial community and soil structure, illustrated in Figure 7.1 as soil architecture, soil chemical environment and soil inhabitants, continue to be a key focus of research for soil scientists. However, more information is needed to fully understand the three-way interaction and feedbacks between soil structure, the soil solution chemistry, and the soil microbial community. For example, there is a limited understanding of how microbial exudates alter soil physico-chemical properties. Investigations of root and seed mucilage and microbial EPS have revealed their significant influence on pore-scale structures and soil hydrological properties, such as water retention, hydrophobicity, and evaporation (Roberson & Firestone, 1992; Carminati *et al.*, 2017; Kroener *et al.*, 2018; Zheng *et al.*, 2018; Benard *et al.*, 2019). Additionally fungal exudates, such as glomalin, have long been suspected to encourage soil hydrophobicity although investigations have been inconclusive (Feeney *et al.*, 2004; Morales *et al.*, 2010). There is also evidence to suggest that changes in physico-chemical conditions, such as pH and electrolyte concentration of the soil solution, can alter microbial processes and products such as the composition of EPS (Or *et al.*, 2007a). Further research is required to understand how soil structure shapes the biological community. The complex heterogeneity of soils should be considered at the aggregate scale, as the distribution of organic matter, substrates and the bacterial communities are influenced by aggregate structure and occlusion (Ranjard & Richaume, 2001; Bach *et al.*, 2018). Research on substrate and microbial distribution at the aggregate scale is necessary to further elucidate the interaction between microbes and microhabitat and the effects on microbial interactions and processes, yet in-situ monitoring remains a key challenge in soil microbial ecology (Bach *et al.*, 2018). To further advance research on aggregate stability, the interactions and feedbacks between microbes and soil physico-chemical properties should be holistically incorporated into understanding of the microbial response to climatic conditions and the effects on biological stabilising mechanisms, aggregate stability, and soil erodibility.

8 Conclusions

This research generated new knowledge on how soil temperature and moisture content affect the soil microbial community, aggregate stability, and soil erodibility. This is the first study, in a new approach, to use aggregate microcosms to examine the effects of temperature and moisture on the microbial community as indicators of shifts in biological stabilising mechanisms.

These are the key findings from the research:

1. Soil temperature and moisture significantly affected aggregate stability, and the soil microbial community which alters aggregate stability, dependent on soil texture (Chapter 3, objective 1).
2. Aggregate stability was not significantly affected by the differences between seasonal treatments representing future climatic scenarios, though it was significantly lower under seasonal treatments, summer-winter, compared to constant season treatments, summer-only or winter-only (Chapter 4, objective 2).
3. Soil erodibility was significantly affected by soil temperature and moisture due to their influence on aggregate stability and the soil microbial community (Chapter 5, objective 3).
4. The simulation of a rainfall event significantly affected the relative abundances of microbial groups, microbial biomass, and respiration in post-rainfall soil (Chapter 6, objective 4). The rainfall event also mobilised differing components of the microbial community in the runoff and infiltrate, dependent on soil temperature and moisture before the rainfall event.

Soil temperature and moisture had significant effects on aggregate stability and the microbial community, and soil texture had a strong influence in the first set of experiments (Chapter 3). Soil microbial properties were significant predictors of aggregate stability, and so the hypothesis that soil moisture and temperature influence aggregate stability through the impact of these climatic conditions on the microbial community was supported. Soil texture altered the response of aggregate stability and soil microbes to climatic conditions and the significance

of microbial properties as predictors of aggregate stability (Chapter 3). The relationship between aggregate stability, microbial properties and soil texture emphasised the importance of soil physico-chemical properties (e.g. particle size distribution, porosity). For the sandy loam, aggregate stability was significantly negatively affected by soil moisture and multiple soil microbial properties were significant predictors for aggregate stability (Chapter 3, Figure 3.2, Table 3.3). For clay aggregates, temperature had a significant positive effect on aggregate stability and the relative abundance of gram-negative bacteria was the only significant microbial predictor for aggregate stability (Chapter 3, Figure 3.2, Table 3.3). The differences in the role of the microbial community for aggregation between soil textures may be a result of a greater influence of physico-chemical aggregating mechanisms with higher clay content and therefore greater proportion of electrostatically charged particles and subsequently stronger physico-chemical particle interactions. Additionally, the variation in physical and chemical properties of the two soil textures, such as clay content, particle size and porosity, influenced the impact of soil moisture content on the microhabitat and therefore generated complex feedbacks between soil architecture and the microbial community.

In Chapter 4, aggregate stability was not affected by differences in the climate scenarios (current, moderate, and high) of the seasonal treatments (summer followed by winter), representing predicted warming and changes in soil moisture content based on UKCP09 emissions scenarios. However, seasonal treatments did affect microbial community composition, biomass, and respiration in clay aggregates, though not in loamy sand aggregates, with the exception of the relative abundance of gram-negative bacteria (Chapter 4). The differences between climate scenario treatments generally did not cause significant microbial change in loamy sand aggregates, and thus may not drive changes in biological aggregating mechanisms and aggregate stability. Therefore, the hypothesis that seasonal conditions under current and future climate scenarios would affect aggregate stability through a climate-driven shift in the microbial community was not accepted. However, in clay aggregates the significant effects of climatic

conditions on the microbial community, but not on aggregate stability, indicated the potential existence of a threshold in the magnitude of change in the microbial community required to impact aggregate stability and soil erodibility (Chapter 4, Section 4.4.1).

Aggregate stability under seasonal treatments of two seasons (summer and winter) was significantly lower compared to the constant season treatments (summer-only or winter-only; Chapter 4). The responses of microbial biomass and respiration to the seasonal treatments and constant seasons varied, with biomass stimulated under the seasonal treatments (summer-winter) in both soil textures. However, microbial respiration was stimulated in the loamy sand, but suppressed in clay aggregates under the seasonal treatments (summer-winter) in comparison to the constant season treatments. As justified in Chapter 4, the seasonal treatments started with summer conditions were expected to place the microbial community under stress, particularly in the high seasonal treatment, followed by winter conditions with high moisture content creating a physical perturbation. However, given the significant differences between the seasonal treatments and constant seasons it would be interesting to further examine the effects of winter-summer incubations and the transition between seasons as discussed in Chapter 7, Section 7.4.1.

Soil temperature and moisture content during incubation were found to significantly influence soil erodibility and erosion (as measured by sediment loss) of aggregate-packed soil trays in Chapter 5. At this scale, soil temperature and moisture content also significantly affected microbial respiration in the loamy sand and microbial community composition in the clay soil. Therefore, the hypothesis that climatic conditions would affect soil erodibility was accepted, with explanatory evidence that climatic conditions affected the soil microbial community and thus aggregate stability and soil erodibility. Another explanatory mechanism may have been in operation; aggregate stability is a key determinant of soil structural properties such as porosity, and therefore also influences hydrological processes such as the generation of runoff and infiltration (Amézketa, 1999; Ding *et al.*, 2019). However, these hydrological processes

were not affected by climatic conditions (i.e. soil temperature and moisture in the current study), therefore the change in sediment load due to soil temperature and moisture can be directly attributed to changes in aggregate stability affecting soil erodibility.

The erosive rainfall event in Chapter 6 had a significant effect on soil microbial composition, biomass, and respiration. In clay, rainfall significantly reduced microbial respiration and biomass in eroded soil compared to the control soil. For both soil textures, generally rainfall increased the relative abundance of gram-negative bacteria and reduced the relative abundance of fungi. The effects of rainfall on the relative abundance of gram-positive bacteria were dependent on soil texture. A differing component of the microbial community was mobilised in the runoff and infiltrate, dependent on the climatic treatment. Soil temperature and moisture may have influenced soil microbial distribution or motility, thus altering the rainfall-mobilised community composition. This observation of the influence of rainfall on microbial distribution has implications for microbial functioning post-rainfall, future erosion events, and the transportation of the mobilised microbial community in the runoff and infiltrate which are discussed in Chapter 7, Section 7.4.1.

The research presented here is at the forefront of investigations at a relevant scale for the microbial stabilisation of aggregates and the upscaling of microbial function to aggregate stabilisation and soil erodibility. Thus, the focus on aggregates in this study has advanced research in critical knowledge gaps on the links between climatic conditions, aggregate stability and the soil microbial community and measured the response of aggregates and microbes at scales relevant to both, microorganisms and soil erodibility.

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APPENDICES

Appendix A General Methodologies

This appendix provides broader information on the methodologies regularly used in this research. Specific details for the methods applied are provided in each chapter.

A.1 Laboratory microcosms:

In order to investigate internal aggregate stabilising mechanisms aggregates were constructed in a single layer microcosm. Custom-made microcosms were created and tested for use in all stages of the experiments based at the aggregate-scale including the aggregate wetting up, treatment period, and aggregate stability testing underneath the rainfall tower. Microcosms were constructed from embroidery hoops with mesh netting to act as the base on which the aggregates rested. Aggregates were held in sufficient contact with the wetting bed to enable slow wetting by capillary action. The mesh used had a regular aperture size of 1.3 mm from Wondermesh. Various materials were tested for the microcosm structure, mesh, and wetting bed, and selections were made based on practicality.

A.2 Temperature and soil moisture content

Soil moisture content was controlled during incubations with the addition of a cotton wool wetting bed to the microcosm. Standardised volumes of deionised water were added to the microcosms dependent on the climate treatment. Moisture content is reported as a percentage and calculated as wet soil/dry soil x 100 (minus crucible weights). This is the equivalent to gravimetric water content, which is (wet weight-dry weight)/dry weight, which gives a decimal figure. For example, where soil moisture content is reported as 37%, gravimetric water content is 0.37.

There were slight variations in soil moisture between soil textures, as physico-chemical properties related to texture adjusted water capillary uptake and soil water storage. Whilst equal matric potential would promote direct comparison between soil textures, in the context of future climate change the input (precipitation) was consistent for both soil textures representing equal precipitation levels and so matric potential will vary in the field naturally.

A.3 Aggregate stability and rainfall simulation

Aggregate stability is broadly accepted as the most suitable indicator for soil erodibility, the susceptibility of soil to erosion processes (Bryan, 1968, 2000; Cantón *et al.*, 2009; Ahmadi *et al.*, 2010; Nciizah & Wakindiki, 2015).

As discussed in Chapter 2, Section 2.1.1.1, there are four main mechanisms of aggregate breakdown including slaking, differential swelling, raindrop impact and dispersion (Le Bissonnais, 1996). Partly as a result of these multiple processes of aggregate breakdown, there have been numerous methods developed to test aggregate stability (Amézketa, 1999). For example, Le Bissonnais (1996) developed the unified framework for stability assessment which combines three treatments; fast wetting, slow wetting and stirring after pre-wetting and measures the resultant fragment size distribution from each method of breakdown. However, methods based on the physical disruption of aggregates through the application of mechanical stresses are far removed from realistic process operating in the field, such as the effects of raindrop impact and dispersion (Almajmaie *et al.*, 2017). Testing aggregate stability by rainfall simulation incorporates the effect of raindrop bombardment and is considered to best represent realistic disaggregation processes (Almajmaie *et al.*, 2017). Therefore, in this research aggregate stability was tested by the rainfall simulation method to best represent field conditions and realistic aggregate breakdown mechanisms.

A.4 Microbial respiration

Microbial respiration was measured by rapid automated bacterial impedance technique (RABIT), based on the protocols by Ritz *et al.* (2006) and Don Whitley Neil, UK. The method monitors the level of conductance in alkaline agar in the RABIT cell over time, which declines as the agar absorbs CO_2 produced by the soil microbes. Temperature has a strong effect on the conductivity of the cells and so was maintained at 25°C for all samples. To reduce methodological error, three replicates were monitored for every sample and the average reading calculated. Readings 1.5 times the interquartile range above or below the first and third quartiles were identified as outliers and removed from the dataset.

A.5 Microbial biomass carbon

When investigating the function of the microbial community, microbial biomass is often measured and used to infer potential changes in activity. Microbial biomass carbon is often the parameter measured and can be evaluated by chloroform fumigation extraction. Chloroform fumigation extraction (CFE) uses chloroform fumigation to kill and lyse microbial cells and thereby releasing previously immobilised carbon. The resulting carbon flush can be monitored and calculated by comparing fumigated and non-fumigated samples. Carbon standards were used to create a calibration curve with 1000mg C/litre stock with potassium hydrogen phthalate mixed with potassium sulphate sodium polyphosphate for 10/20/30/40/50 mg C/litre calibration curve.

A.6 Phospholipid fatty acid analysis

Biomarker methods use signature molecules as indicators of microbial biomass or community structure. Several prerequisites have been identified for biochemical compounds to be used as biomarkers. Firstly, the measured compounds must be present at known concentrations (Frostegård *et al.*, 2011). Secondly, biomarker molecules must exhibit a high level of taxonomic specificity to enable identification (Ruess & Chamberlain, 2010). Thirdly, the compounds must be unstable outside the cell in the soil in a free state and must represent only living organisms (Frostegård *et al.*, 2011). This requires the biomarker molecule to exhibit rapid degradation. Finally, the measured compounds must be accurately and precisely extracted from soils to allow quantification. Microbial membrane components and fatty acids can be used as biomarkers. Phospholipid fatty acids (PLFAs) are present within the cell membrane. PLFAs are hydrocarbons with a hydrophilic phosphate head and hydrophobic fatty acid tail; it is the atomic structure of the tail that is used to distinguish different PLFAs. PLFA analysis uses these molecules as biomarkers based upon the assumption that different PLFAs correspond to different microorganisms and their phenotypic response to environmental conditions. The presence of PLFAs can be identified from pure cultures; however the specificity of PLFAs can sometimes be questionable (Ruess & Chamberlain, 2010). The mass profile produced shows the phospholipid classes present and their relative abundance. PLFA analysis can therefore be used to monitor changes in the whole community or in specific groups of microorganisms (Frostegård

et al., 2011). PLFAs generally fulfil the criteria established for use as biomarker molecules. Aside from those within microorganisms, the concentration of lipids within the soil is usually low. Additionally, it is thought PLFAs are rapidly turned over following cell death, although there is a call for more empirical data supporting this**.** Methods for sample preparation have largely been standardised and analysis often completed using gas-chromatography (Ruess & Chamberlain, 2010).

PLFAs were extracted from 10g of soil with the addition of 20 ml of Bligh and Dyer solution, sonication and centrifugation. The supernatant was mixed with 4 ml of chloroform and citrate buffer and left overnight at 4°C in dark conditions to separate. The upper layer was then removed with a vacuum pump and the lower layer dried under nitrogen and resuspended in 1 ml of chloroform. The extracts were then loaded on to solid phase extraction cartridges washed twice with 2 ml chloroform. Elutions with 5 ml chloroform and 12 ml acetone removed non-target lipids, then phospholipids were eluted with 8 ml of methanol and dried under nitrogen. The extracts were then methylated and centrifuged. The supernatant with the phospholipids was resuspended in hexane, filtered through glass wool, and dried under nitrogen. Phospholipid extracts were then resuspended in 200 µl hexane ready for analysis by gas chromatography with flame ionisation detector (GC-FID) and identified by retention time. PLFAs were assessed as the relative abundance of the total area of identified peaks and grouped to represent the relative abundances of fungi, gram-negative bacteria, and gram-positive bacteria (Table A.1).

Table A.1: Microbial groups identified by PLFA peaks

Microbial group	PLFAs	Reference
Gram-positive bacteria	i15:0, ai15:0, i16:0, 17:0 i17:0 and ai17:0	Bardgett et al., 1996; Ruess and Chamberlain, 2010; Zelles, 1999
Gram-negative bacteria	Trans16:1ω11, cis16:1 ω7, cyc17:0iso, cis17:1ω8, cis17:0, trans17:1ω8, cis19:0	Bardgett et al., 1996; Ruess and Chamberlain, 2010; Zelles, 1999
Fungi	16:1ω5, 18:2ω6, cis18:1ω9	Frostegård et al., 2011, 1993; Frostegård and Bååth, 1994
Other measured PLFAs	14:0, 15:0, i16:1, 16:0, Me17:0iso, Me17:0iso2, cyc17:0iso. 17:0br, 17:1ω 7, 17:0(12Me), 18:1ω7t, 18:1ω13, 18:0, 19:1ω6, 18:0(Me), 20:4(5,8, 4, 11,14), 20ω5(3), 20:0.	

Appendix B Supplementary information for Chapter 4

B.1 Climate projections

The climate projections from UKCP09 were superceded by UKCP18 projections (Lowe *et al.*, 2018), but are still aligned. In Chapter 4, the moderate climate treatment is based on the medium emissions scenario in UKCP09 and aligns with the SRESA1B projection from the UKCP18, and the high treatment, based on the high emissions scenario in UKCP09, aligns with the RCP8.5 projection from the UKCP18, and is most similar to SRESA1FI (Lowe *et al.*, 2018; Murphy *et al.*, 2018).

Table B.1: Corresponding future climate projections from UKCP09 and UKCP18.

Seasonal treatment in Chapter 4	Corresponding future climate projection in UKCP09 (Jenkins *et al.*, 2009)	Corresponding future climate projection in UKCP18 (Lowe *et al.*, 2018; Murphy *et al.*, 2018)
Current	n/a	n/a
Moderate	medium emissions scenario	SRESA1B
High	high emissions scenario	RCP8.5 (SRESA1FI)

B.2 Summer midpoints for seasonal treatments

The seasonal treatments in Chapter 4, were initially held under summer conditions in three climate scenarios (current, moderate, and high). The seasonal treatments lasted two and four weeks with the initial summer period lasting one and two weeks, respectively. Therefore, the microbial community after the initial summer period represents the mid-point of the seasonal treatments.

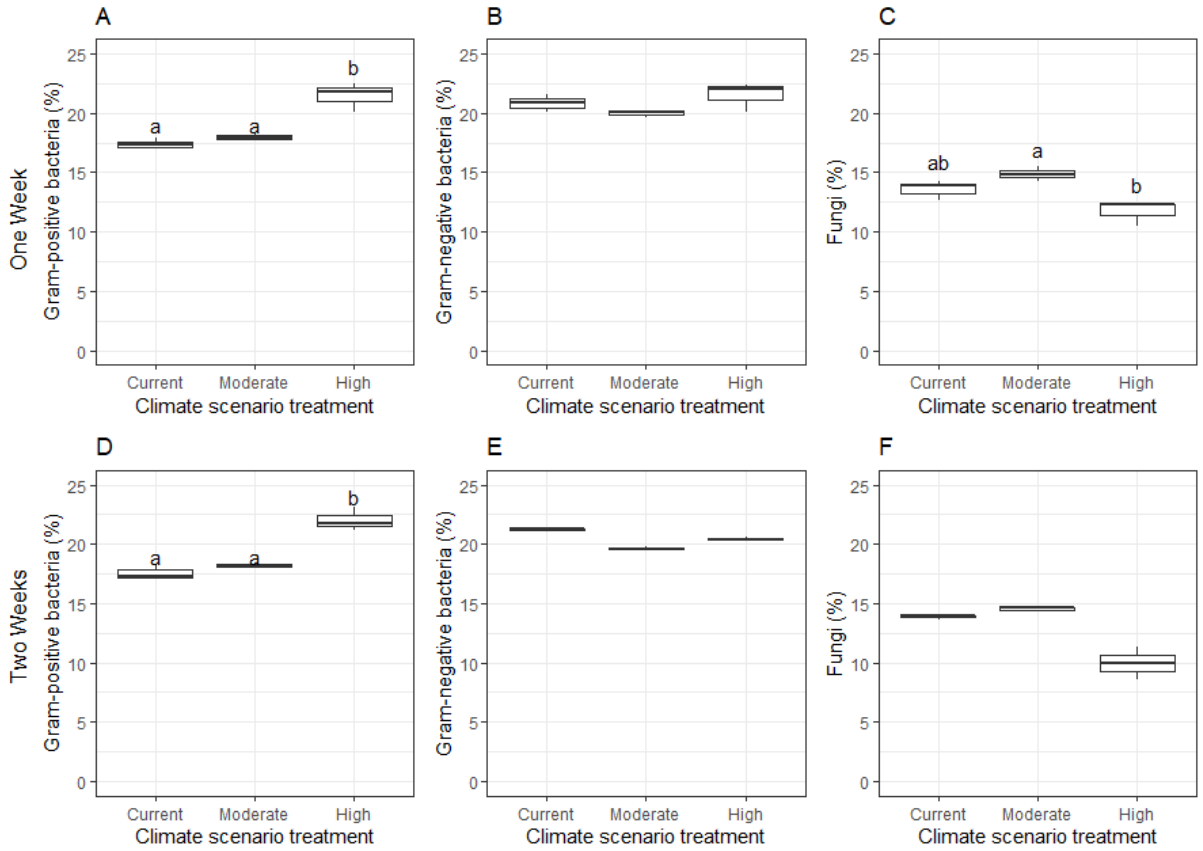

Figure B.1: The relative abundances for gram-positive bacteria, gram-negative bacteria, and fungi in the loamy sand under summer conditions after one week incubation (A-C), and two weeks incubation (D-F).

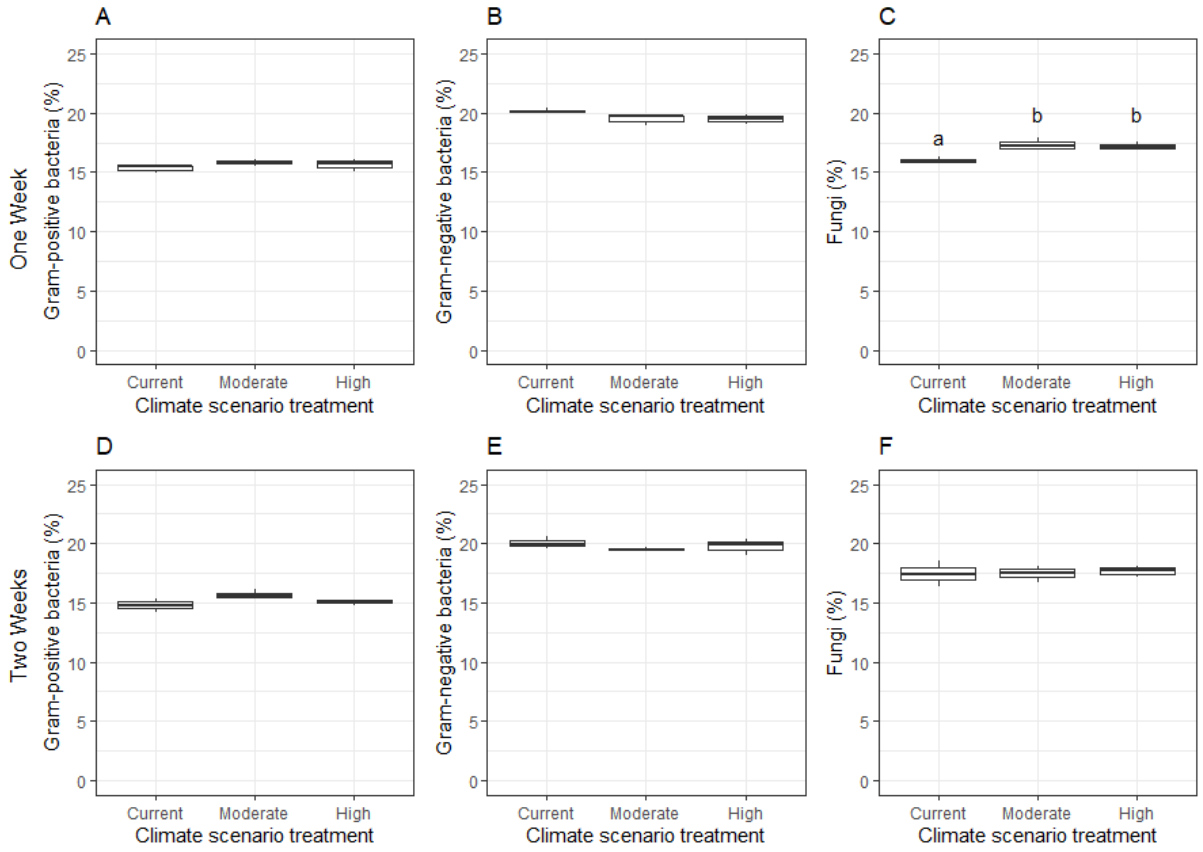

Figure B.2: The relative abundances for gram-positive bacteria, gram-negative bacteria, and fungi in the clay under summer conditions after one week incubation (A-C), and two weeks incubation (D-F).

Appendix C Baseline soil sampling

Fresh soil was collected in advance of each experiment (Table C.1). As the laboratory experiments spanned three years, these samples were roughly taken one year apart. The baseline soil properties, such as particle size distribution, organic matter content, soil pH, aggregate stability, microbial community composition, respiration, and biomass were measured to monitor the starting point for each experiment. Whilst all effort was made to ensure that soil was collected from the same location, there was some variability which means that baseline soil properties and so the starting point for experiments were slightly varied. Plus, intra-annual variability in climatic conditions or short-term weather patterns could have influenced the composition and ecological strategies of the microbial community.

Table C.1: Summary of baseline physical, chemical and biological characteristics for two soil textures in each chapter.

Chapter	Soil Classification	Sand content (%)	Silt content (%)	Clay content (%)	Organic matter content (%)	pH	Aggregate stability (%)	Microbial biomass	Microbial respiration	Relative abundance of gram-positive bacteria (mol%)	Relative abundance of gram-negative bacteria (mol%)	Relative abundance of fungi (mol%)	Collection date
Ch. 3	Sandy loam	68.93	19.76	11.31	6.0	5.22	81.5	276.9	0.35	18.15	20.07	11.22	September 2017
Ch. 3	Clay	41.52	14.95	43.53	6.0	7.93	90.1	232.4	2.80	14.58	21.18	16.70	September 2017
Ch. 4	Loamy sand	87.44	1.74	10.82	3.9	6.75	58.6	27.48	2.97	15.49	21.33	18.48	November 2018
Ch. 4	Clay	13.69	12.31	74.00	7.1	8.10	88.9	156.54	1.63	13.74	20.84	19.88	November 2018
Ch. 5	Loamy sand	86.38	4.81	8.81	4.0	5.48	N/A	41.16	0.77	18.22	20.56	14.15	August 2019
Ch. 5	Clay	13.05	21.01	65.94	6.7	8.40	N/A	57.62	2.27	12.67	22.49	19.47	August 2019