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IMPACT OF CLIMATE CHANGE ON SOIL RESPIRATION AND RESILIENCE

By

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Dedication

I dedicate the entire research work to Almighty God, the source where every blessing and understanding have come to me.

Declaration

This work was carried out by me and the use of materials from other works have been duly acknowledged.

Signature:

Date: 21st May, 2021.

Abstract

Understanding the mechanisms by which soil respiration responds to climate change is critical to predicting and mitigating future global warming. Changes in temperature and moisture are known to influence soil processes like soil organic matter decomposition and heterotrophic respiration which are mediated by soil microorganisms. For better predictions of microbial respiration, there is a need to understand the importance of microbial community composition and incorporate the processes they mediate in predictive climate models. However, the accuracy of such models could be enhanced by accounting for high frequency temperature fluctuations (e.g. daily maximum and daily minimum temperature). This study explored the complex mechanisms by which soil microbial communities react to temperature change both in the laboratory and the field. The overall aim was to examine how the legacy effect of soil temperature conditions affect the temperature sensitivity of soil respiration.

In a pilot study, sieved or intact soil cores from arable, grassland and woodland land use types were incubated for 42 days and soil CO_2 flux measured over time. The results showed that physical disturbance did not significantly influence soil respiration. Differences in soil respiration rate between land use types were due to contrasting soil water holding capacity and the quantity and stoichiometry of soil organic matter.

While our climate warms, a reduced Diurnal Temperature Range (DTR) has been observed over the last 50 years as daily minimum temperature has increase more than daily maximum temperature, due to global climate change. However, the relative importance of these short-term diurnal temperature oscillations, compared to the overall increase in daily average temperature, is unclear. Especially since most laboratory measurements of soil respiration are made at constant temperatures. The same grassland soil used in the pilot experiment was incubated at four different temperature regimes including constant incubation at temperatures representing the daily minimum (5°C), daily mean (10°C), daily maximum (15°C), and diurnal oscillation between average daily minimum and maximum (5-15°C) temperature of the area the soil was collected from for 17 weeks and CO_2 flux measured over time. CO_2 released from the oscillating incubation was similar to that from the maximum incubation temperature, not the average incubation temperature. Daily maximum temperature dictates the composition of soil microbial community. Changes in soil biological and chemical properties due to temperature change was consistent with apparent thermal acclimation. It was therefore

concluded that daily maximum rather than daily average temperature determines the flux of CO_2 from soil, thus challenging the justification for researchers performing experiments by incubating samples at the daily mean temperature without oscillation.

It was unclear whether the importance of daily maximum temperature was due to its influence on intracellular or extracellular enzyme activity rates. Extracellular enzymes are considered to catalyse the rate limiting step in organic matter decomposition. To investigate the temperature sensitivity of extracellular and intracellular enzymes, two extracellular enzymes (β-glucosidase and chitinase), intracellular enzyme activity (glucose-induced respiration), and basal respiration were assayed at a range of temperatures (5°C, 15°C, 26°C, 37°C and 45°C) after the same grassland soil used in the previous experiments was pre-incubated at 5°C, 15°C, or 26°C. The aim was to assess whether both extracellular and intracellular enzyme activities are equally sensitive to temperature and whether pre-incubation temperatures, which should create a gradient of acclimation, influences these processes. The result revealed that pre-incubation temperatures influenced the temperature sensitivity of both extracellular and intracellular enzyme activities, and that these two enzyme-mediated processes were not equally sensitive to temperature. While intracellular enzyme had higher temperature sensitivity in the range 15°C - 26°C, extracellular enzymes had higher temperature sensitivity in the range 26°C - 37°C. Thermal acclimation was also observed and attributed to temperature-induced changes in stoichiometry (C/N ratio) of soil organic matter and soil chemistry (pH).

Finally, attempt was made to observe the impact of soil warming on the thermal adaptation of the soil microbial community under field conditions. The aim was to examine whether the addition of soil organic matter through cover crop residue incorporation mitigates the effect of simulated climate change (winter warming) on the resilience of the soil microbial community to wet and dry cycles. Custom designed Open Top Chambers (OTCs) were deployed and demonstrated to effectively, but inconsistently, warm soils above the ambient temperature. However, the laboratory component of the work could not proceed due to COVID-19 pandemic.

Overall, I can conclude that (i) soil microbial communities thermally adapt to changing temperature, (ii) changes to daily maximum temperature are more important than changes in daily average temperature, and (iii) this is because extracellular enzymes activity is the rate limiting step in organic matter decomposition and this step is more sensitive to temperature changes during warm days than cool days. (v) Temperature driven changes in soil chemical and biological properties influenced the thermal adaptation of soil microbial respiration.

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Chapter 1

1.0 General Introduction

1.1 Introduction

Rising temperatures and an increase in the frequency and magnitude of precipitation events have been experienced globally, and yet further rises are predicted for the future as a result of global climate change (Meyer et al., 2018; Knapp et al., 2015; Ye et al., 2018). During the 21st century, temperature is expected to rise between 1.1 °C and 6.4 °C, globally (Brevik, 2012). Climate change has resulted in a decrease in the number of cold days and nights and an increase in the number of warm days and hot nights experienced in most land areas globally (Jia et al., 2019).

The use of meaningful daily temperature data (e.g. daily maximum and minimum temperature of an area) is recommended when designing regional or global climate change experiments, analysing climate model projections, and when studying the response of the terrestrial ecosystem to warming (Ye et al., 2018). This recommendation is based on the fact that the use of daily average temperatures alone cannot effectively represent the extremes of temperature (cold or warm) experienced by an ecosystem. However, a plethora of ecological studies investigating the effects of climate change on community-level interactions within ecosystems incubate mesocosms to a constant temperature that represents the mean temperature of the study area to gain insights into the temperature sensitivity of the activity or functions of ecological communities (Thompson et al., 2013; Barton and Schmitz, 2018).

Our changing climate will influence soil ecosystems because soils have a complex interaction with atmosphere-climate system through carbon, nitrogen and hydrological cycles (Brevik, 2012). Soil is the largest terrestrial carbon pool (Brevik, 2012; Lal et al., 2018; Lal, 2020), but it also provides a habitat for diverse and complex communities of organisms (Bardgett and Putten, 2014). Soil acts as a huge potential source of volatile carbon and a potential sink for additional carbon. Soil can therefore buffer CO_2 losses into the atmosphere, depending on the balance between photosynthesis, autotrophic respiration (respiration by plants), and heterotrophic respiration (decomposition of organic matter by soil heterotrophic microbes) (Dungait et al., 2012; Jia et al., 2019) . This balance exerts major controls on the biogeochemical interactions between land and atmosphere leading to exchange of greenhouse gases like CO_2, CH4 and N20 (Jia et al., 2019), the losses of which could cause positive feedbacks in our climate system (Crowther et al., 2015; Bradford et al., 2016). While the

response of autotrophic respiration to changing climates has been well research, predicting changes to the carbon sink in soil due to climate change has been a major source of uncertainties in projections because, although it is known that increasing temperature can stimulate microbial degradation of soil organic carbon thereby increasing the atmospheric concentration of CO_2 (Bardgett et al., 1999; Bardgett et al., 2008; Bradford et al., 2016), the magnitude of this positive feedback is unclear.

Soil microbial respiration remains an important flux of CO_2 into the atmosphere since it contributes about half of the annual C emissions (119 Gt C) from terrestrial ecosystems (Auffret et al., 2016; Jia et al., 2019). Making proper quantitative or mechanistic predictions of how various environmental factors influence this flux of carbon remains difficult (Hartley and Ineson, 2008; Dungait et al., 2012; Jia et al., 2019). Although there is much research data quantifying the impact of warming on soil microbial respiration, the magnitude of responses to warming varies among the studies (Jia et al., 2019), and remains the subject of research due to large uncertainties that persist (Kirschbaum, 2006; Moinet et al., 2020). It is generally understood that the rate of soil respiration approximately doubles for each 10°C increase in temperature (Davidson and Janssens, 2006; Paz-Ferreiro et al., 2012). However, this generalisation neglects the possibility of acclimation of the soil microbial community to higher temperatures. Furthermore, the generalisation also contrasts with observations that soil temperature increases soil microbial respiration up to a maximum (optimum) temperature, after which soil microbial respiration declines with further increases in temperature (Carey et al., 2016). The decline in soil respiration above such optimum temperature has been attributed to the decomposition of readily decomposable (labile) organic carbon, leaving only recalcitrant carbon remaining at higher temperatures (Auffret et al., 2016). Earlier studies have shown that over the longer term, the temperature sensitivity of recalcitrant carbon will contribute more to carbon losses from soils than labile carbon (Hartley and Ineson, 2008) and a recent study showed that temperature sensitivity will decrease with increasing carbon stability over seasonal and daily timescales, against Arrhenius kinetics (Moinet et al., 2020).

The inconsistencies in research findings open windows to further research into the mechanisms by which microbial contributions to CO_2 emissions respond to both short- and long-term temperature changes, the extent to which microbial communities thermally adapt to new temperature regimes, and how this adaptation contributes to positive feedbacks of warming on the flux of CO_2 to the atmosphere.

1.2 General aim

The aim of the current study is to examine how legacy effects of soil temperature conditions affect the temperature sensitivity of soil respiration. In this study, we define legacy effect as the previous (historical) condition to which soil was exposed to (e.g. a previous temperature regime).

1.3 Research questions

Does historical temperature regime influence future soil respiration?

What are the mechanisms by which historical temperature regime influences soil respiration?

1.4 Overall hypothesis

Previous (historic) conditions alter soil microbial community structure and influence the response of soil respiration to future conditions.

1.5 Specific hypotheses addressed in each individual chapter:

- Chapter 3: Physical disturbance of soils increases the short-term soil respiration rate.
- Chapter 4: Diurnal oscillation of soils between daily minimum and daily maximum temperatures results in different temperature sensitivity of soil respiration, compared to incubation at daily mean temperature.
- Chapter 5: A previous incubation temperature influences the temperature sensitivity of extracellular depolymerisation and intracellular catalytic enzyme activity.
- Chapter 6: The addition of soil organic matter through cover crop residue incorporation mitigates the effect of simulated climate change (winter warming) on the resilience of the soil microbial community to wet and dry cycles.

1.6 Outline of the thesis

In Chapter 2, a review of recent literature on the impacts of climate change on soil respiration was carried out to form a knowledge base upon which the project was carried out.

In Chapter 3, a laboratory experiment was conducted to examine the effect of sieving on *ex situ* soil respiration from soils collected from three major UK land uses (arable, grassland and woodland). The overall objective was to help make initial decisions on the choice of assay methods (i.e. intact cores or sieved soils) and sampling strategy and identify possible differences between land use types. The chapter has been published as a paper in the *Journal of Soil Science and Plant Nutrition* (https://doi.org/10.1007/s42729-020-00177-2)

In Chapter 4, the grassland soil used in Chapter 3 was resampled and incubated under a variety of different constant and diurnally oscillating temperature regimes representing the average daily minimum, average daily maximum, average daily mean, and diurnal oscillation between average daily minimum and average daily maximum temperatures of the site from which the soil was sampled. Specifically, the temperatures include constant temperatures at 5 °C, 15 °C, 10 °C and diurnal oscillation between 5 °C and 15°C. Respiration of each of these soils incubated at these various temperature regimes (incubation temperatures) were then measured periodically at three measurement temperatures (5 °C, 10 °C and 15 °C). Soil microbial community composition (using PLFA) and range of soil chemical properties were then analysed from the soils at each incubation temperature after 17 weeks (119 days) of incubation. The chapter is formatted for submission to *Global Change Biology Journal*.

In Chapter 5, intracellular and extracellular enzyme assays were performed on the same grassland soil used in Chapter 4 at a range of assay temperatures (5 °C, 15 °C, 26 °C, 37 °C and 45 °C) after the soil was acclimatized to three pre-assay temperatures (5 °C, 15 °C and 26 °C) for 60 days. The temperature co-efficient (Q10) of each assay on soils pre-incubated at those three temperatures was calculated to assess whether the temperature sensitivity of both the extracellular and intracellular component of soil organic matter decomposition were equally sensitive to temperature and whether pre-assay temperature influenced temperature sensitivity. I also examined temperature sensitivity using Arrhenius activation energy and derivatives of Macromolecular theory (MMRT). The chapter is formatted for submission to *Soil Biology and Biochemistry*.

In Chapter 6, I introduced Open Top Warming Chambers (OTCs) to warm the soil surface of a field plot experiment in which cover crops (single species monocultures and 4-species polycultures) were grown over the summer in between autumn sown cash crops in a cereal rotation. The aim of the experiment was to assess the legacy effects of winter warming and cover crop incorporation on soil microbial community composition, soil properties and the resilience and resistance of soil respiration to repeated drying and rewetting perturbations. However, due to the COVID19 pandemic, I could not conclude the experiment. The chapter presents only the proposed idea and the temperature data collected from data loggers installed in the field to quantify the warming that occurred under the OTCs.

In Chapter 7, major findings from the results of the experiments are discussed and general conclusions are made.

Chapter 2

2.0 Literature review

2.1 Carbon Cycle

The carbon cycle (Figure 2.1) is a variety of processes that take place over time scales ranging from hours to millennia. Processes occurring on a short term (hourly, daily or seasonal) basis include photosynthesis, respiration, air-sea exchange of carbon dioxide, and organic matter accumulation in soils (Berner, 2003). They are continuous phenomena whereby, plants take up carbon dioxide from the atmosphere in the process of photosynthesis to create glucose, starch, and other carbohydrates which humans and animals consume as food, or dwell in as shelter to sustain life. When plant leaves and roots are shed or senesced, dead organic matter is formed (detritus) in a process called decomposition. The detritus is a substrate that supports both animal and microbial lives. In their metabolic process, animals and microbes respire (heterotrophic respiration) to release CO_2 into the atmosphere (Waring and Running, 2007). Although carbon is being naturally released into the atmosphere through oceanic and geological processes, human activities, such as the burning of fossil fuels, can also enrich the atmosphere with carbon (CO_2). Both the oceanic and geological processes and the other activities involving plants, animals, humans and other natural systems returns carbon to the atmosphere and then renews the cycle.

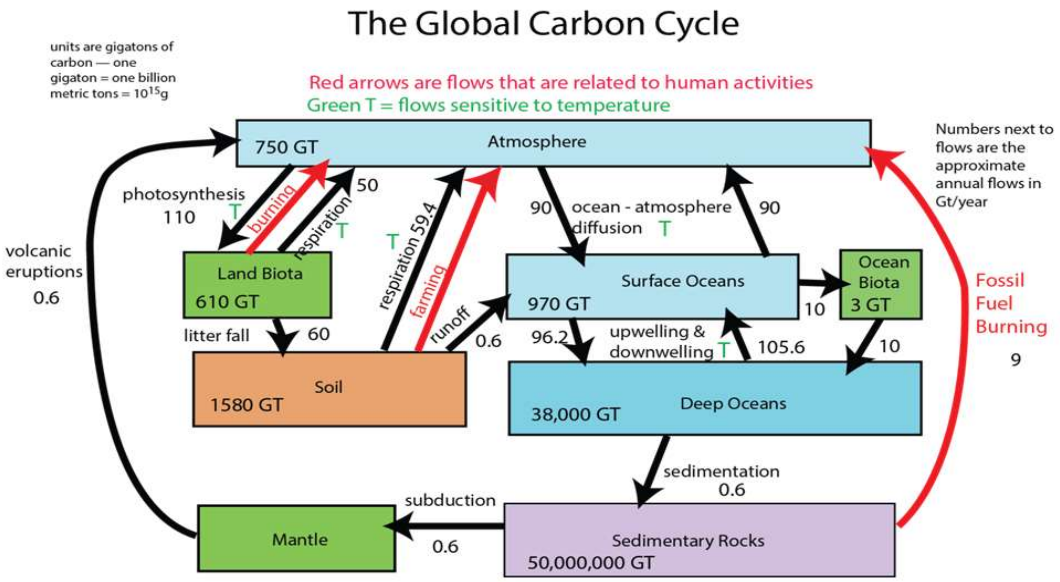

Figure 2.1 Diagram showing the size of carbon reservoirs (GT) and the fluxes between reservoirs (GT y^{-1}) in the global carbon cycle. (Source: Bice, 2017)

The atmosphere, terrestrial vegetation, soils, surface and deep ocean (including their biota), and rocks or geological sediments are all reservoirs in the carbon cycle (Figure 2.1). These reservoirs may be either a sink or a source at any time as the carbon cycles between these reservoirs through a myriad of physical, chemical and biological processes that transfer or transform carbon (Dilling et al., 2006).

2.2 The carbon cycle and land use

Soil is essential to nutrient cycling and carbon storage and also harbours most of the diverse terrestrial microbiomes (Bahram et al., 2018). Globally, soil (organic and inorganic carbon) remains the major sink of terrestrial carbon as it stores nearly 85% (i.e. about 2293 Pg) of C within the top 1 m of the soil profile, and around 3600 Pg within the top 2 m of the soil profile (Lal, 2020). Soil contains 4.4 times more carbon than the atmosphere and 5.8 times more carbon than the biotic (vegetation) pools (Lal, 2020). The atmospheric pool is about 820 Pg C and the biotic pool is about 620 Pg C. Therefore a slight change in soil C stocks can result in a significant increase in the concentration of CO_2 in the atmosphere, with consequences for global climate change (Smith et al., 2020;Lal, 2020). Combustion of fossil fuel, tropical deforestation and other land use changes have been identified as major causes of anthropogenic emission of C into the atmosphere (Lal et al., 2018; Lal, 2020). Between 2009 and 2018 CO_2 emissions have been estimated to be up to 11.0 Pg C and land use change and deforestation have emitted 235±75 Pg C between 1750 and 2017 (Friedlingstein et al., 2019; Lal, 2020).

It has been demonstrated experimentally that, ecological mechanisms like CO_2 fertilization, N deposition and climatic variability have a short term influence on the physical and chemical processes controlling the amounts of carbon in the atmosphere (Houghton, 2002). Recovery from past changes in land use and land management have control over whether terrestrial ecosystems become a carbon sink or source. However, globally, carbon sinks are predicted to decline and lead to a net release of C in the future. Using bottom–up estimates for regional land carbon flux distribution, Sarmiento et al., (2010) reported that the major source of CO_2 to the atmosphere is tropical land use change. A study by (Dang et al., 2014) assessed climate and land use on soil organic carbon in China and found that historical climate warming and a slight reduction in precipitation accounted for 19% of the change in soil organic C (a reduction of 0.05 Pg C) while land use change from cropland to grassland (which increased soil organic C by 0.14 Pg C) accounted for 55% of the change.

Global land use changes where forests (natural systems) are being replaced with croplands or grasslands due to increasing human population (Gries et al., 2018) or urban and mined lands (Lal et al., 2018), are contributing greatly to an imbalance in global C budget (Lal, 2020). An imbalance in C budget from agricultural soils could result from low C input (especially root biomass), enhanced decomposition and mineralization of SOC (due to changes temperature and moisture resulting from changes in vegetation cover), higher risk of erosion, and leaching of dissolved organic matter (Lal, 2020). Agricultural activities such as intensive soil tillage, wetland drainage, biomass burning, subsistence and resource-based farming, or practices that reduce soil fertility and or organic carbon pools could further enhance carbon emission into the atmosphere (Lal, 2004).

It is necessary to sequester carbon to replace lost carbon, enhance agricultural productivity and mitigate climate change (Smith, 2004; Lal, 2004; Feller *et al.*, 2012). Soil organic carbon sequestration involves using plants to capture atmospheric carbon and then returning plant residues to the soil pool, representing an input of biomass-C and thereby creating a positive soil/ecosystem C balance (Lal, 2020). Both the arable lands and grasslands have been identified among those habitats that have capacity to hold more carbon if properly managed (Chamberlain et al., 2010; Alonso et al., 2012). Adopting certain regenerative agricultural practices that permit such a win-win scenarios have been proposed by many authors (Paustian *et al.*, 1997; Lal, 2002; Smith, 2004; Lal *et al.*, 2018). Such practices include but are not limited to (i) conversion from plough till to no-till, (ii) frequent use of winter cover crops in rotation, (iii) elimination of summer fallow, (iv) integrated nutrient management, and (v) the use of improved crop varieties with large root biomass (Lal, 2002). Understanding how soil systems under these practices are resilient or resistant to future climate change impacts is very important for future prediction of soil use and management under predicted climate change scenarios.

2.3 Carbon flow through terrestrial ecosystem

2.3.1 Sources of Carbon into the soil: Photosynthesis

Photosynthesis is as the major source of food and oxygen for all forms of life on earth, thereby providing the basis for the global food chain (Johnson, 2016). It is a process by which light energy is converted into chemical energy in the form of organic compounds (Ruban, 2015). In this process, photon energy (from the sun) is being converted into glucose when the carbon of carbon dioxide (originating from atmospheric CO_2) is reduced by the electrons taken from the oxygen of water (H_20), as depicted in the equation below:

$6CO_2 + 2H_2O + 18ATP + 12NADPH \rightarrow C_6H_{12}O_6 + 18ADP + 18P_i + 12NADP^+ + 12H^+ + 6O_2$. Photosynthesis is therefore the major means by which carbon enters into the soil (see also Figures 2.1 and 2.2). When plants photosynthesise carbon is added to the soil because, when the plant dies and decomposes, the carbon and nutrients within its tissues enter the soil (Craggs, 2016). In addition, root exudates from growing plants offer another means by which photosynthesised carbon can increase the carbon concentration in soil, thereby making plant photosynthesis a natural way of reducing atmospheric CO_2, and consequently sequestering carbon and mitigating climate change. In addition to green plants, photosynthesis can also occur in algae, cyanobacteria and some specialized groups of bacteria called photosynthetic bacteria (Horwath, 2015).

Through the process of photosynthesis, about 110 Pg of CO_2-C is converted into organic compounds annually. However, about half of this carbon is lost into the atmosphere through respiration (see figure 2.2) by photoautotrophs and heterotrophs (Horwath, 2015). It has been reported that the flux of photosynthates exert a major control on soil respiratory losses of carbon to the atmosphere (Högberg et al., 2001).

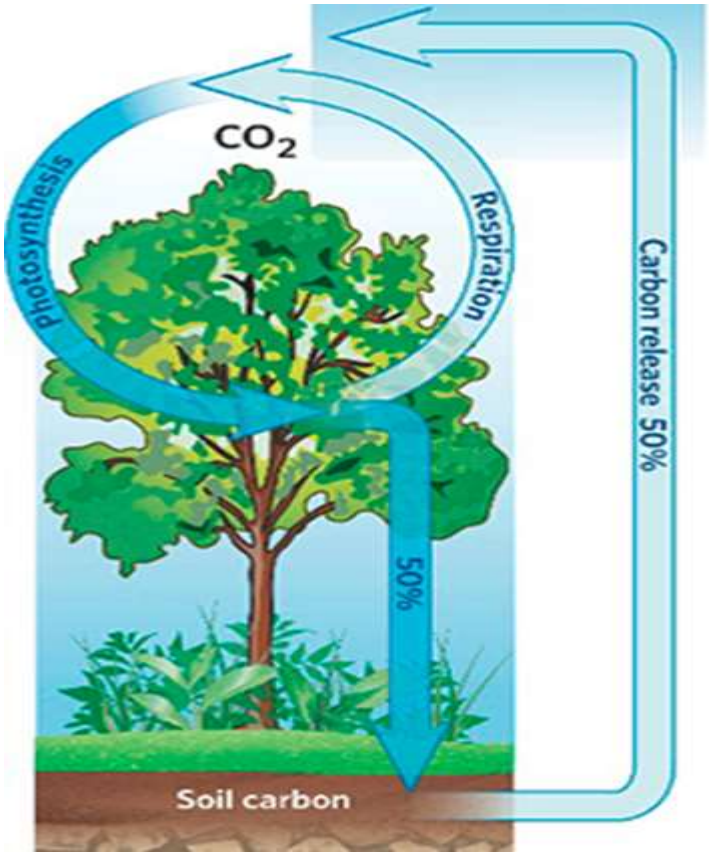

Figure 2.2 – Carbon sequestration through photosynthesis – adapted from Lehmann, (2007)

2.3.2 Sources of carbon loss from the Soil: Soil respiration

Soil respiration is the release of carbon dioxide (CO_2) into the atmosphere either through respiration of plants root and their associated organisms (autotrophic respiration) or through the decomposition of plant biomass, soil organic matter, litter and soil animals (heterotrophic respiration) (Yu et al., 2015). About 98 billion tons of carbon (i.e. 98 GT C) are released from soils into the atmosphere per year (Yu et al., 2015), resulting in a positive feedback into the atmosphere which may further exacerbate global climate warming (Lai et al., 2012). Biological factors like the community composition of soil microbes and their activities are important mediators of soil respiration, while temperature and moisture are among the abiotic factors influencing soil respiration which can be consequential (Yu *et al.*, 2015; Lai *et al.*, 2012). For instance, temperature can influence both the plant roots, microbial communities in soil and the respiratory enzymes, thereby influencing all aspects of soil respiration. Thermal acclimation in enzyme function can influence the overall soil heterotrophic respiration (Conant et al., 2011), and current enzyme activity is a result of the long term microbial activity and activity of the currently active microbial population (Ahmed, 2017). The response of soil respiration to temperature and other abiotic variables can vary from one land use or ecotype to another (Lai et al., 2012). Soil respiration is also temporally dynamic since soil respiration can fluctuate or oscillate on centennial, annual, seasonal, weekly, and diurnal time scales. These fluctuations make predicting the impact of changing temperature on soil respiration particularly challenging, thereby requiring more research attention by the soil scientists to uncover the mechanisms by which soil respiration respond to pattern of changing temperatures.

2.4 Climate Change

Climate change, as defined by Nwankwoala, (2015), is the long term change in the average weather pattern over a period of time and region. It expresses the change in the statistical distribution of weather patterns over a period of time which may be for decades or millions of years. Therefore, global warming is a component part of climate change. Average temperatures of the earth have increased since the pre-industrial era, and 17 out of the 18 warmest years recorded so far have occurred during the 21st century.

Generally, increased maximum and minimum temperatures, reduced diurnal temperature range and fewer cold days over nearly all land areas have been predicted during the 21st century. Also, there will be an increase in summer continental drying with its attendant risk of drought, increases in the number of hotter days and heat index, and high or intense precipitations,

especially in winter seasons(IPCC, 2001). Increasing temperatures have been forecast across the globe, across all seasons and regions. Global average surface temperatures have risen by 0.74 °C from 1906 to 2005 (IPCC, 2007). Temperature is predicted by the IPCC to increase by as much as 4.8 °C with regional and latitudinal variations by the end of this century (21st Century) (Collins et al., 2013). Studying the impact of climate change will therefore require the use of climate models to guide strategic research to elucidate the mechanism by which these changes may have impacts across the globe.

In the UK, a future climate projection has been made in UKCP09; it is expected that, there will be overall warming which will be accompanied by intermittent wetter winters and drier summers (Jenkins et al., 2009). For example, average summer precipitation is expected to reduce by 23 % across the UK by 2080 and average summer temperature is expected to increase by 3.9 across south East England (Defra, 2009). These changes in seasonality will impose varying adverse effects on different habitats. For instance, grasslands which account for approximately 70 % of the UK agricultural land and where about 32 % of UK's agricultural products are produced are sensitive to drought (Hopkins and Davies, 1994;Wilkins, 2000; Knapp et al., 2015). Projected droughts in the summer months due to climate change will influence grass growth, especially in soils with low available moisture contents (Clair et al., 2009). The reduced grass growth will, in turn, reduce the soil carbon input and biodiversity of the grassland ecosystems. Projected wetter winters can lead to water logging which may indirectly influence the release of dissolved organic matter into the system. This is because extremes of dryness (water stress) and wetness (anoxia) due to changes in precipitation could limit decomposition (Balser et al., 2010a). A shift in short-term climatic extremes, such as the frequency of hot and wet days, is predicted to have a more severe impact on the environment than the effects of mean monthly or seasonal climates (Jenkins et al., 2009).

2.4.1 Effects of projected future climate on diurnal temperature oscillations

Different possible patterns of diurnal temperature variation due to the influence of global climatic warming have been predicted (Speights et al., 2017; Barton and Schmitz, 2018). There could be (i) a rise in maximum day time temperature which increases diurnal temperature range, (ii) a rise in both maximum and minimum temperature, which maintains the magnitude of the diurnal temperature range or (iii) a rise in the minimum night time temperature which decreases the diurnal temperature range (Easterling et al., 1997; Barton and Schmitz, 2018). These different possible patterns of diurnal temperature oscillation could therefore influence

the activities of soil organisms and then affect their community structure. However, much of the understanding through field or laboratory research has resulted from observations on the impact of elevated temperatures on soils maintained at a constant temperature. Scientists have raised the need to consider (and not ignore) the possible asymmetrical warming (warming effects partitioned into day and night temperatures) as this may enhance the accuracy of predictions of the net effects of climate change on soil ecosystems and ecosystem processes (Xia et al., 2009). This is because; daytime and night-time warming could exert varying but opposite effects on the ecosystems as climate changes (Speights et al., 2017).

2.4.2 Influence of climate change on soil respiration rate

Global change has been predicted more than a century ago, but despite this prediction a doubling of the global atmospheric CO_2 concentration and rise in temperature of around 1.3^{o}C has occurred. Apart from rising global warming, there is now a considerable shift in weather patterns, ocean acidification, and the potential loss of species which have varying impacts on soil quality and productivity, land use and land cover changes, and ecosystem processes (IPCC, 2001; Balser et al., 2010a). Most prominent of the ecosystem processes as a result of climate change is its impact on soil carbon cycle, because of its influence on CO_2 emission which further increases the extent of global warming. While there are a whole lot of factors that can influence carbon flux, the major example of the physical, and the biological factors in this case are; soil temperature, moisture, biological properties (Feng et al., 2014). To properly document the global carbon flux between the terrestrial ecosystem and the atmosphere, both the physical and biological factors affecting the flux must be considered.

Terrestrial carbon cycle feedbacks to the climate system are large, complex and poorly understood (Melillo et al., 2017a). Though the magnitude of terrestrial carbon cycle feedbacks to the climate system varies depending on the location, increases in future atmospheric concentrations of carbon dioxide (CO_2) and temperature have been predicted in most of the carbon-climate model simulations (Dillings et al., 2006). Carbon- climate models have predicted that carbon cycle feedbacks could either slow or accelerate climate change over the 21st Century (Mellilo, et al., 2017). The consequent acceleration of global warming may result in a further increase in soil carbon loss into the atmosphere (Bradford *et al.*, 2016). This is based on the caveat that, warming tends to stimulate a microbial mediated decay of soil C to CO_2 which further enriches the atmospheric concentration as the rate of warming increases

(Bradford *et al.*, 2016), making soil a CO_2 source rather than sink. Despite much research, there is currently no consensus on the magnitude of warming induced changes on soil carbon loss due to limited evidence on combination of factors that affect soil carbon dynamics. In the global contexts, research is now focusing on feedbacks between the changing carbon cycle, changing climate and their future implications (Dilling et al., 2006).

2.4.3 Moisture changes and soil respiration

Although a large body of evidence has identified soil temperature and soil moisture as individual factors influencing soil respiration, the two physical factors interact to influence soil respiration, resulting in seasonal and spatial differences in different ecosystems (Qiu et al., 2005; Chang et al., 2014). Warming-induced soil moisture losses may cause a decrease in soil CO_2 release by eliminating the stimulating effects of warming (Wang et al., 2014). However, low soil water content can inhibit soil respiration, especially when temperature is kept constant and there is no spatial heterogeneity, as found in most laboratory studies (Davidson et al., 2000). If soil moisture exceeds optima, mediated by other hydrological properties of soils like water holding capacity, a reduction in CO_2 evolution from soil is observed (Davidson *et al.*, 2000; Herbst *et al.*, 2016). Determining the optimal soil water content resulting in the CO_2 production capacity of a soil system under a given condition will enhance our ability to assess the impact of soil water changes (e.g. as a result of precipitation or warming) in the future. While there are few studies that have related soil respiration with moisture conditions under varying temperature regimes, the studies that have been undertaken have yielded contradictory results, and most have not compared different land uses.

Even though soil temperature has always been regarded as the primary abiotic control of soil respiration, and soil moisture the secondary, soil moisture could become the dominant factor that influences soil respiration. For example, Chang et al., (2014) reported a study where they examined how moisture content can moderate the effect of temperature on respiration. They found that daily mean soil respiration correlated with temperature above a particular moisture threshold. They also found that warming reduced soil respiration at low moisture content. They concluded that there could be a switch of control between temperature and moisture at a threshold temperature. Moinet et al., (2016) reported another scenario where soil respiration did not relate with either soil moisture or temperature but with dissolved and particulate organic carbon. Their results identify microbial access to substrate as being more important than soil moisture or temperature in controlling soil respiration.

Temperature-induced soil drying is common when soil is exposed to high temperatures and such conditions are characterized by low microbial activities due to water limitation (low water availability). Such conditions are typical in drier regions (Zheng et al., 2017) but predicted to become more frequent during the summer seasons of temperate regions under climate change. Using historical records of air and soil temperature and soil moisture, Huang et al., (2011) estimated the soil respiration through temperature, moisture and temperature/moisture models between 1980 and 2000. Their results showed that, rises in temperature from 1980 decreases soil moisture both annually and seasonally and this, in turn, inhibits soil respiration. Thus, increases in the accumulation of soil organic carbon are expected during droughts since reduced CO_2 emissions will accompany decreasing soil moisture under climate warming thereby preserving carbon stocks in drier soils or ecosystems. That is, under dry condition, as envisaged to become more frequent in summers of most arid and semi-arid environments, soil respiration is expected to decrease. Monitoring changes in moisture and nutrient levels in soil can give more detail information about the direction and magnitude of such changes as climate warms, this may be clearer by exploring the ability of soil to hold water rather than the gravimetric moisture content commonly reported.

Water availability is an important abiotic factor regulating microbial properties. In soils with sufficient water availability increases in microbial biomass and microbial respirations are observed because water supports the diffusion of dissolved organic C and inorganic N in water films, which helps stimulate the activities of extracellular enzymes (Zhao et al., 2016). Ding et al., (2016) reported on how temperature sensitivity of soil CO_2 release is linked to substrate, environmental, and microbial properties across alpine ecosystems and found that, though precipitation was the major driver of topsoil CO_2 release rate in alpine ecosystems over a broad geographic scale, both the substrate properties and environmental variables account for 52% of the changes in soil CO_2 release rate across all grassland sites, and 37% and 58% of changes in temperature sensitivities across the steppe and meadow sites, respectively. While assessing the effects of biotic and abiotic variables that influence spatial variation of soil respiration in secondary oak and planted pine forests, Luan et al., (2012), found that both the water filled pore spaces and water holding capacity significantly and positively correlated with soil respiration. Since changes future climate projection will alter temperature events, a better understanding of the relationship between soil carbon processes and soil hydrological properties will be needed (Castellano et al., 2011).

2.4.4 Effect of increasing temperature on soil respiration and microbial community composition

Temperature can directly or indirectly influence soil respiration under the future climate change scenarios. The temperature of the soil is related directly to the air temperature and it impacts most of the important ecosystem processes like plant growth, species distribution and capacity to store C (Michelsen-Correa and Scull, 2005). Increasing temperature (warming) is expected to accelerate the rate of chemical reactions and lead to enhanced soil biological activities (Dang et al., 2009). These enhanced soil biological activities can lead to a profound direct or indirect impact on ecosystem processes like primary production and decomposition. Increases in temperature are capable of influencing soil respiration, which is responsible for the largest flux of CO_2 from terrestrial ecosystem into the atmosphere; thus increasing the atmospheric concentration of CO_2 (Wang et al., 2014). A 10 ºC increase in temperature will lead to a doubling in the capacity to release carbon into the atmosphere (Michelsen-Correa and Scull, 2005).

A model simulation by Jones et al., (2005) showed that, increasing global temperature will still result in an enhanced soil respiration rate and will therefore reduce global soil carbon stocks. It was evidenced in their results that this effect will be greater than that of increased soil organic matter input predicted due to enhanced vegetation growth. Results of a 26 year old experiment which examined the impact of soil warming on soil carbon feedbacks to the climate systems in a forest ecosystem in America identified an inconsistency in the response of soil carbon flux to warming (Melillo et al., 2017). They reported a period of substantial carbon fluxes to the atmosphere and loss of soil organic matter, alternating with periods when there was no loss. They attributed these changes in pattern of carbon flux to depletion of microbially-accessible carbon pools, a shift in carbon use efficiency and microbial community composition, and a reduction in microbial biomass and activity. As a result of warming, a global loss of approximately 190 Pg C from the upper 1 m of the soil profile over the 21st century was predicted from their study. They then concluded that two thirds of the predicted carbon losses were due to microbial activities in soil, raising the need to intensify effort in researching the factors mediating soil microbial (heterotrophic) respiration. In a bid to separate responses of heterotrophic and autotrophic soil respiration to warming using a synthesis from several studies, Wang et al., (2014) reported that temperature increase by 2 °C raised heterotrophic respiration (by 21 % above autotrophic) continually throughout the warming duration disproving the hypothesis that soil microbial communities thermally acclimate to warmer

temperatures. Because their result was obtained by synthesis through meta-analysis, it may not have considered the variation in warming pattern (elevated, increasing or diurnal temperatures) from several studies. There may be the need to further validate their claim through laboratory or field studies. The above claims further complicate the ability to properly predict the magnitude of soil respiration under continuous warming, raising the need to generate more empirical data.

Changes in temperature and or moisture are known to influence soil processes like soil organic matter decomposition and soil heterotrophic respiration which are mediated by soil microorganisms. A shift in microbial community composition has been identified to be stimulated as a result of temperature changes (Zhou *et al.*, 2017). Temperature also regulates the strength of biotic interactions. Microbial populations separate into a varying thermal ecotypes and community composition as they respond to environmental temperature which brings about differences in genotypic and species fitness. As a result, temperature changes therefore directly influence not only the microbial respiration of soil but also the biomass, community physiology, and composition (Bradford, 2013).

The quantity and quality of soil organic matter has been identified as one of the dependent factors influencing soil microorganisms since soil organic matter supplies C (substrate) and energy. A strong interrelationship between temperature and quantity of C available has been documented (Panikov, 1999). Increasing temperature can reduce both the quantity and quality of organic matter during decomposition process. The initial stage of SOM decomposition is characterized by fungal growth (Feng and Simpson, 2009), thereby leaving the bulk of decomposition to be undertaken by bacterial communities and, when the environmental conditions changes and become unfavourable, the bacterial groups are found to express various survival strategies or select for substrate preferences. For example, bacteria which are grouped according to their cell wall composition (Strickland and Rousk, 2010) are divided into Gram positive, which are well adapted to conditions of limited substrate and Gram negative bacteria that prefer fresh organic materials (Landesman and Dighton, 2010) thus creating hot spots of decomposition in soil. Change in relative abundance of Gram negative and Gram positive bacteria due to increasing temperature have been evidenced in literature and this has been attributed to shift in substrates availability (Zogg et al., 1997; Balser et al., 2010) Therefore, varying degrees of substrate limitations in soil could result in temporal and spatial differences in the relative abundance of fungal and Gram negative/Gram positive bacterial populations as temperature increases. Due to functional diversity and selection to environmental condition,

we may expect functional redundancy (which implies that not all the microbes performs a given function at a particular time), and increased resistance and resilience of soil microbial community to perturbation (Nielsen et al., 2015).

2.5 Soil food web and climate changes

Soils harbour a large community of living organisms which play many important roles in ecosystem processes (Classen et al., 2003; Gupta et al., 2011; Eilers et al., 2012; Grandy et al., 2016; Shaw et al., 2016). Living organisms in soil perform roles such as soil formation, organic matter decomposition, nutrient cycling, infiltration, purification and storage of water, carbon and nitrogen fixation, and sequestration (Collof, 2011). Soil organisms are also involved in the degradation of agro-chemicals and disease incidence and suppression (Gupta et al., 2011). Soil organisms are often grouped based on their functions or sizes (Figure 2.3). Based on their sizes, the soil living organisms include microflora (such as bacteria, fungi, archaea and actinomycetes) and microfauna (e.g. protists and nematodes) with body widths less than 0.1 mm; mesofauna (e.g. mites, springtails, enchaetraeids) which has less than 2 mm body widths and macro and megafauna (e.g. earthworms, ants, millipedes). Of these four classes, microflora are perhaps the most important as they form the base of the soil food web and play a significant role in ecosystem processes (Nielsen et al., 2015). Among many others, microbes in soil perform ecosystem services like cycling and recycling of nutrient and wastes and detoxification (Aislabie et al., 2013). Fungi and bacteria dominate the microbial community as they represent the greatest percentage of the belowground biomass and soil biodiversity pool. There is a growing need to deepen our understanding of the roles of soil food web in shaping or regulating the below ground processes of decomposition and nutrient cycling (Shaw et al., 2016; Grandy et al., 2016). This rising interest may be due to the fact that, biological processes in soils are regulated in part by soil temperature (Fraser et al., 2013), and that variation exists in the way the soil food web regulates the ecosystems functioning across geographical locations and environmental gradients in relation to abiotic factors such as climate (de Vries et al., 2012; de Vries *et al.*, 2013). Scientists have raised the need to better assess the dynamics of soil food webs because of their likely sensitivities or responses to climate and other drivers in the face of the current global environmental changes (Wardle et al., 2004; Gilbert et al., 2014).

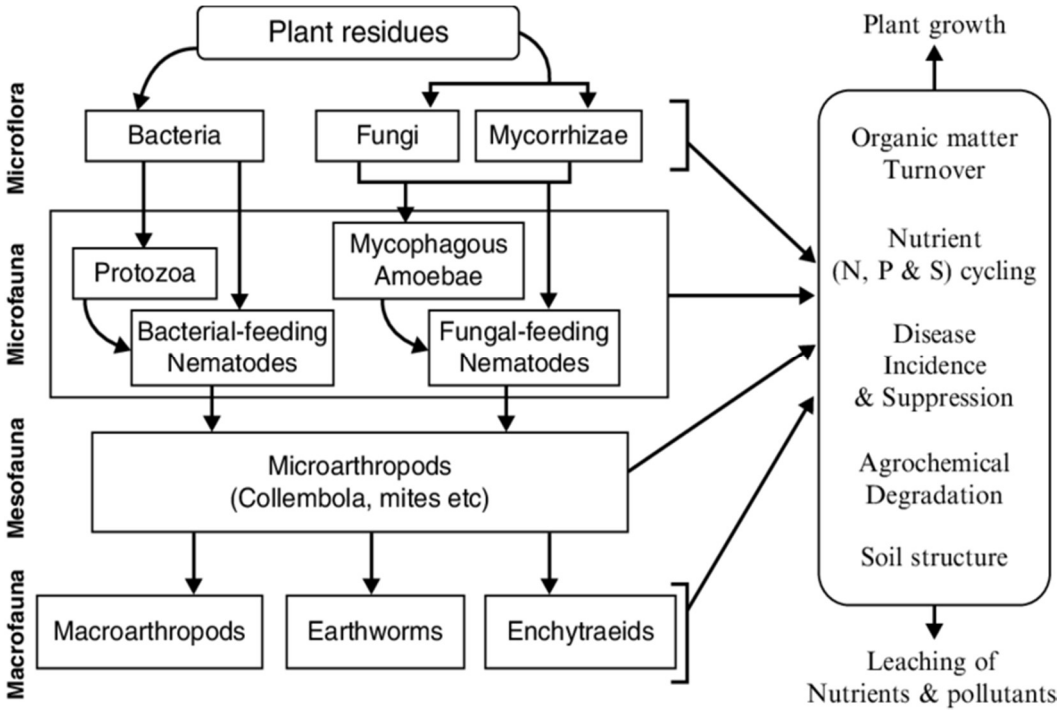

Figure 2.3: Soil food web with detritus showing trophic levels and the role of the web in soil biogeochemical processes. Source: (Gupta et al., 2011).

2.5.1 Role of soil food webs in soil ecology

Traditional understanding of soil biogeochemical processes dictates that there are two major input channels (originating from roots and aboveground detritus) by which energy moves through the soil food web and both of them contribute to the third substrate source which is the older soil organic matter (Gilbert et al., 2014). The detrital channel consisting of labile and recalcitrant C pools was further sub-divided into fungal and bacterial channels with the understanding that this energy channel dominates C supply into soil food web (de Vries and Caruso, 2016; Bradford, 2016). In contrast, it has been evidenced in recent studies that living root systems supplying labile inputs like exudates dominate as the energy supply channel in soil systems (Pollierer et al., 2012; Gilbert et al., 2014; Bardgett et al., 2014; Bradford, 2016). This observation may be due to the fact that a higher proportion of the C input to the soil in these systems is supplied by root rather than leaf litter (Gilbert et al., 2014). Also, the old understanding that labile C is only consumed through the bacteria channel has being updated. de Vries and Caruso, (2016) demonstrated that, both fungal and bacteria populations can exist together, and that higher consumption of labile C or transfer of recalcitrance to labile C pool is carried out by the fungal group. Morriën, (2016) further demonstrated a soil food web with a shift in carbon flow from a bacterial dominated channel to a more fungal dominated carbon

flow due to land use succession changes. This updated understanding unified the traditional and recent understanding of the carbon flow through soil food webs. Although, scientists have unified both the traditional and the recent views on the carbon flow through the soil food web, the facts remains that, the bacterial- and fungal base compartment which forms the later channel by which carbon flow through soil food web differs greatly in their substrate use, community composition and response to certain disturbances which consequently drives their relative dominance and determines their carbon and nutrient cycling rates and storage (Bradford, 2016).

2.6 Influence of climate change on soil biological communities

Climate change can exert varying levels of impacts on soil biological communities. Direct effects of climate change on the activities of soil microorganism include the impacts on soil microbes and greenhouse gas production due to altered temperature and precipitation and events of climate extremes (Bardgett et al., 2008). Climate-driven alterations in processes like plant productivity and diversity are identified as the indirect effects of climate change on soil microorganisms. Such processes directly influence changes in soil physical and chemical properties, carbon supply to soil, microbial community structures and activities (Bardgett et al., 2008). Measurement of indirect impacts of climate change is therefore more critical to our understanding than the direct impacts (Balser et al., 2010). Climatic variables like precipitation and temperature are environmental variables which underpin the growth of microorganisms. These variables are modulators rather than resources because they can only influence the activities of organisms and are not necessarily used for growth or biomass production (Balser et al., 2010). Changes in the modulators can therefore affect changes in the whole soil communities due to their influence on resource availability and the entire soil system. For example, a decrease in available substrate, which is a resource, can be as a result of decrease in water potential, which is a modulator, altering diffusion and thus enhancing the hyphal strategists' success in soil microbial communities (Balser et al., 2002; Balser et al., 2010). Substrate limitation will reduce the rate of soil respiration and subsequent release of CO_2 into the atmosphere. This can make the measurement of impacts of climate change on soil biological community to be more critical, and the need to account for all avenues by which both direct and indirect impacts of climate change can influence soil carbon cycling and the possible feedbacks through manipulative experiments.

It has been noted that climate change impacts on soil microbes or microbial communities will have negative implications on the soil food web compositions and this will, in turn, influence

the ecosystem services and functions that they perform (de Vries and Bardgett, 2015). Changes in within group diversity due to climate change is expected to exert less of an impact on ecosystem functioning compared to changes in soil food web composition. For instance (de Vries et al., 2012) reported that the composition (e.g. fungal to bacterial) of a soil food web determines the extent of C and N loss after a drought event and that land use change can alter resilience of soil food web to drought. Also, changes in the ratios of fungi-to-bacteria, or their energy channel is a determinant to the subsequent C and N cycling process (de Vries et al., 2013) and fungal based soil food webs can be more efficient in C and N cycling processes. In Handa et al., (2014), a reduction in functional diversity reduced litter decomposition across all ecosystem types and there was high litter decomposition rate in the presence of a more complete decomposer group.

A shift in climate events could impact soil biological communities and then influence their physiology, composition and structure and alter the rate of important biogeochemical processes. Climate change can shape microbe-to-microbe interactions which may alter their resilience under warming or moisture stress. An example of such shifts is the type of interaction reported due to substrate use or life history strategy under climate change scenario. de Vries and Shade, (2013) documented controls on soil microbial community stability under climate change. Their result, which drew from re-analysing already published data, showed that relative abundance of copiotrophs and oligotrophs (classified based on life history strategies; copiotrophs have high growth rates, but low resource use efficiency and oligotrophs have low growth rate but high resource use efficiency), resource availability, and the abundance and diversity of higher trophic levels shifted the composition of microbial communities to perturbations, due to climate change. Such responses reflect the mechanisms by which microbial communities adapt to varying environmental conditions through selection.

Climatic warming is an example of abiotic stressors that can change the diversity of the microbial community and the processes that they mediate. Specific patterns of microbial response to altered temperature regimes can add to or interact with the predicted warming-induced changes in soil community structures. Specific shifts in community structure due to warming could be because microbes that are used to warm environment will adapt well to increased or elevated temperature This was demonstrated by Dang et al., (2009) who observed that fungal species that are known to dominate the community in the summer also dominate at higher temperature. However, the direct effect of warming could bring about changes in soil microbial community physiology (or structure), biomass and composition (Bradford, 2013).

Shifts in microbial community composition are expected when there is a change in soil temperature (e.g. freezing) as global climate changes. This may be due to changes in substrate availability or use by microbial population decomposing the organic substrate. While optima temperature may not directly relate with average local temperature, substrate availability or limitation may do and this may as well drive the changes in microbial community response to warming (Lipson, 2007; Balser et al., 2010). For instance, bacteria dominance in the alpine tundra in summer was replaced by a fungi dominance at temperatures near or below freezing, due to altered substrate use (Henry, 2008). The dominance of fungal populations at low temperature points to higher carbon storage potential, and this has been evidenced by low fungal/bacteria ratio at lower temperatures in several studies (e.g. Malik et al., 2016; Bai et al., 2017).

The presence of microbial grazers or organisms from higher trophic levels can further complicate the response of soil microbial organisms to moisture or temperature changes. It has been observed recently that plant roots, substrate or resource availability and microbial grazers can individually influence the stability of soil microbial community (de Vries and Shade, 2013; Kong et al., 2018). Research on how predicted temperature increases or weather extremes will impact this phenomenon is not common and whether abundance or presence of microbial grazer correlates with relative abundance or diversity of microbial groups remains unclear. Studies have shown that a diverse range of soil organisms from different trophic levels in the food web can increase the various levels of interaction and enhance resilience after disturbance (Uvarov et al., 2006; de Vries and Shade, 2013). A particular example that is gaining the attention of the ecologist in recent times is that of interaction between the soil microbes (fungi and bacteria) that decompose soil organic carbon and the microbial grazers which feed on the primary decomposers (de Vries and Shade, 2013). Microbial grazers (nematode, protozoa, collembolan, earthworm, etc.) are members of higher trophic levels (Figure 2.3). The interactions between trophic levels of the soil food web has been found to be of significant importance in ecosystem processes that relate to soil CO_2 release under climate change scenarios (Uvarov et al., 2006). In a warming experiment Uvarov et al., (2006) reported on the response of soil microbial activities to temperature, moisture and litter addition and found that the presence of earthworms enhanced soil respiration under temperature fluctuation treatment in the field. However, evidence abounds that resilience to climate induced perturbation of both the microbes and microbial grazers depends on land use type (de Vries et al., 2012). Another study showed that the combination of root and heterotrophic respiration was higher in grassland

soil where there was an abundance of earthworms and CO_2 flux also correlated positively with earthworm biomass (de Vries et al., 2013). Understanding the temperature regimes, in combination with other soil and enivromental factors, which favour both the microbial grazers and microorganisms will enhance our ability to properly predict microbial contribution to various ecosystem processes.

2.6.1 Response of soil microbial community to altered resource availability due to climate change

Altered resource availability due to climate change can influence soil microbial community composition and functioning. Differences in water and nutrient availability in terms of dissolved organic C and dissolved inorganic N can cause variations in microbial community composition (Zhao et al., 2016), but this can be moderated under warming. Bacterial and fungal components of the soil population are the major groups responsible for the decomposition of organic matter; of these two, fungal populations are more tolerant to drought. This is possible because of their ability to transfer moisture from water-filled micro pores to drained pore spaces, as opposed to bacteria which need more water for substrate diffusion and motility (Zhao et al., 2016). The microbial response to resource addition (moisture and organic matter addition) in a salt affected soil was documented by Van Horn et al., (2014) where organic matter treatment was added as a leachate to the same moisture content as the moisture treatment. Their results showed that, microbial and enzyme activities increased with resource addition when the salt stress is low to moderate, but not at high salinity. Also, bacterial community composition was altered as *Proteobacteria* and *Firmicutes* increased in abundance with water and organic matter additions at the low- and moderate-salinity sites and a near dominance of *Firmicutes* was observed at the high-salinity site. Their results demonstrated how resource limitation could mediate soil microbial community composition and functioning under salt stress. Qiu et al., (2005) had earlier documented differences in microbial respiration from upland soils and lowland sediment under warmer and wetter condition due to litter leachate. They also found that litter leachate enhanced heterotrophic soil respiration of moisture and nutrient stressed upland soils. However, substrate limitation could partition soil microbial community composition and their functioning under differential warming, therefore influencing the effects due to soil moisture or temperature alone. Understanding whether thermal adaptation of microbial decomposer communities is due to C depletion or moisture and temperature effects is very pertinent in predicting the future climate change effects on ecosystem processes.

2.7 Diurnal temperature oscillations and soil respiration

Much research on the way that ecosystems respond to changing climate are based on the assumption that the future global warming will arise from simultaneous increases in daily maximum (day time), daily minimum (night time), and daily average temperatures. However, evidence from recent studies showed that mean warming arises disproportionately from increasing night time temperatures (Barton and Schmitz, 2018). Over the last 50 years, negative trends in Diurnal Temperature Range (DTR) have been observed due to an approximately 0.9 $^{\circ}$C increase in daily minimum and only 0.6 $^{\circ}$C increase daily maximum temperature (Braganza et al., 2004). Therefore, while the climate warms, we have a reduction (i.e. narrowing) of DTR. There is now consensus that the decreasing trend in DTR is attributed to increases in the amount of cloud (due to the effects of aerosols), precipitation, and water vapour but that there will be deviations from this global trend as different regions will experience different changes in cloud cover, precipitation and water vapour (IPCC, 2001). Increases in both the average daily maximum and daily minimum temperatures with seasonal and regional variations have been predicted in the UK (Jenkins et al., 2009). For instance, under the medium emissions scenario, we expect summer average daily maximum temperature to increase by a magnitude of between 2.2 and 9.5 °C in parts of southern England but winter increases by a magnitude of between 1.5 and 2.5 °C across the country. We also expect average daily temperatures to increase by a magnitude of between 2.1 and 3.5 °C in winter, and between 2.7 and 4.1 °C in summer, with higher warming in southern parts of the country than in the northern parts of the country (Jenkins et al., 2009). Jenkins et al., 2009 noted that projected changes to daily climate are expected to be more significant for many climate effects than changes in monthly or seasonal averages. Climate models have predicted that this trend may continue (Davy et al., 2017; Barton and Schmitz, 2018) throughout the 21st century. The understanding that increased annual average temperature globally will increase soil respiration rate is well documented in the literature. The impact of narrowing or decreasing the diurnal temperature range on soil respiration and soil microbial community structure and physiological functions remains largely unknown. Ecological studies that examine how community level interactions will be influenced by climate change usually warm mesocosms continuously to constant temperatures that matches the future average temperature of the study location (Barton and Schmitz, 2018). Most of these studies do not account for temperature sensitivity of soil respiration during diurnal temperature oscillations, especially in the upper few centimetres of soils (e.g. Hicks Pries et al., 2017).

Daily maximum and daily minimum temperatures, as defined by the American Meteorological Society, are the highest or lowest temperature reported over a continuous time interval of 24 hours. The temperature indicated by thermometer as it is exposed to the air in a place sheltered from direct solar radiation (about 2 m above earth surface) is regarded as the surface temperature (Lin and Hubbard, 2008). The temperature of the soil is related directly to the air temperature and it affects most of the important ecosystem processes like plant growth, species distribution, and the capacity to store (Michelsen-Correa and Scull, 2005) or release carbon. Understanding the magnitude and direction of both the rising average temperature and the DTR has then become necessary in predicting the impacts of future climate change on terrestrial ecosystems. It is well established that increases in temperature will accelerate the depletion of soil organic matter and then expose microbes to starvation stress (Feng and Simpson, 2009; Bérard et al., 2015; Liu et al., 2019). Such indirect temperature effects may be more pronounced under the scenario of climate change where average temperature are increasing, but whether this will be consistent with the narrowing of DTR (a faster increase in minimum temperature, compared to maximum temperature) predicted as climate changes remain unclear.

Alongside the increase in the mean temperature predicted in climate models, temperature variations, which occur at various time scales, have been identified to contribute to the large repercussions predicted by climate change scenarios. These include the regular diurnal oscillations between night time temperature and daytime temperature (Dang et al., 2009). It is important to consider such temperature gradients because, short term temperature fluctuations have greater consequence on processes that are driven by microorganisms like bacteria and fungi (Dang et al., 2009). An example of such processes includes the decomposition of organic matter and the consequent release of CO_2 through microbial respiration. In a bid to elucidate the overestimation of closed-chamber soil CO_2 effluxes at low atmospheric turbulence in temperate forest, Brændholt *et al.*, (2017) reported a diurnal pattern of soil respiration across all seasons and that soil respiration was higher during the night than during day period. This pattern showed that soil warming at night may trigger higher CO_2 flux than warming during daytime. Their result did not separate autotrophic from heterotrophic soil respiration. Instead their automated chamber method accentuated a diurnal pattern, thereby overestimating the effects on the annual CO_2 efflux. In a field experiment, Xia et al., (2009) reported that effects of diurnal warming on soil respiration are not equal to the summed effects of day and night warming even though night warming showed significantly higher respiration than day warming. Their result also showed that, both day and night warming influenced the daily mean

soil temperature; the daily mean soil temperature was higher under night than day warming scenarios. Higher respiration during night than day evidenced in the two studies above could be a result of diel hysteresis where there is a certain time lag in the response of soil respiration to temperature change (Bahn et al., 2008; Phillips et al., 2011). Researchers have thought that, environmental variables like carbon substrate that oscillate out of phase as soil temperature change could be one reason for diel hysteresis (Phillips et al., 2011). Understanding the mechanisms by which daily or seasonal high and low temperature or even the cycle between them exert varying impacts on CO_2 release from soil through microbial respiration will help to better interprete the response of soil respiration to climate warming.

Studies dedicated to examine the effects of diurnal temperature on soil respiration are very rare and their scopes are also limited. Zhu and Cheng, (2011) assessed the temperature sensitivity of soil organic carbon decomposition under constant temperature and a diurnally varying temperature regime. Their results showed that temperature sensitivity under constant temperatures were consistently higher than those under diurnally varying temperatures. In their experiment, the diurnal temperature treatment was performed by alternating high and low temperatures rather than cycling between high and low temperatures, as would be experienced in the environment. In a field and laboratoratory experiment, (Uvarov et al., 2006) observed a similar cummulative and average respiration from constant and fluctuating temperatures under laboratory condition. This result was attributed to the fact that, soil microbial orgamisms responsible for the process may have acclimatised to the average temperature, thereby saving the cost of adaptation to fluctuating temperature. In general, they did not find a significant difference due to the absence or presence of a diurnal temperature oscillation, although temperature sensitivity varied between the two temperature gradients. In the field where the diurnal variation was just a little above the constant (1.0 – 2.5 ^{o}C) as against a large seasonal diurnal changes in the laboratory (±5 ^{o}C), increased soil CO_2 respiration was observed. They concluded that there may be need to further explore the factors driving the mechanisms of soil microbial respiration under diurnal fluctuating temperature in the laboratory.

2.7.1 Impacts of land use changes on soil temperature and diurnal temperature range

Although climate change has been forecasted to reduce DTR, soil temperature patterns can also be moderated by land use and land use changes. Wei et al., (2018) observed a decline in DTR under croplands, forests or mutual change between croplands and grasslands land use types, but an increased DTR when the land use change from grasslands to forests types, though there

was a seasonal variation in DTR according to their result. The observed decrease in DTR when land used remain as grasslands was as a result of both decreasing maximum (daytime) temperature and increasing minimum (night-time) temperatures (Wei et al., 2018). Arevalo et al., (2010) observed that variability in soil respiration among different land use types is due to soil temperature differences. These differences may explain lower CO_2 emission from forest soils, compared to grassland soils (e.g. Adekanmbi et al., 2020), though this will depend on the nature of soil properties (Tian et al., 2017). Studies that incubate soils from grasslands and woodlands have reported higher CO_2 emissions from grassland than woodland soils (Raich and Aydin, 2000; Lang et al., 2011; Gritsch et al., 2015; Nazaries et al., 2015). It has been demonstrated that soil microbes adapt to the temperature of their original site, thus expressing a legacy effect (Hawkes and Keitt, 2015). Evidence of such legacy effects will be reviewed in Section 2.8.

It has been argued that, the response of soil respiration, especially heterotrophic respiration, to increasing temperature may be transient or short lived (Eliasson et al., 2005) depending on the standing carbon stock (Crowther et al., 2016a). This is because, continuous depletion of soil carbon substrate as a result of increasing temperature can reduce the magnitude of CO_2 release and therefore acclimate at a particular time (Tingey et al., 2006; Auffret et al., 2016). Karhu et al., (2014), documented the results of a 90 day incubation study where they tested the temperature sensitivities of soil respiration to microbial responses from different land use types. They found that there was a lower effect of a temperature change on soil respiration rate in arable soils and soils with low C content, compared to other land uses. The greater effect of warming on respiration in other land uses was attributed to the loss of readily decomposable C rather than microbial community level responses. This observation has generated further argument by many authors in recent studies. For example, Hicks Pries et al., (2017) in a field study assessed the whole carbon flux through the soil profile in response to warming and claimed that soil respiration did not acclimate in response to temperature or become substrate limited since temperature sensitivity did not decline over 27 months of warming. Their results have demonstrated soil respiration from deeper soil which reflects similar temperature sensitivity down the profile and did not account for surface flux between 0-15 cm soils depths. This is the depth where most of the organic carbon and soil microbial activities are concentrated.

2.7.2 Soil depth as a model for soils with different diurnal temperature ranges

The variation in soil temperatures with depth exhibits a similar pattern as the expected air or surface temperatures forecasted in future climate scenarios. For instance, de Farias et al., (2018) assessed hourly, daily and monthly soil temperature from 5, 10 and 20 cm depths and found that, soil temperatures fluctuate diurnally down the profile, depending on month of the year. They also found a decreasing amplitude in DTR with depth (from 5 cm to 20 cm) due to higher diurnal temperature fluctuations at the surface compared to lower layers. They showed that, thermal diffusivity and soil moisture do not relate with one another due to differences in soil texture down the profile. Since soil textural differences with depth can influence thermal diffusivity, texture may influence the temperature sensitivity of soil processes occurring at various depth. Whereas soil respiration is usually measured in the laboratory from samples of soil collected at a defined soil depth range, or in the field as the integrated net flux from the entire soil profile (Graf et al., 2008), there could be variation in the temperature response of soil CO_2 release as a result of thermal or CO_2 diffusivity down soil depth in field measurements (Subke and Bahn, 2010), which are not accounted for in most studies.

It has been observed that soil respiration is rarely constant with soil depth. The variability can be daily (diel) or seasonal. One reason may be because of the changes in biotic and abiotic conditions with soil depth. For example, the amplitude of diurnal temperature ranges (de Farias et al., 2018), texture, organic carbon (Fang and Moncrieff, 2005), abundance and distribution of heterotrophic soil microbial groups (Eilers et al., 2012) and enzyme activities (Schnecker et al., 2015) all vary with soil depth. Schnecker et al., (2015) documented a situation where different drivers control the pattern of enzyme activities at surface and subsurface horizons. Their results showed that SOM content and microbial community composition controls enzyme activities of topsoil while soil pH, water content and microbial community composition controls enzyme activity in subsoils.

Soil microbial properties in deeper soil horizon (mineral horizons) depend largely on climatic variables and bedrock properties, while those of the surface (more organic) horizons are more controlled by the overlying vegetation. (Klimek et al., 2016) assessed the functional diversity of soil microbial community from boreal and temperate regions under Pine forests and found that, there were no differences in the microbial properties measured in O horizon, but the A horizon showed a different pattern in functional diversity. In the A horizon, soil microorganisms were more active and more functionally diverse in temperate forest than boreal

forests. Their results are indicative of the importance of both the vegetation cover, soil depth and climate in determining soil microbial functional diversity.

2.8 Soil microbial responses to temperature changes moderated by site (soil) history

Soil microbial resistance and resilience to disturbances are controlled by the physico-chemical structures of the soil, but history of the site can also influence the process (Griffiths and Philippot, 2013). The microbial response to climate change might be limited by the history of the local climate, resulting in larger, more variable, or less predictable, responses when soils are incubated outside of their historical temperature range (Keitt et al., 2015). Wu et al., (2010) reported historical and incubation temperature effects on soil microbial biomass and community structure based on phospholipid fatty acid (PLFA) analysis. Their results showed that decreasing soil microbial biomass was found as temperature increased, depending on the temperature history of the site. Decreasing microbial biomass might be a result of thermal denaturation that increased mortality of soil microbes at higher temperature. Their result portrays how organisms from low temperature environments could not survive high temperatures, but those from high temperature could.

Similarities between initial or first disturbances and subsequent disturbances can influence the response of soil microbial processes and communities to disturbances (Griffiths and Philippot, 2013; Hawkes and Keitt, 2015). Soils earlier exposed to more than 20 years of either 0 or 750 kg copper ha^{-1} with low (4.0) or neutral (6.1) pH caused reduction in soil respiration (induced with Lucerne meal powder) after the subsequent lead or salt stress was imposed (Tobor-Kapłon et al., 2005). Addition of lead which is also a metal caused greater inhibition of soil respiration in copper contaminated soil compared to soils not contaminated with lead. The observed higher inhibition of soil respiration was greater at low pH and higher copper concentration soils where increased bioavailable copper concentration was also encouraged after lead addition. These authors concluded that reduced stability due to environmental impact of a single stress will negatively affect community stability and that subsequent stress will exact stronger impact than undisturbed conditions. In another study, Tobor-Kapłon et al., (2006) demonstrated that soil respiration or bacterial growth in metal contaminated soils with long term zinc and cadmium due to proximity to a zinc smelter differed in their response to the subsequent lead, salt (NaCl), or heat stress. Soil respiration in polluted soils showed low stability to heat and salt stresses, compared to lead, implying that lead exacted least effect on soil respiration compared to heat and salt stresses. This weak effect of lead on soil respiration was common to both historically

polluted and non-polluted control soils. However, in terms of heat and salt stresses, non-polluted soils showed greater resistance to stresses than polluted soils. Heat stress exacted a greater effect on soil respiration than lead or salt stresses. These authors believed that historical metal pollution led to the development of a lead tolerant community that utilized the carbon in dead cells of previously impacted microbial organisms thereby resulting in greater soil respiration. Also, they attributed the higher effect of heat stress to lack of adaptation of the soil microbial community to temperature since the land use type of study site was a forest type with litter layers which protect the soil against temperature fluctuations. This observation is contrasted by a similar study where pH stresses were imposed to agricultural soils with higher temperature fluctuation and known history of copper (Tobor-Kapłon et al., 2006a).

Land use history can create a gradient of increasing intensity to disturbance which, in turn, can lead to variations in physical and chemical characteristics of soils that modify the soil microbial community composition (Steenwerth et al., 2003). Total or organic carbon, total or inorganic nitrogen, ratio of C/N, and available P have been identified as chemical properties that influence soil respiration (Luan et al., 2012; Wang et al., 2013; Qiu et al., 2005; Lal, 2004). Although these physical and chemical properties exert varying effects on ecosystem processes, the magnitude and direction of such effects depends on the soil or land use type under varying temperature regime. Steenwerth et al., (2003) assessed soil microbial community composition and land use history in cultivated and grassland ecosystems. They found that grassland soil had higher total PLFA (biomass), higher C, higher N, and lower pH compared to cultivated soils. They attributed the differences in total microbial biomass detected to the effect of labile organic matter on microbial composition, thus projecting the impact of land use change on substrate availability and quality on microbial composition. Jangid et al., (2011) demonstrated that, land use history had a stronger impact on soil microbial community composition than above ground vegetation and soil properties. Their results portrayed that the legacy effects of several decade's land use history were the determinants of the microbial community composition. Matsushita et al., (2007) also observed variations in soil microbial community structure from different forest ecosystems. Their results showed variation in the magnitude of change in community structure along the soil depth gradient; where the impact was more obvious in the surface layer (0-5 cm) than the deeper layer, suggestive of the legacy effects of land use and depth gradients on soil microbial community. They did not attribute the differences observed to changes in soil chemical characteristics.

Pettersson and Bååth, (2003) reported on the temperature dependent changes in the soil bacterial community in limed and unlimed soil. Using the Thymidine incorporation method, Pettersson and Bååth, (2003) assessed the response of soil microbial activities on bacteria extrated from soils. Bacterial communities in soil earlier incubated at higher temperatures adapt well to higher temperature compared to those earlier incubated at low temperature. Also, PLFA analysis showed that samples earlier incubated at higher temperatures (20 ^{o}C and 30 ^{o}C) showed only a small change in pattern when moved to a lower temperature (5 ^{o}C) for another 35 days of incubation. However, higher temperatures led to a higher rate of activitiy and community changes due to higher turnover rate compared to lower temperature. They further demonstrated that temperature changes may lead to phenotypic acclimmation where shift in community composition appears before changes in activities.

It has been noted that changing temperature can lead to a selection pressure, thereby altering microbial communities (Pettersson and Bååth, 2003; Bárcenas-Moreno et al., 2009). Therefore, microbial species that adapt well to the new temperature condition have tendency to grow faster and outcompete those that cannot adapt well to the new condition thereby yielding a shift in microbial community composition and function (Pettersson and Bååth, 2003). The magnitude of temperature change determines how much selection pressure will be expressed and this may subsequently influence the rate of microbial community shifts or adaptation.

2.8.1 Soil microbial response to temperature change as influenced by life strategy

It is a general notion that soil microbes can inhabit almost any kind of soil habitat, including extremely hot or cold, extremely acid or alkaline, salt affected regions, or areas with high concentrations of pollutants like polychlorinated biphenyls, hydrocarbons, pesticides etc. This remarkable ubiquity is because soil microorganisms have a high adaptive capacity to survive and tolerate such conditions. However, soil habitats even in relatively benign ecosystems can be regarded as extreme environments for soil microorganisms due to the fluctuating conditions they experience during episodes of dramatic environmental changes, as predicted under climate and global changes. Such fluctuations could result in high metabolic cost to survive the stress and eventually cause their death (Torsvik and Øvreås, 2008). However, in such conditions we yet still find soil microbes, which is because they have very high physiological and ecological plasticity which enables them to tolerate sudden and dramatic environmental changes. Such conditions could be extremes of temperature gradients, moisture and nutrient levels, or the

presence of toxic substances (Torsvik and Øvreås, 2008). For example, extremophiles can thrive or tolerate conditions that limit other organisms even though they normally grow best under more moderate conditions.

Examples of extremophiles include those organisms that can adapt to low temperatures (psychrophiles or psychrotrophs) and those that can adapt to high temperatures (thermophiles or thermotrophs). They also include those organisms whose nature of adaptability at extreme or fluctuating conditions could span different areas of limitation or extremity (e.g. the thermophiles and xerophiles). Such organisms are found in desert or arid environments with low precipitation, highly variable temperatures and where soils are deficient in nutrients. Warming yields a positive selection effect from psychrotolerant to mesophilic sulphate reducing bacterial in an arctic soil (Robador et al., 2009) under warming condition compared to ambient temperatures. Future climate change is expected to bring about shifts or fluctuations in the normal or prevailing environmental conditions with high temperature and low precipitation. Thus, soil microbes may require physiological adaptation to such conditions if they are to survive in an increasingly climatically extreme environment.

Physiologically different microbial groups compete for similar C substrates under similar environment conditions, and thus form their niche based on their preference for survival (life strategy). Life strategy is defined as the ability of organisms to survive and grow under climate driven stress and competition (Evans and Wallenstein, 2014). Such strategy has been classified according to the influence of environmental circumstances occurring in nature. They include the L-selection under adverse environmental condition, r-selection under state of temporary ecological vacuum (i.e. at pioneer stages of succession) and K-selection which are at the climax stage (i.e. under most intensive competition) (Panikov, 1999). Soil microbiologists also define r–strategist microbes as those with high growth rate and low resource use efficiency (copiotrophs) and K–strategist microbes as those with low growth rate and high resource use strategy (oligotrophs) (de Vries and Shade, 2013). There are two groups of L-selected organisms. One group of L- selected organisms such as psychro-, thermo-, halo-acid-, and alkalotolerant organisms adapt to unfavourable or harsh environments, while the other group have the capacity to adapt under starvation by growing structures like cysts, spores, etc. which enables them to enter dormancy during starvation. L-selected organisms therefore will be more resilient under conditions that relate either the harshness of extreme or fluctuating temperature with C limitation as climate changes. A community dominated by L-selected microorganisms will exhibit high functional diversity (Panikov, 1999). Increasing ratios of fungal to bacterial

abundance, increasing ratios of Gram positive bacterial to Gram negative bacterial relative abundance, and increasing C/N ratios of microbial biomass are indicative of the abundance of K-strategists (oligotrophs). In contrast, communities with high abundance of bacteria are known to be r-strategist (copiotrophs) communities, which are more resilient than K strategists. K strategists are known to be less resilient but more resistant than r-strategists under climate change scenarios. A frequently disturbed soil community will have more oligotrophs, compared to a soil maintained under constant conditions, thereby supporting the resilience of the soil microbial community (de Vries and Shade, 2013). Metabolic quotients (ratio of soil respiration rate per microbial biomass) have also been used to identify the presence of K-strategists. Lower quotients suggest substrate depletion during decomposition processes (Mamilov and Dilly, 2002), indicative of a soil dominated by K-strategists.

2.8.2 Soil microbial response to temperature changes through dormancy

The functional response of a community could be as a result of the physiological breadth of an individual taxon or a diversity of physiologies among taxa. Short term changes in soil temperature or moisture could lead to functional plasticity in soil microbial communities. Another form of functional plasticity is dormancy, a life strategy that enables members of a community to avoid environmental stress for a temporary period, followed by resuscitation when the conditions become more favourable, contributing to functional resilience. Whether this will lead to resilience depends on the persistency of the dormant propagules and the nature of the new environment (Keitt et al., 2015). Soil microbial communities may respond to soil warming or cooling by entering into dormancy (reversible state of reduced metabolic activity) and this phenomenon may influence how microbial communities adapt or deliver functions during or after extreme climatic events.

The dormancy of microbial communities due to warming varies with the magnitude and direction of warming. For instance, the relative abundance of bacterial (and/or fungal) populations that contribute to various biogeochemical processes could vary through time when there are fluctuations in environmental conditions (Aanderud et al., 2015). Dormancy enhances the capacity of soil microbial groups to maintain diversity by helping communities to build ‘seed banks’ of microbes that can resuscitate after a period of inactivity under a more favourable environmental conditions, reduce strengths of species interactions by encouraging co-existence due to storage effects, or to protect taxa from extinction (Aanderud et al., 2015). It should be noted that not all microbial taxa/species participate in all microbially-mediated soil processes, some microorganisms have been found to remain inactive or dormant due to stresses

such as desiccation or reduced availability or diffusion of substrates. A flush of CO_2 evolution after rewetting events reported by several authors supports the hypothesis that short-term changes in soil microclimate can cause dormant microbial groups to resuscitate and dominate the activity of the community after the change. The impact of drying-rewetting has been used to demonstrate how fluctuations in environmental condition promote dormancy in soil microbes. However, little is known on how this phenomenon changes under fluctuating temperatures.

Lennon and Jones, (2011) reviewed the ecological and evolutionary implications of microbial dormancy and noted that unfavourable environmental changes in abiotic factors like osmotic pressure (moisture) and temperatures could result in microbial dormancy which is known to be more common to soil than any other ecosystem. Direct or indirect effects of such abiotic factors could result in changes in resource availability, perturbation regime, predation, and residence time. In general, resource limitation and starvation are the common causes of dormancy and they often result in a stringent response whereby there is reallocation of resources to survival rather than growth due to a depletion of intracellular amino acids, fatty acids, and other carbon compounds. Theoretically, when environmental conditions are stable, there is a spontaneous switch from active to a dormant state which helps organisms to avoid the cost required to be sensitive to their environment, thereby generating a subpopulation of the microbial group or individuals that can respond to stochastic perturbation events (Kussell and Leibler, 2005; Lennon and Jones, 2011). However, under fluctuating conditions responsive switching may be possible.

Although it is very difficult to quantify dormancy, phenotypic characteristics like altered quantity and composition of lipids and fatty acids (Linder and Oliver, 1989; Kieft et al., 1997), reduced DNA and RNA content (Dell'Anno et al., 1998; Lebaron et al., 2001; Suzina et al., 2004), and changes in the stoichiometry of biologically important elements (Fagerbakke et al., 1996; Mulyukin et al., 2002) can be measured to ascertain the presence of dormancy under a perturbed system. For example, measures such as changes in C, N, or C/N ratio can be used to determine changes in stoichiometry, while changes in the ratios of trans-/cis-monoenoic fatty acids, changes in the ratios of cyclopropyl precursors/monoenoic precursor fatty acids and changes in the ratios of saturated/unsaturated fatty acids or reduction in total lipids or enzyme activity can be used to detect altered quantity and compositions of lipids and fatty acids when assessing phenotypic characteristics of dormant microbes (Lennon and Jones, 2011).

Owing to the diversity and overlapping functional traits within microbial communities, resistance and resilience of microbial communities could vary under forces of environmental changes. Response or stability of microbially mediated ecosystem processes therefore depends on the biodiversity of the microbial communities under stress. Biodiverse communities can enhance the stability of ecosystem processes through functional redundancy when there are overlapping functional traits among many species. However, this functional redundancy depends on whether the functionally redundant groups are able to respond differently to varying environmental conditions or perturbation. Quantifying the extent of dormancy can help identify the stability of microbial communities under such conditions.

The need to differentiate the active microbial biomass and total microbial biomass is of great importance due to our understanding that some members of soil microbial communities may go into dormancy in response to a perturbation (and then become active when conditions become suitable again) and that not all the total microbial populations are actively involved in ecosystem processes under a given environmental condition at a given time. Salazar-Villegas et al., (2016) examined the respiration of soil microbial communities and discovered that soil respiration correlates with the active microbial pool rather than the total microbial pool under a short-term changes in soil temperature and moisture parameters. They concluded that using the total microbial pool to explain soil respiration response may lead to underestimation of CO_2 release capacity of soil and that the concepts of active and total microbial biomass will help account for the active and dormant portions of the community that always accompany the response of soil microbial communities to environmental changes. Their results imply that the impact of temperature changes on soil respiration are primarily due to their impacts on the active microbial pool rather than the total microbial pool. This view remains new and under-exploited. In order to capture dormancy as a common adaptive strategy used by soil microbes He et al., (2015) proposed a microbial-enzyme decomposition model that includes dormancy while investigating controls on soil respiration and dormancy. They found a better representation of soil respiration at field scale and a more realistic magnitude of microbial biomass soil respiration from regional modelling when dormancy was included in the model than when it was not. They also found that while temperature and moisture were found to exert local and temporal controls on dormancy, C/N ratio was found to regulate dormancy at regional scale.

2.8.3 Response of soil microorganisms to changes in temperature as moderated by microbial physiology

Temperature determines to a large extent both the temporal and spatial variation in the distribution and abundance of organisms because it separates organisms into niches, depending on their physiological tolerance (Bradford, 2013). Most microorganisms around the temperate zone could be categorised as either psychrophilic or psychrotrophic (psychrotolerant). De Maayer et al., (2014) noted the differences between psychrophilic *sensu stricto* and their psychrotolerant counterparts. Psychrophilic bacteria are extremophiles; organisms which have an optimum temperature for growth of 15 ^{o}C or below and their maximum and minimum temperatures at about 20 and 0 ^{o}C. Psychrophiles represents the most diverse, most widespread, and most abundant extremophiles on the surface of earth. By contrast, psychrotrophs have the capacity to grow at low temperatures, but their maximum and optimum temperatures are above 20 and 15 ^{o}C respectively (Moyer et al., 2017). Above their maximum temperature psychrophiles may tend to lose their ability to take up oxygen, but at optimal or supra-optimal levels they produce more protein or rRNA and the organisms grow rapidly or maintain their membrane fluidity at low temperatures (Moyer et al., 2017). Inability to take up oxygen impairs respiration during their metabolism, thereby limiting soil respiration.

The effects of changes in temperature on psychrophilic and psychrotrophic microorganisms can be examined using PLFA physiological profiles. Changes to the lipid composition of the cell membrane in response to temperature can be observed using this technique. The changes to the fatty acids within the lipid membrane include shifts between poly-unsaturated or unsaturated, branching or cyclization, chain length or combinations of these features, but the exact nature of membrane alterations will depend on the bacterium involved (Moyer et al., 2017). For instance, psychrophilic and psychrotrophic bacteria contain unsaturated, polyunsaturated, short chain, branched and cyclic fatty acids within their cell membrane. When the optimum temperature required for the growth of psychrophiles decreases, it leads to increases in their levels of poly unsaturated phospholids and neutral lipids to maintain membrane fluidity at low temperatures. While psychrophiles are extremophiles suited to cold environments, psychrotrophs are found mainly in fluctuating environments and are therefore able to alter their fatty acid composition more easily. Decreasing temperatures are characterised by lipid desaturation, thus increasing the amount of unsaturated lipids, though temperature dependent changes may follow thereafter and this helps to maintain the membrane's capacity to perform function at low temperatures.

Low temperatures in cold environments can cause a severe physicochemical limitation on the function of microbial cells which may negatively impact the integrity of cell, water viscosity, solute diffusion rate, membrane fluidity, enzyme kinetics and interactions of macromolecules. Psychrophiles and psychotrophs have various mechanisms by which they can adapt to low temperatures thereby counteracting temperature stress under such environmental conditions. Examples of stress associated with low temperature, especially in cold environments, includes desiccation, radiation, excessive UV, high or low pH, high osmotic pressure and low nutrient availability (De Maayer et al., 2014). These stress factors are among the independent environmental factors that influence microbial physiology and molecular biology of soil organisms (Panikov, 1999). Assessing the microbial response to such factors is therefore a research priority as climate changes.

2.8.4 Changes in phospholipids fatty acid (PLFA) profiles due to temperature changes in soil.

Temperature is the major environmental factor that has a direct impact on the phospholipids of soil microbes, which are an important part of their cell membranes. Soil ecologists have used the profile of phospholipids fatty acids (PLFA) extracted from soils to assess the impact of environmental factors (including temperature changes) on soil microbial communities because the technique provides a 'finger print' of the community phenotype (Petersen and Klug, 1994).

Lipids representing the abundance of fungal and bacterial groups, or their ratios, are indicative of the rate of carbon cycling (Oates et al., 2017). Variation in fungal to bacterial lipids can also be used to determine soil capacity to store carbon due at low soil temperature (Malik et al., 2016). For instance higher fungal PLFA abundance and greater fungal/bacterial ratios are evidence for soil carbon storage characteristics at lower temperature, but decrease with warming in most studies (Bai et al., 2017). The response of fungi and bacteria to changing temperatures could be expressed as a decline in the relative abundance of fungal or bacterial PLFAs at high temperature or an increase in the relative abundance of fungal or bacterial PLFAs at low temperature (Strickland and Rousk, 2010). Total lipids is a measure of microbial biomass size and it's ratio with rate of soil respiration (i.e. CO_2 rate/total PLFA) is an important measure of qCO_2 (microbial metabolic quotient) (Bai et al., 2017).

The ratio of Gram positive to Gram negative bacterial PLFAs can be used to indicate carbon availability and whether we have more labile or more recalcitrant carbon in the system after a disturbance (Fanin et al., 2019). More abundant Gram negative bacteria, compared to Gram

positive, implies that there is more labile carbon compared to recalcitrant carbon. Warming is expected to increase the concentration or ratio of certain biomarkers used in determining various environmental stresses (Kaur et al., 2005). Stress indicators like the ratios of cyclopropyl/unsaturated monoenoic precursors (i.e. cy-/pre-) and Gram positive/Gram negative bacteria will also increase with increasing temperatures especially due to nutrient limitation imposed by warming (Bai et al., 2017). Increased temperature will result in higher ratio of trans to cis isomers of monounsaturated and cyclopropyl fatty acids (Kaur et al., 2005) It is generally expected that increasing temperature will lead to a decrease in cis-unsaturated fatty acids and brached-chain fatty acids (Kaur et al., 2005), there by increasing the concentration of straight-chain saturated fatty acids, which may be due to membrane lipid turnover or growth.

Bai et al., (2017) assessed temperature sensitivities of PLFA-distinguishable microbial communities incubated under varying and constant temperatures and found that diurnally varying temperatures (Varying between 10 and 30 °C) led to increases in the ratio of fungal/ Gram positive, fungal/ Gram negative, and fungal/actinomycetes PLFAs, but constant temperatures reduced these ratios, though the reduction was higher at high temperature regimes. PLFA biomarker ratios indicating stress conditions (i.e. cyclopropyl/monoenoic, Gram positive / Gram negative bacteria) were significantly higher under constant temperature but were reduced under varying temperature regimes. Their results point to the fact that fluctuating temperature conditions favour K-strategists (e.g slow growing fungal groups) and a less stressed microbial community that has greater membrane fluidity, compared to the constant temperatures.

Zhou et al., (2017) reported on the individual and interactive effects of soil temperature and moisture on the composition of the soil microbial community and found that, though the interactive effects of moisture and temperature was significant, the effect of temperature alone significantly increased fungal/bacterial ratio and decreased Gram positive / Gram negative bacteria due to increasing temperatures. Fungal PLFA were generally less abundant at higher temperatures but not affected by moisture. Also, the bacterial PLFAs were generally more abundant at high temperatures and moderate moisture contents. The dissolved organic carbon, inorganic nitrogen, and temperature factors explained about 30 % of the variation reported in their study, indicating the higher importance of temperature and temperature induced changes on soil properties. This importance was further confirmed in the correlation analysis which

showed a correlation between the altered microbial community composition and soil properties. Their results are indicative of the impact of climate change on soil microbial functions.

Zogg et al., (1997) assessed compositional and functional shifts in microbial communities due to warming in an incubation experiment and found increased microbial respiration due to increases in the pool size of C metabolised by microbes at high temperatures due to a shift in microbial community composition. Their results revealed that the substrate pools for microbial respirations at higher temperature triples that of the lower temperatures. They also reported a decreasing unsaturation, greater chain length, and greater numbers of cyclo-propyl fatty acids and a decreasing total PLFA at higher temperature. Furthermore, they revealed that low biomass at higher temperatures metabolize more substrate. Impacts of temperature and substrates on microbial PLFA composition was assessed in a laboratory study (Feng and Simpson, 2009). It was established from this study that, without substrate limitations, PLFA biomarkers for fungi and Gram negative bacteria reduced compared to Gram positive bacteria in soils incubated at higher temperatures. This was attributed to fast depletion of available nutrients due to continuous incubation at higher temperature. Also, their results showed a linear relationship between the incubation temperatures and microbial stress biomarkers such as the ratios of cyclopropane PLFA-to-monoenoic precursor i.e. cy17:0/16:1ω7c, and cy19:0/18:1ω7c and mono/sat PLFAs. This relationship was attributed to the combined effects of temperature and temperature induced substrate limitations. Finally, there may be a shift in microbial community composition due to warming without a corresponding effect on the rate of decomposition. This may be a result of functional redundancy on the part of the decomposing community.

2.9 Summary and conclusions

Soil respiration represents the largest flux of carbon from the terrestrial pool to the atmospheric pool in the carbon cycle. This flux can be in form of autotrophic respiration through the plant roots and root associated organisms or heterotrophic respiration through the decomposition of plant biomass, soil organic matter, and soil animals by heterotrophic soil organisms. The role of soil in the carbon cycle is enormous owing to its large reservoir of carbon and the host of micro and macro organisms that it provides a habitat for. Carbon flows through the food web first as primary substrate (food) for the heterotrophic microbes (e.g. fungi, bacteria and mycorrhizae) and subsequently through these channels into higher trophic levels. Soil

respiration is a product of the activities of these microbes. It a processes that can fluctuate or oscillate hourly, daily, seasonally, annually, or be subject to changes over millennial time scales.

Soil respiration can be influenced by many natural and anthropogenic factors. These include changes in abiotic variables like climate, land use and other environmental factors as well as biotic factors like the community composition of soil microbes, the production and activity of enzymes, and substrate quantity or quality. There could also be interactions between or among biotic and abiotic factors that cause changes in soil respiration.

The effect of climate change on soil respiration has become an important issue globally due to the fact that when climate warms, it stimulates CO_2 release into the atmosphere and enhances the greenhouse effect, thus causing further global warming. Most important among the climate variables that influence soil respiration is temperature. While measures of soil respiration (or even organic matter decomposition) in response to changes in temperature have been thoroughly researched, the mechanisms by which soil respiration, enzymes and the soil microbial community respond to temperature change remains uncertain. One possible reason for the uncertainty is how such studies have been designed. Previous efforts have typically incubated soils at constant temperatures that represent the mean seasonal or annual temperature to assess the mechanisms underlying the temperature sensitivity of soil respiration in laboratory or field warming experiments. This inadequately represents the expected rise in temperature predicted to occur as a result of climate change. Average annual temperature fails to take into account the daily and seasonal temperature fluctuations and the asymmetric increases in daily maximum and minimum temperature that are expected to occur. Increases in both daily maximum and daily minimum temperature and a reduction in the diurnal temperature range have been predicted due to climate change. However, experiments designed to understand how such asymmetrical warming will influence the mechanisms underlying temperature sensitivity of soil respiration are rare.

Generally, increasing temperature (warming) will result in the depletion of organic carbon, reduce total microbial biomass or dormancy of microbial cells, shift the soil microbial community composition, reduce soil moisture, increase soil enzyme kinetics, and change soil chemistry (e.g. pH). Research investigating the effects of warming on soil microbial communities and their subsequent respiration show high levels of inconsistency and uncertainty. While some studies have reported thermal acclimation, some did not find evidence

of thermal acclimation. We know that thermal acclimation in enzyme function can influence the whole soil heterotrophic respiration, and current enzyme activity results from a combination of the long-term microbial activity and the activity of the currently active microbial population. Enzyme activity catalyses the decomposition of organic matter that brings about soil respiration. We therefore propose that cumulative effect of warming and the asymmetric changes to daily or seasonal fluctuations of temperature will affect soil respiration differently to what might be predicted by an increase the average temperature. This asymmetric warming may influence the resilience of soil microbial community or their function to further stress from climatic extremes. Therefore, the design of future experiments should consider exposing soils to appropriate daily temperature oscillations to adequately predict the effect of climate change on soil respiration and resilience.

Chapter 3

This chapter is a paper published in *Journal of Soil Science and plant Nutrition*

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3.1 Abstract

This study aims to investigate effect of sieving on *ex-situ* soil respiration (CO_2 flux) measurements from different land use types. We collected soils (0 – 10 cm) from arable, grassland and woodland sites, allocated them to either sieved (4 mm mesh, freshly sieved) or intact core treatments and incubated them in gas-tight jars for 40 days at 10 ºC. Headspace gas was collected on day 1, 3, 17, 24, 31 and 38 and CO_2 analysed. Our results showed that sieving (4 mm) did not significantly influence soil respiration measurements, probably because micro aggregates (<0.25 mm) remain intact after sieving. However, soils collected from grassland soil released more CO_2 compared to those collected from woodland and arable soils, irrespective of sieving treatments. The higher CO_2 from grassland soil compared to woodland and arable soils was attributed to differences in the water holding capacity and the quantity and stoichiometry of the organic matter between the three soils. We conclude that soils sieved prior to *ex-situ* respiration experiments provides realistic respiration measurements. This finding lends support to soil scientists planning a sampling strategy that better represents the inhomogeneity of field conditions by pooling, homogenising, and sieving samples, without fear of obtaining unrepresentative CO_2 flux measurements caused by the disruption of soil architecture.

3.2 Introduction

CO_2 flux from soil not containing plant roots represents the heterotrophic respiration of soil organisms (Gabriel and Kellman, 2011). Soil respiration releases more CO_2 into the atmosphere annually than all anthropogenic sources combined (Marland, 2008) and a small change in CO_2 flux from soils, globally, can greatly alter the concentration of atmospheric CO_2 and influence our climate (Schurgers et al., 2018). Measurement of soil respiration is therefore important for quantifying the flux of CO_2 to the atmosphere from soils. Soil CO_2 flux also represents the

activity of soil biological communities, thereby serving as a valuable indicator of soil health (McGowen et al., 2018).

Soil respiration measurements undertaken in the field and laboratory often yield contrasting results (Davidson et al., 1998). CO_2 flux measured at a single location *in situ* may not be representative due to soil heterogeneity at the field scale. Excavation of soil, followed by *ex-situ* measurement of soil respiration under controlled conditions is often performed to compare soils or test specific hypotheses (Gutinas et al. 2013; Zhou et al. 2014; Bao et al. 2016; Yan et al. 2017), while minimising confounding factors. Whereas *in-situ* measurements are more representative of the actual field conditions (Gabriel and Kellman, 2011), *ex-situ* measurements can be used to apply treatments in a systematic manner and are thus very useful. Soil respiration can be measured *ex-situ* on intact cores (Hangs et al. 2016; Meyer et al. 2019), fresh soil sieved with mesh sizes ranging 2 mm to 5 mm (Thomson et al. 2010; Datta et al. 2014), or (most frequently) air-dried soil sieved to 2 mm (Mathur and Sanderson 1978; Valerie and Cook 1983; Thuries et al. 2000; Thomson et al. 2010; McGowen et al. 2018). Sieving and homogenisation prior to incubation (often favoured by researchers to create replicates appropriate for statistical analysis) disrupts the original architecture of the soil experienced by decomposer organisms (Baveye et al., 2018) and may influence the soil respiration measurement. Few studies have previously examined the effects of sieving on soil respiration. While one of them (Stenger et al., 2002) revealed no significant differences in glucose-C mineralisation between intact and sieved soil, another (Herbst et al., 2016) observed that air-drying and sieving influenced the nature of the relationship between soil moisture and soil respiration.

It is often believed that disturbing soil by sieving will expose occluded organic matter to microbial degradation and break fungal hyphae, thereby generating a flush of CO_2 (Datta et al., 2014). We aimed to assess the effect on soil respiration of sieving soils collected from a woodland, a grassland, and an arable field representing the major land uses in the UK (Table 3.1). We hypothesised that sieving would increase short-term soil respiration and that this difference would be greater for soils less affected by previous physical disturbance (i.e. woodland soils).

3.3 Materials and Methods

Twelve soil cores were collected using 98 cm^3 bulk density rings (inner diameter 5 cm, height 5 cm), similar to that used by Comeau et al. (2018), from woodland (Latitude 51º28.678′, Longitude 000 º53.739′), grassland (Latitude 51º28.564′, Longitude 000 º54.198′) and arable

(Latitude 51º28.577′, Longitude 000 º53.970′) soils on the University of Reading research farm at Sonning, Berkshire, UK. Sonning soils are classified as Chromic Endoskeletic Luvisols, containing freely draining slightly acidic loamy soils predominantly and the site location matches Sonning 2 soils description (Cranfield University, 2019) which are flinty coarse loamy soils over a gravelly typical paleo-argillic brown earths. The three land uses represent the most common land use types in UK, and around the world. While the grassland is mainly used for grazing by dairy cattle, the arable land was planted to wheat which was at seedling stage at the time of sampling. The woodland (comprising of mixed deciduous species) has been established for over 80 years.

Table 3.1: Location and physical and chemical properties of the three different soils from different land uses.

	Land Use		
	Arable	Grassland	Woodland
Easting	476503	476187	476710
Northing	175919	175861	176060
%Sand(50-2000µm)	41.46	43.01	51.11
%Silt (2-50µm)	50.46	48.86	40.76
%Clay (<2µm)	8.08	8.14	8.13
Texture	Silt loam	Loam	Loam
%Water Holding Capacity	48.88(1.11)	55.00(1.05)	40.54(0.87)
pH in Water	6.30 (0.02)	6.23 (0.37)	3.86 (0.03)
NO_3-N (mg g^{-1})	13.48 (1.56)	6.32 (0.73)	12.52 (1.40)
NH3_N (mg g^{-1})	0.53 (0.21)	0.58 (0.25)	1.87 (0.07)
Total N (g kg^{-1})	1.88 (0.08)	2.59 (0.06)	2.07 (0.04)
Total C (g kg^{-1})	19.05 (0.26)	26.31 (0.54)	28.16 (0.63)
C/N ratio	10.12 (0.29)	10.17 (0.06)	13.62 (0.05)

Values in parenthesis are standard deviations, n = 3

Soil samples were stored immediately at 4 °C for 3 days before the start of the experiment. Six cores per land use were left intact within the rings used to collect them (intact cores treatment) and the remaining six were sieved, moist, to 4 mm, re-packed back into bulk density rings, and the residue on the sieve discarded. Each ring was placed in a 320 ml gas-tight jar customised to include a gas sampling port and covered with Parafilm® to reduce moisture loss (but allow

gas exchange) when not in use (Figure 3.1). Six empty rings (without soil) incubated in jars served as blanks similar to the method in Winkler et al. (1996) instead of the time zero measurement to correct for the initial flux. Samples were incubated at 10 °C for 45 days. The temperature was chosen to reflect the average temperature of topsoil (10 cm) in Reading, UK between 1990 and 2017, which was 10.48 °C (University of Reading, Meteorology Department Weather Station). The moisture content in the soils were 21.6, 13.4 and 22.8 % (w/w) in arable, woodland and grassland, respectively. The moisture content of the soil in each jar was adjusted to 23 % (w/w) to maintain the set up at constant temperature and moisture. On days 1, 3, 17, 24, 31, and 38 after the start of the incubation, jars were sealed with a Suba-Seal® Septa for one hour and a 10 ml headspace gas sample was taken from each jar using a syringe and hypodermic needle, transferred into pre-evacuated Labco® exetainer vials and analysed with gas chromatography (Agilent 7890B). A 24 hour pre-incubation was adopted, following the method in Meyer et al. (2019), but unlike the 14 days pre-incubation used in Comeau et al. (2018). A short pre-incubation prevents fast depletion of organic carbon, thereby preserving the carbon in near-field conditions. Moisture loss was corrected by gravimetric addition of deionised water after each gas sampling. The universal gas law was used to determine the amount of CO_2 (µmol CO_2/ mol air) in our incubation jar; µg C-CO_2 was calculated as: µg C-CO_2 g^{-1} soil = mmol air * ppm CO_2 (µmol C / mol air) * (10-3 mol/mmol) * (12 µg C/µmol C) / weight of oven dried soil (g). Cumulative CO_2 (µg C-CO_2 g^{-1} soil) was calculated from flux rate, as reported elsewhere (Lang et al., 2011), after deducting the blank CO_2 concentration from each treatment. Prior to undertaking the experiment described above, we collected CO_2 headspace gas from both the sieved and intact cores from each of arable, grassland and the woodland soils after incubation for 30, 60, 90 and 120 minutes to confirm a linear relationship between CO_2 concentration and incubation time. Soil particle size distribution was measured using laser granulometry and converted from % volume to % mass, as described elsewhere (Yang et al., 2015a).

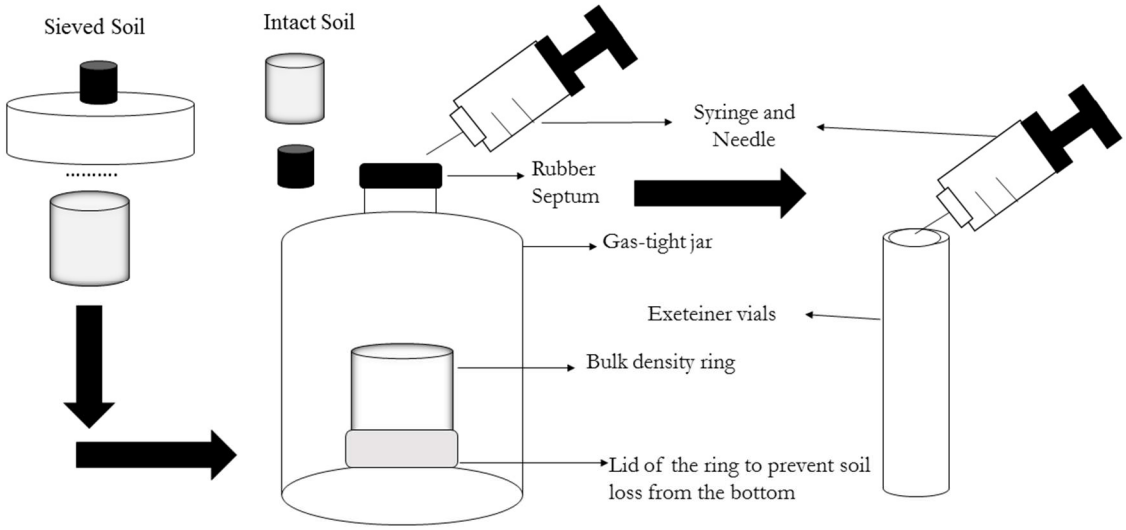

Figure 3.1 Diagram depicting experimental methodology.

Standard laboratory methods were used to analyse the soil characteristics presented Table 3.1. The particle size distribution of soils was determined using a Malvern Mastersizer 3000 Laser Granulometer after dispersing the soil in a solution containing 3.3 % sodium hexametaphosphate + 0.7 % sodium carbonate. Soil pH was determined by shaking soil samples with deionised water (1:2.5 mass/volume ratio) for 10 minutes and leaving the mixture to stand for 2 minutes before pH was measured using a digital type DMP-2mV/pH meter (Thermo Orion). Total N and C concentrations were determined using C/N Elemental Analyser (Thermo Flash 2000 EA). The C/N ratio was then calculated from total C and N. Nitrate and ammonia were extracted in 1M KCl and then analysed using Continuous Flow Analyzer (San^{++} Automated Wet Chemistry Analyzer - SKALAR). Moisture content and loss on ignition were determined by weight loss at 105 °C and 500 °C, respectively. Soil water holding capacity was determined using saturation and drain method by submerging a 30g air-dried sample in a plastic cylinder with a mesh bottom in water for 12 hours to ensure complete saturation and then allowing the water to drain for another 12 hours. The drained soil was then oven-dried at 105 $^{\circ}$C for 24 hours and the dried weight recorded.

Repeated measures Analysis of Variance (ANOVA) was performed in GenStat (10^{th} edition) to assess the variation in cumulative soil CO_2 due to sieving and land use changes on different sampling days. Data showing negative fluxes were observed in one out of six replicates under

disturbed grassland and were removed before the analysis. Correlations between the cumulative C-CO_2 flux and soil properties were explored.

3.4 Results and Discussion

The effect of soil sieving on cumulative CO_2 flux is presented in Figure 2.2. Repeated measures ANOVA revealed no significant effect of sieving on cumulative C-CO_2 ($p > 0.05$), even though intact cores emitted more CO_2 than sieved arable and grassland (but not woodland) soil. These observations challenge the assertion (Datta et al., 2014) that sieving soils stimulates a short term CO_2 flux, and our hypothesis that the decomposer soil microbial community under physically disturbed systems (e.g. arable soil) is more resistant to soil sieving compared to those of previously undisturbed systems (e.g. woodland soils). Performing our laboratory incubation study between 20 °C and 25 °C (which are often used in lab incubations) may result in different concentrations of CO_2 reported, but we believe that the temperatures selected here best represent the respiration of the soils in the field since we adopted the average daily mean soil temperature of the area from which the soils were collected. Our observations support the findings of others who also observe no significant difference in soil respiration between intact and sieved soils (Stenger et al. 2002; Thomson et al. 2010). However, the abovementioned studies analysed the effect of sieving on only pasture soils whereas soils from three different land-use types are compared here in our experiment. Further, the former (Stenger et al., 2002) reported only on the long-term (6 months) effect of sieving on soil respiration, while we report short-term-effects in this study.

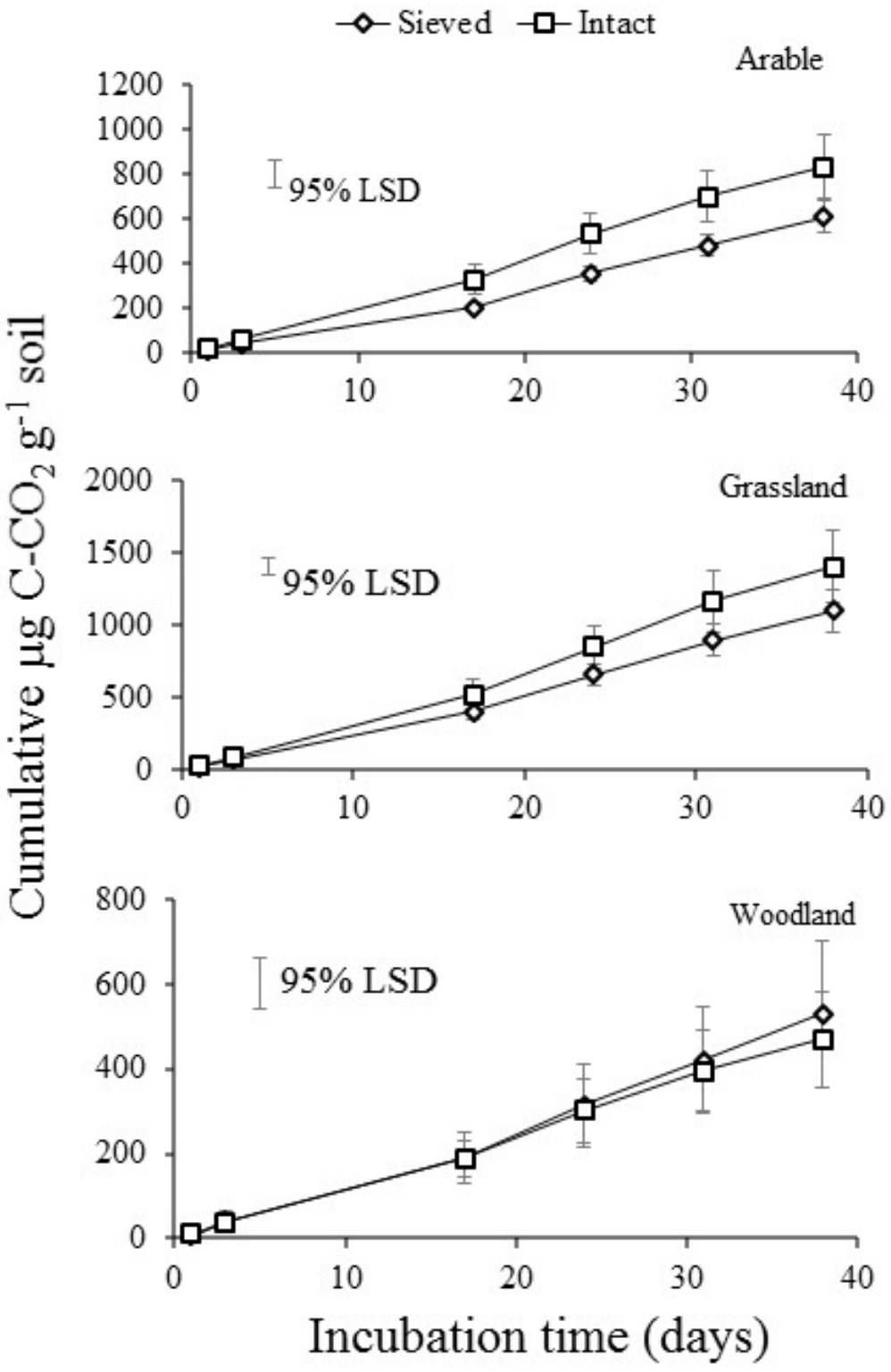

Figure 3.2 Cumulative CO_2 emissions of sieved soils and intact cores from arable, grassland and woodland soils. Error bars represent standard errors of mean, n = 6. 95% LSD = Least Significant Difference at the 95 % level.

Although previous studies have shown that macroaggregates (>0.25 mm) can protect a small fraction of soil organic C from mineralization, occlusion of C is likely to be more significant within microaggregates (<0.25 mm) that are present within the macroaggregates (Pulleman and Marinissen, 2004). Our sieving treatment using a 4 mm mesh would only have disrupted the largest macroaggregates, leaving microaggregates intact. This phenomenon might explain why we obtained no significant effect of sieving on CO_2-C flux. The use of a large (4 mm) mesh size for sieving also minimised the residue to be discarded to only an insignificant amount of small stones or woody material making the contents of the intact and sieved cores to be similar.

Clear differences were observed between soils from different land uses, irrespective of the sieving treatments. Cumulative C-CO_2 from grassland soil was 59.4 % higher than woodland and 42.1 % higher than arable soils, respectively. Similar results have been reported (Gutinas et al., 2013) where grassland soils emitted higher CO_2 compared to arable and woodland after 42 days of incubation. However, our results contrast with the earlier study (Lang et al., 2011), which examined greenhouse gas emissions from forest and grassland soils and revealed that woodland soils emitted more CO_2 than grassland soils, concluding that the global warming potentials of woodland soils are greater than that of grasslands.

The grassland soil had a higher water holding capacity, higher organic matter content, and lower C/N ratio (Table 3.1), whereas the woodland soil had a higher total carbon content, but lower total nitrogen than the grassland and arable soil (resulting in a higher C/N ratio). Thus, the differences in respiration between the three soil types could be explained by both the quantity and the stoichiometry of the organic matter. These differences can be further explained by considering the different water holding capacities of the three soils. All the soils were incubated at the same moisture content (23 %), but the woodland soil had a lower water holding capacity, compared to grassland and arable soils (Table 3.1). As a result, the grassland, arable and woodland soils were incubated at 41.4 %, 46.6 %, and 56.2 % of their respective water holding capacities, perhaps resulting in proportionally more water-filled pores in the woodland soil. However, we found no significant correlation between the cumulative C-CO_2 flux and any soil properties, including water-filled pore space ($R^2 = 0.116$; $p = 0.501$).

3.5 Conclusions

Our experiment reveals that, although soil respiration varies with land use type, soil sieving has no significant impact on *ex situ* CO_2 flux measurements. Thus, we conclude that soils sieved (4 mm) prior to ex-situ respiration experiments provides realistic respiration

measurements. However, we urge careful consideration when choosing a method of soil sampling and preparation prior to incubation for measuring soil respiration since both soil sieving and the use of intact cores have advantages and disadvantages. If soils are sieved prior to ex-situ soil respiration measurements, then a soil sampling strategy that better accounts for the overall inhomogeneity of field conditions can more easily be adopted (since samples from multiple locations can be pooled, homogenised and assigned to treatments). While sieving may help in achieving multiple similar homogenous replicates, leading to increased reproducibility, incubation of soils in intact undisturbed cores better represents the soil architecture under field conditions. As a result of this understanding, and considering that the data shown were obtained for only three soils (arable, grassland and woodland from Sonning, England), our results provide evidence to help resolve an important dilemma for soil ecologists planning *ex-situ* CO_2 flux measurements to determine the influence of imposed treatments on soil respiration. However, we acknowledge that there could be different results obtained when using soils from geographical regions, land use types, or soil management systems outside of those investigated in this study.

Chapter 4

This chapter is formatted as a paper to be submitted to *Global Change Biology*

Adekanmbi, A.A., Shu, X., Zhou, Y., Shaw, L.J. and Sizmur, T., (In prep). Legacy Effect of Constant and Diurnally Oscillating Temperatures on Soil Respiration and Microbial Community Structure. *Global Change Biology*

4.1 Abstract

Laboratory incubation studies evaluating the temperature sensitivity of soil respiration often use measurements of respiration taken at a constant incubation temperature from soil that has been pre-incubated at the same constant temperature. However, such constant temperature incubations do not represent the field situation where soils undergo diurnal temperature oscillations. We investigated the effects of constant and diurnally oscillating temperatures on soil respiration and soil microbial community composition. A grassland soil from the UK was either incubated at a constant temperature of 5 ℃, 10 ℃, or 15 ℃, or diurnally oscillated between 5 ℃ and 15 ℃. Soil CO_2 flux was measured by temporarily moving incubated soils from each of the abovementioned treatments to 5 ℃, 10 ℃ or 15 ℃, such that soils incubated at each temperature had CO_2 flux measured at every temperature. We hypothesised that, irrespective of measurement temperature, CO_2 emitted from the 5 ℃ to 15 ℃ oscillating incubation would be most similar to the soil incubated at 10 ℃. The results showed that both incubation and measurement temperatures influence soil respiration. Incubating soil at a temperature oscillating between 5 ℃ and 15 ℃ resulted in significantly greater CO_2 flux than constant incubations at 10 ℃ or 5 ℃, but was not significantly different to the 15 ℃ incubation. The greater CO_2 flux from soils incubated at 15 ℃, or oscillating between 5 ℃ and 15 ℃, coincided with a depletion of dissolved organic carbon and a shift in the phospholipid fatty acid profile of the soil microbial community, consistent with the thermal adaptation of microbial communities to higher temperatures. However, diurnal temperature oscillation did not significantly alter Q10. Our results suggest that daily maximum temperatures are more important than daily minimum or daily average temperatures when considering the response of soil respiration to warming.

4.2 Introduction

Soils harbour the largest actively cycling pool in the carbon cycle (Harden et al., 2018). Depletion of soil organic matter releases CO_2, a greenhouse gas, into the atmosphere, contributing to global warming. The resulting increase in global temperatures is expected to stimulate heterotrophic soil respiration (Bardgett et al. 2008; Walker et al. 2018), thus causing a positive feedback that releases more CO_2 into the atmosphere. The annual release of CO_2 from soils by heterotrophic microorganisms is about 8 to 9 times higher than anthropogenic emissions from the burning of fossil fuels (Dutta and Dutta, 2016), so reducing the uncertainty concerning the magnitude of the positive feedback under future climate change scenarios deserves attention (Davidson and Janssens, 2006).

The impact of environmental change on soil carbon can be simulated using soil carbon models (e.g. ECOSSE (Dondini et al., 2016), DNDC (Gilhespy et al., 2014) and CENTURY (Parton, 1996). These models divide the soil organic matter into pools which have different mean residence times in soil, related to their chemical recalcitrance. The rate of decomposition of each pool is subject to a first-order decay process, dependent, among other parameters, on the soil temperature, according to Arrhenius kinetics (Schimel and Weintraub, 2003). Other models (e.g. CEM (Foereid et al., 2014), CASA (Potter et al., 1993) and TEM (Raich et al., 1991) use a fixed Q_{10} value that represents the increase in soil respiration that occurs after a 10 °C increase in soil temperature (Meyer et al., 2018). These approaches have received criticism (Davidson and Janssens 2006; Schmidt et al. 2011) and, as a result, a new generation of soil carbon models are under development that better represent the physical and biological processes mediating soil organic matter turnover (Todd-Brown et al. 2012; Wieder et al. 2013; Abramoff et al. 2017). Soil carbon models generally operate on a monthly time step, using average monthly temperature as an input variable (Kirschbaum 1995; Yokozawa et al. 2010; Karhu et al. 2014), although there has been attempts to model daily time steps, using daily average temperature as an input variable (Gilhespy et al. 2014). In all of the abovementioned models, no consideration is made concerning the extent to which soil temperature oscillates diurnally, the influence this may have on the inherent temperature sensitivity of soil respiration, or whether daily or monthly average temperatures are adequate to capture the temperature sensitivity of soil respiration to the changes in temperature that we actually expect soils to experience (Mitra et al., 2019). There is a lack of experimental evidence highlighting the

importance of diurnal temperature range, daily maximum temperature, or daily minimum temperature on soil respiration.

Assumptions on the relationship between soil temperature and soil respiration and their interpretation are often arbitrary (Subke and Bahn, 2010b). One such facet that is arbitrary in nature is the selection of two temperatures for which the temperature coefficient (Q10) is determined during ex situ measurements (Graf et al., 2008). Often, the two temperatures, 10°C apart, that are chosen do not fall within the daily temperature ranges that soil microbial communities were previously exposed to in the field. To improve the accuracy with which temperature sensitivity (Q10) is determined in laboratory assays, estimating Q10 using temperature that reflect field conditions has been suggested (Pavelka et al., 2007;Graf et al., 2008). A similar diurnal oscillation of CO_2 efflux and temperature (Akinremi et al., 1999) confirms the need for such recommendations. Estimating temperature sensitivity Q10 using the daily maxima and minima temperature may provide a better estimate of the relationship between soil temperature and soil respiration for modelling purposes.

The wide range of Q_{10} values reported in the literature may also be explained by differences in the laboratory and field procedures that have been used to measure soil respiration (Smith et al., 2018). A possible reason for this wide range may be because the temperature sensitivity of soil respiration is often assessed without considering that temperature oscillates diurnally (Ross and Täte, 1993; Winkler et al., 1996; Conant et al., 2008; Chen et al., 2009). This omission may be the reason why differences in seasonal Q10 of soil respiration earlier reported did not represent differences in the temperature sensitivity of soil microbial metabolism (Curiel Yuste et al., 2004; Gritsch et al., 2015). Periodic fluctuations in air temperatures can influence the soil temperatures and affect the underlying soil microbial activities (Chang et al., 2011) which, in turn, may influence soil respiration. How temperature fluctuations alter soil microbial community assemblages and how this may influence soil microbial function is important in predicting the impact of climate change on soil respiration (Uvarov et al. 2006; Hawkes and Keitt 2015).

Oscillating temperatures are uncommon in laboratory experiments, where soils are often incubated under constant temperatures for several months (von Lützow and Kögel-Knabner, 2009; Yan *et al.*, 2017). Diurnal variation in the rates of soil microbial functions that release atmospheric gases such as CO_2, N_2O and CH_4 have been reported in both the field (Zhou et al. 2015) and laboratory (Xu et al., 2016) studies. Such diurnal variation has been attributed largely to temperature oscillations (Shurpali et al., 2016). In addition to the abovementioned direct impacts of temperature on soil respiration that are due to increases in the activity of soil microbial communities, indirect effects on microbial activity could occur due to long term shifts in soil microbial community composition as a result of thermal adaptation (Luo et al. 2001; Davidson et al. 2006; Bradford et al. 2008; 2010; Buysse et al. 2013). Understanding how the legacy effects of changes in temperature regimes influence the structure and function behaviour of soil microbial communities is currently among the most important areas of investigation in the field of microbial ecology (Antwis et al. 2017). This area is important because, along with a future increase in global mean temperatures, we also expect a dampening of the diurnal temperature range, with daily minimum temperatures expected to increase more than daily maximum temperatures (Braganza et al. 2004; Zhou et al. 2009). Thus, soil microorganisms may become thermally adapted to a narrower diurnal temperature range and respond differently to temperature increases than the communities that currently inhabit soils.

Measuring soil respiration in soils that are incubated at diurnally oscillated temperature may create conditions that are more similar to those experienced by soil microbial communities in nature (Thiessen et al., 2013). However, previous attempts at measuring soil respiration under controlled oscillating temperatures are rare. A few studies have achieved this simulated oscillation by moving soils from one temperature to another and holding them at these constant temperatures for longer than may occur in nature (e.g. between 9 and 12 hours), during which soil respiration measurements were made (e.g. Fang et al. 2005; Thiessen et al. 2013). Unfortunately, these studies did not report how microbial community composition changed as a result of these oscillating soil temperatures. Past temperature regimes already experienced by soil can influence both the soil microbial community (Wu et al., 2010) and substrate availability through depletion (Pold et al., 2017). It is uncertain how current respiration and temperature sensitivity of respiration depends on the interaction between changes in soil microbial community and substrate depletion due to the legacy effect of the past temperature regime already experienced by the soil. Comparing the soil microbial community composition and its

function *ex situ* under both constant and diurnally oscillating temperatures that mimic real diurnal temperature oscillations may offer a better understanding of how soil microbial communities may change under future environmental change and help us to better predict the magnitude of the positive feedback of CO_2 flux into the atmosphere.

We designed and executed a laboratory incubation experiment to examine the effects of constant and diurnally oscillating temperatures on soil microbial community structure and function. The temperature treatments were chosen to reflect average daily minimum, daily average, and daily maximum temperatures in Reading, UK. Soils were incubated at these three constant temperatures (5 °C, 10 °C or 15 °C) alongside soils that were oscillated between daily minimum (5 °C) and daily maximum (15 °C) temperatures. Soil CO_2 flux was measured by temporarily moving incubated soils from the abovementioned treatments to 5 °C, 10 °C or 15 °C, such that soils incubated at each temperature had CO_2 flux measured at every temperature. Our approach used incubation and measurement temperatures as statistical factors to explore the influence of incubation temperature on the respiration at the measured temperature. Our aim was to determine whether soil samples incubated under different temperature regimes exhibit different respiration rates, even if the measurements of respiration are all made at the same temperature. This approach availed us the opportunity to calculate Q10 using the 5 °C and 15 °C measurement temperatures. We hypothesised that 1) respiration measurements made at higher temperatures would result in greater respiration rates, 2) incubation temperature will induce changes in soil microbial community structure and the availability of soil C and N, and that 3) these changes in community composition and biogeochemistry would lead to different respiration rates from soils incubated under different temperature regimes, even when respiration was measured at the same temperature. Our null hypothesis was that soil respiration of soils diurnally oscillating between 5 °C and 15 °C would be similar to respiration from soils incubated at 10 °C, thus confirming the suitability of daily or monthly average temperatures as input variables in soil carbon models.

4.3 Materials and Methods

4.3.1 Site selection and Soil Sampling

Soil was collected at 0-10 cm depth from a permanent grassland field (Latitude 51º28.564′, Longitude 000 º54.198′) on the University of Reading experimental farm at Sonning, UK. The

soil was identified as a Chromic Endoskeletic Luvisol. Details of the soil description and land use history are provided in Adekanmbi et al. (2020). Multiple subsamples from an area of approximately 10 m^2 were bulked together to obtain a composite sample. The fresh soil was sieved to 4 mm, thoroughly mixed, and then stored at 4°C until the start of the experiment. A subsample of approximately 500 g was air-dried to characterise soil texture, water holding capacity (%WHC), pH in water, total carbon (TC), total nitrogen (TN), and available NO_3^- and NH_4^+ (Table 4.1). The methods for each of these analyses are reported in Appendix 1.

Table 4.1 Physical and chemical properties of the soil used in the experiment

Parameters	Values
%Sand	46.8
%Silt	45.0
%Clay	8.16
Texture	Loam
%WHC*	43.6
pH in water	6.52
Total N (g^{-1} kg^{-1} soil)	2.53
Total (C g^{-1} kg^{-1} soil)	26.4
C/N ratio	10.4
NH_4^+ (mg^{-1} kg^{-1} soil)	0.53
NO_3^- (mg^{-1} kg^{-1} soil)	57.3
Total extractable N (mg^{-1} kg^{-1} soil)	57.8

*WHC = Water Holding Capacity.

4.3.2 Experimental Design

The experiment was a 4 x 3 factorial design comprising of 4 incubation temperatures (5 °C, 10 °C, 15 °C, or diurnally oscillating between 5 °C and 15 °C) and 3 measurement temperatures (5 °C, 10 °C, and 15 °C), with four replicates (as in Table 4.2), resulting in 12 treatments and 48 units (Treatments 1 – 12 in Table 4.2). Each week of the experiment the soil samples were incubated in controlled environment chambers for six days at their allocated incubation temperatures before moving to their allocated measurement temperatures 24 hours prior to respiration measurement, and then returned to their allocated incubation temperature after

measurement of CO_2 flux (Figure 4.1A and 4.1B). Two blank (without soil) incubation jars were incubated at each measurement temperature as a blank to correct for background atmospheric CO_2 concentration in the mesocosms and accounted for while calculating the CO_2 flux.

Four extra cores were both incubated and measured in an environment diurnally oscillating between 5 ℃ and 15 ℃ (See treatment 13 in Table 4.2). Measurements of CO_2 flux were made when the environment was at 10 ℃ while the temperature was decreasing during the diurnal oscillation. The addition of this treatment meant that, at the end of the experiment, we had soils that had remained (without movement) at 5 ℃, 10 ℃, 15 ℃, and diurnally oscillating between 5 ℃ and 15 ℃ (representing 4 treatments, and 16 experimental units). These units were used for post incubation soil chemical and biological analysis.

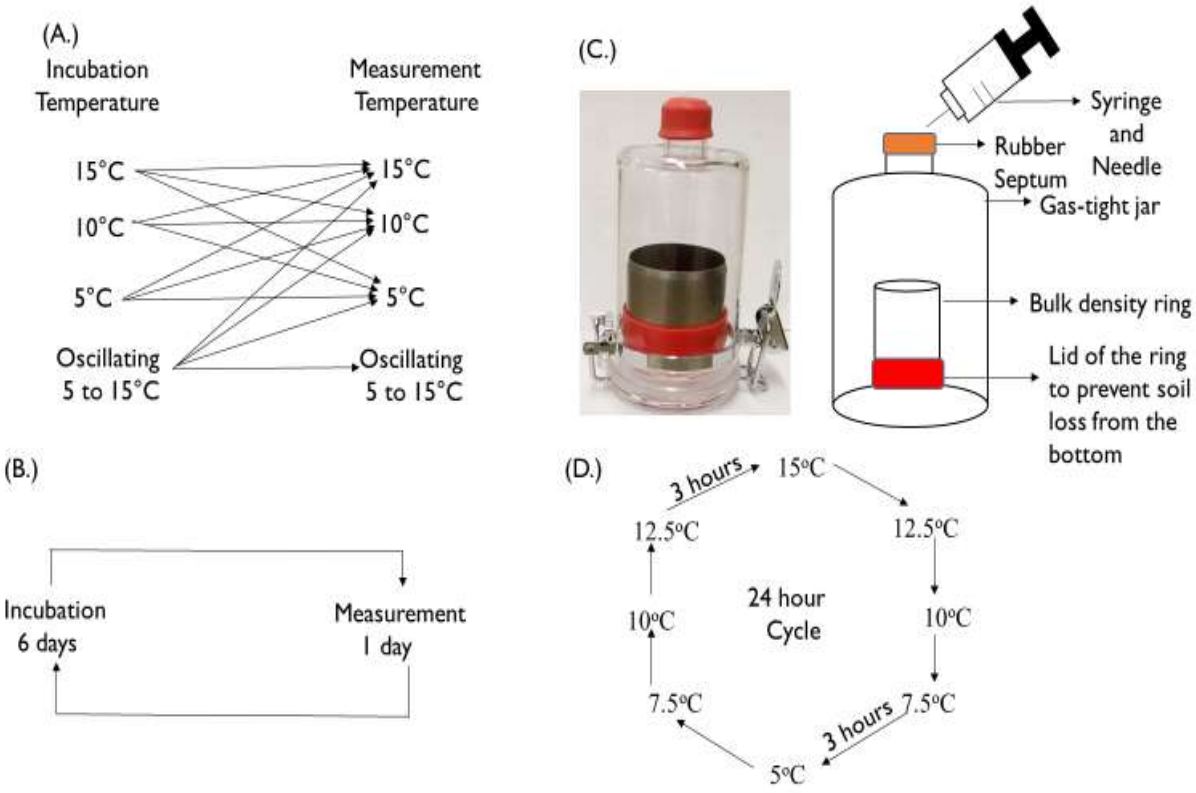

Figure 4.2 Experimental design including (A) a graphical depiction of the experimental treatments showing how individual treatments were moved from their incubation temperature to their measurement temperature prior to CO_2 flux measurements; (B) the weekly schedule for moving soils from their incubation temperatures to their measurement temperatures; (C) the design of the incubation containers and the method by which headspace gas samples were collected from the soil incubation containers; and (D) the daily temperature regime that the soils assigned to the 5 ℃ to 15 ℃ oscillating treatment were exposed to, with the temperature held for three hours at each temperature step.

Table 4.2 Experimental design highlighting the incubation and measurement temperatures and the analysis undertaken on experimental units assigned to individual treatments.

Treatment Number	Incubation Temperature	Measurement Temperature	Replicates	CO_2 flux	Post incubation analysis
1	5°C	5°C	4	✓	✓
2	5°C	10°C	4	✓	
3	5°C	15°C	4	✓	
4	10°C	5°C	4	✓	
5	10°C	10°C	4	✓	✓
6	10°C	15°C	4	✓	
7	15°C	5°C	4	✓	
8	15°C	10°C	4	✓	
9	15°C	15°C	4	✓	✓
10	Oscillating (5°C -15°C)	5°C	4	✓	
11	Oscillating (5°C -15°C)	10°C	4	✓	
12	Oscillating (5°C -15°C)	15°C	4	✓	
*13	Oscillating (5°C -15°C)	Oscillating (5°C -15°C)	4	✓	✓

* Treatment 13 was not included in statistical analysis for CO_2 flux

4.3.3 Experimental setup and CO_2 flux measurements

Field moist soil samples of 70 g fresh weight (equivalent to 56.51 g dry weight) were weighed into a 5 x 5 cm cylinder (height x diameter; volume = 98.22 cm^3) and placed in a 320 ml gas-tight container (Figure 1C). The containers were modified to allow gas collection ports, which were covered with Parafilm® to reduce moisture loss (but allow gas exchange) when not in use, following Adekanmbi et al. (2020). The soils were adjusted to 60 % of their water holding capacity, as described by Yang et al., (2017). All the soil samples were pre-incubated for 7 days at their respective incubation temperature to allow the sieving/re-wetting induced flush in respiration (Liu et al., 2018) to subside before the first CO_2 flux measurement was made. The temperatures selected for our experiment were the average daily minimum (5 °C), average daily maximum (15 °C), and average daily mean (10 °C) temperatures measured over a 28 year (1 January 1990 – May 2018) period at the University of Reading Meteorological station, situated approximately 2.5 miles from University of Reading experimental farm at Sonning, where the soil for this experiment was collected. We set the oscillating treatment to oscillate diurnally between the average daily minimum (5 °C) and average daily maximum (15 °C) by programming a growth chamber to spend three hours at each of eight temperatures per day (5 °C, to 7.5 °C, to 10 °C, to 12.5 °C, to 15 °C, to 12.5 °C, to 10 °C, to 7.5 °C, and then back to 5 °C), as shown in Figure 4.1D.

The experiment lasted for 119 days (17 weeks). Soil respiration was measured as CO_2 flux every week up until the third week (day 21) and then at two-week intervals thereafter until the 17^{th} week. Prior to each CO_2 flux measurement, the Parafilm® was removed to allow the gas in the gas-tight container to mix with the atmosphere. During CO_2 flux measurement, containers were sealed with a Suba-Seal® Septa and kept at the measurement temperature for one hour before a 15 ml headspace gas sample was taken from each container using a syringe and hypodermic needle and transferred into a pre-evacuated Labco® exetainer vial. After each sampling, the septum was removed and the Parafilm® replaced to reduce moisture loss. The gas samples were analysed using an Agilent 7890A (Agilent Technologies, Wilmington USA) gas chromatograph fitted with a flame ionization detector. The moisture content of the soil in each container was adjusted back to 60 % of their water holding capacity after collecting gas and before returning samples back to their incubation temperatures by addition of deionised water to compensate for mass loss due to evaporation.

4.3.4 Laboratory analysis of soil chemical and biological properties

At the end of the experiment (after 17 weeks), soil samples were taken from the 16 containers that had remained (without movement) at 5 ℃, 10 ℃, 15 ℃, or diurnally oscillating between 5 ℃ and 15 ℃ for the entirety of the experiment to examine soil chemical and biological properties. A 10 g sub-sample of soil was extracted immediately for determination of available NO_3^- and NH_4^+. A further 5 g was used to determine the gravimetric water content and adjust the results of the NO_3^- and NH_4^+ analysis for soil moisture so that they could be expressed on a dry mass basis. A 5 g sub-sample was freeze-dried prior to phospholipid fatty acid (PLFA) analysis. A 15 g sub-sample was air-dried for chemical analysis to determine TC, TN, and hot and cold water extractable carbon (HWEOC and CWEOC).

Soil microbial community structure was assessed using PLFA profiles (Tunlid and White, 1992). Freeze-dried soils (2 g per sample) were extracted using Bligh and Dyer solvent (Bligh and Dyer, 1959) following Frostegård and Bååth (1996). Extracted phospholipids were derivatised, as described by Dowling *et al*. (1986), and analysed as fatty acid methyl esters by gas chromatography (Agilent 6890N, flame ionization detector and a 30 m x 0.25 mm capillary column with a 0.25 µm film of 5 % diphenyl, 95 % dimethyl siloxane) following Frostegård *et al*. (1991). Individual fatty acid methyl esters were identified and quantified according to the retention times and peak area in using quantitative and qualitative standards (26 bacterial FAMEs, C11 to C20 and 37 FAMEs, C4 to C24; Supelco, Supelco UK, Poole, UK). Individual PLFAs were attributed to microbial groups according to (Kaur et al., 2005; Willers et al., 2015; and Quideau et al., 2016). The assignment of individual fatty acid biomarkers to microbial groups or stress indices is described in Table S-1 in the supplementary material (Appendix 1).

Total C and N were analysed using a C/N elemental analyser (Thermo Scientific Flash 2000 EA). Available NO_3^- and NH_4^+ were analysed by extracting 10 g moist soil with 50 ml of 1M KCl for 1 hour, filtering (GF/A 15.0cm diameter), and analysing using a Skalar SAN++ flow injection auto-analyser. HWEOC and CWEOC were analysed as described by Ghani, et al., (2003). Approximately 3 g air-dried soil samples of known moisture content were accurately weighed into 50 ml polypropylene centrifuge tubes. Thirty ml of ultra-pure water was then added to each tube before mixing on a rotary shaker at 30 rpm for 30 min at 20℃. This was followed by centrifuging at 3500 rpm for 20 min at 20 ºC. The supernatants were then removed using polypropylene syringes and passed through 0.2 µm cellulose nitrate membrane filters into polypropylene universal tubes, discarding the first 3 ml of the filtrate each time. A further

30 ml of ultra-pure water was then added to each centrifuge tube before vortexing for 10 seconds and leaving in an 80 ℃ water bath overnight and the supernatants removed, as described above. Both supernatants were analysed for CWEOC and HWEOC, respectively using a Shimazu TOC analyser.

4. 3.5 Q10 Determination

The temperature sensitivity was determined by calculating the temperature coefficient (Q10) using the equal time method (Lin et al., 2015; Zang et al., 2020):

$$Q10 = \left(\frac{\mathrm{RT2}}{\mathrm{RT1}}\right)^{\frac{10}{T2-T1}}$$

where RT2 is CO_2 flux measured at 15 °C, RT1 is the corresponding CO_2 flux measured at 5 °C, T2 is 15 °C, and T1 is 5 °C.

4.3.6 Statistical Analysis

Differences in soil respiration due to incubation and measurement temperatures over the period of 17 weeks were tested using repeated measures analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test was used for pairwise comparisons in Genstat (10^{th} edition). Two way ANOVA was conducted to assess effect of incubation temperature and incubation week on Q10. Non-metric multi-dimensional scaling (NMDS) ordination derived from Bray-Curtis similarities was used to separate soil microbial community structures of samples subjected to different incubation temperatures using the *vegan* package (Oksanen, 2017). The distance was Bray Curtis, performed in 2 dimensions, with stress factor of 0.05370305. PERMANOVA was used to assess whether incubation temperature influenced the soil microbial community distance. R v3.5.1 (R Development Core Team, 2018) was used to perform the NMDS. One-way ANOVA was also used to assess the differences in the NMDS 1 and 2 after establishing that there was a significant difference in community distance due to temperature to examine the direction of temperature impact on soil microbial community. One-way ANOVA was used to test the differences in soil properties and PLFA biomarkers due to incubation temperatures.

4.4 Results

4.4.1 Effects of measurement and incubation temperatures on soil CO_2 flux

The CO_2 flux data for each individual treatment are presented in Figure S-1 and Figure S-2 of the supplementary material (Appendix 1). Repeated measures ANOVA of the data revealed that both incubation ($P < 0.001$) and measurement ($P < 0.001$) temperature had a significant effect on the CO_2 flux measured (Table 4.3). However, there was no significant interaction between incubation and measurement temperatures ($P = 0.680$). Irrespective of measurement temperature, incubating soil at 15 ℃, or oscillating between 5 ℃ and 15 ℃, released significantly ($P < 0.001$) more CO_2 compared to incubating at 5 ℃ or 10 ℃ (Figure 4.2A, Table 4.3). As expected, CO_2 flux, when measured at 15 ℃, was significantly ($P < 0.001$) greater than CO_2 flux measured at 5 ℃ or 10 ℃ (Figure 4.2B, Table 4.3). However, counter to expectations, it was observed that soils measured at 5 ℃ released slightly (but not significantly) more CO_2 compared to soils incubated at 10 ℃ (Figure 4.2B). Irrespective of measurement temperature, CO_2 flux from the soils that oscillated between 5 ℃ and 15 ℃ was not significantly different ($P > 0.05$) from the soils incubated at 15 ℃, but was significantly ($P < 0.001$) greater than the soils incubated at 10 ℃ or 5 ℃ (Figure 4.2A).

Repeated measures ANOVA of the data also revealed that incubation week ($P < 0.001$) had a significant effect on the CO_2 flux measured (Table 4.3), as indicated by a slightly elevated CO_2 flux measured between week three and week eleven (Figure 4.2C). There was also a significant interaction ($P < 0.001$) between incubation week and incubation temperature, and between incubation week and measurement temperature on soil CO_2 flux (Figure 4.2D and 4.2E, Table 4.3). The abovementioned elevated CO_2 flux measured between week three and eleven was more pronounced in treatments incubated at 15 ℃ or oscillating between 5 ℃ and 15 ℃ (Figure 4.2D). Although CO_2 flux measured at 5 ℃ was lower than that measured at 10 ℃ in week 1 of the experiment, it was greater in soils measured at 5 ℃ for the remainder of the experiment (Figure 4.2E).

Table 4.3 Summary table for two-way repeated measures ANOVA for soil respiration; Incubation and Measurement temperatures were the main (subject) factors.

Source	df	F Value	P Value
Incubation Temperature (I)	3	9.21	**0.001**
Measurements Temperature (M)	2	9.01	**0.001**
Incubation Week (W)	9	12.54	**0.001**
I *M	6	0.66	0.680
W * I	27	3.81	**0.001**
W * M	18	3.43	**0.001**
W * I * M	54	1.25	0.180

Values in bold letters are significantly different at P < 0.01

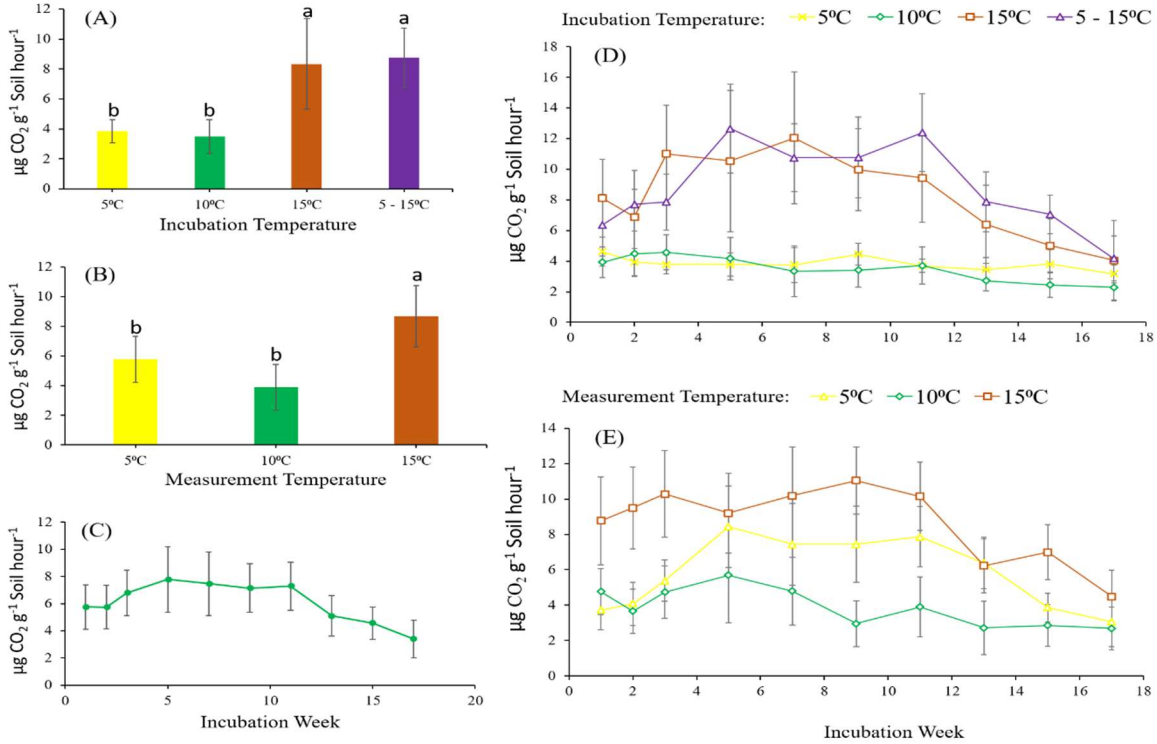

Figure 4.2 Effects of soil incubation temperature (A), measurement temperature (B), Incubation Week (C), Incubation Week x temperature (D) and Incubation Week x Measurement temperatures (E) on soil CO_2 flux from soil. Error bars represent standard errors of the mean. Bars with the same lower case letters are not significantly different from each other ($P > 0.05$). For (A) n = 120, for (B) n = 160, for (C) n = 48, for (D) n = 12, and for (E) n = 16.

4.4.2 Effects of incubation temperature on temperature sensitivity (Q10) of Soil respiration

The result on the effects of incubation temperature on the Q10 of soil respiration is presented in Figure 4.3. The two-way analysis of variance (data not shown) showed that, incubation temperature ($P < 0.0001$), but not incubation week ($P = 0.504$), significantly influenced the calculated Q10. Irrespective of incubation week, incubating soil at 5 °C led to a significantly higher Q10 compared to soil incubated at 10 °C. Constantly incubating soil at 15 °C or at temperature oscillating between 5 and 15 °C had similar Q10 and this showed an intermediate Q10 between soil incubated at either 5 °C or 10 °C. The Q10 derived from incubating soil diurnally oscillating between 5 and 15 °C was not significantly different from that obtained using soils incubated constantly at 5, 10, or 15 °C.

4.4.3 Effects of incubation temperature on soil carbon and nitrogen

The concentrations of chemical fractionations of C and N in soils from the 16 experimental units that remained (without movement) at the same temperature for the duration of the experiment (Table 4.2) are presented in Figure 4.3. CWEOC (Figure 4.4 A; Table 4.4), was significantly ($P < 0.05$) higher in soils incubated at 5 ℃ and 10 ℃ compared to those in incubated at 15 ℃ or oscillated between 5 ℃ and 15 ℃. Furthermore, soils incubated at 5 ℃ had a significantly ($P < 0.05$) higher HWEOC than all other incubation temperatures (Figure 4.4B; Table 4.4). TC (Figure 4.4C; Table 4.4) was significantly ($P < 0.05$) higher in soils incubated at 5 ℃ or 10 ℃, compared to those in incubated at 15 ℃ or oscillated between 5 ℃ and 15 ℃. Total extractable N (TEN) significantly ($P < 0.05$) increased with incubation temperature (Figure 4.4D; Table 4.4). Soils oscillated between 5 ℃ and 15 ℃ had a similar TEN to soils incubated 15 ℃, but significantly ($P < 0.05$) greater than soils incubated at 5 ℃ or 10 ℃. The TN concentration was significantly ($P < 0.05$) greater in soils oscillated between 5 ℃ and 15 ℃, compared to all other treatments incubated at constant temperatures (Figure 4.4E; Table 4.4). The C/N ratio significantly ($P < 0.05$) decreased with increasing incubation temperature, but, unlike TEN, soils oscillated between 5 ℃ and 15 ℃ had a significantly ($P < 0.05$) different (lower) C/N ratio than soils incubated at 15 ℃ (Figure 4.4F; Table 4.4).

Table 4 : Summary of ANOVA on the impact of temperature on Carbon and Nitrogen

Sources	df	*F* Values	*P* Values
CWEOC µg C g-1 Soil	3	9.38	**0.002**
HWEOC µg C g-1 Soil	3	4.33	**0.028**
TC g kg-1 Soil	3	5.55	**0.013**
TEN mg kg-1 Soil	3	45.53	**0.0001**
TN g kg-1 Soil	3	11.24	**0.001**
C/N	3	53.56	**0.0001**

Values in bold letters are significantly different at $P < 0.05$; C/H-WEOC = Cold/Hot water extractable carbon; TEN = Total extractable N.

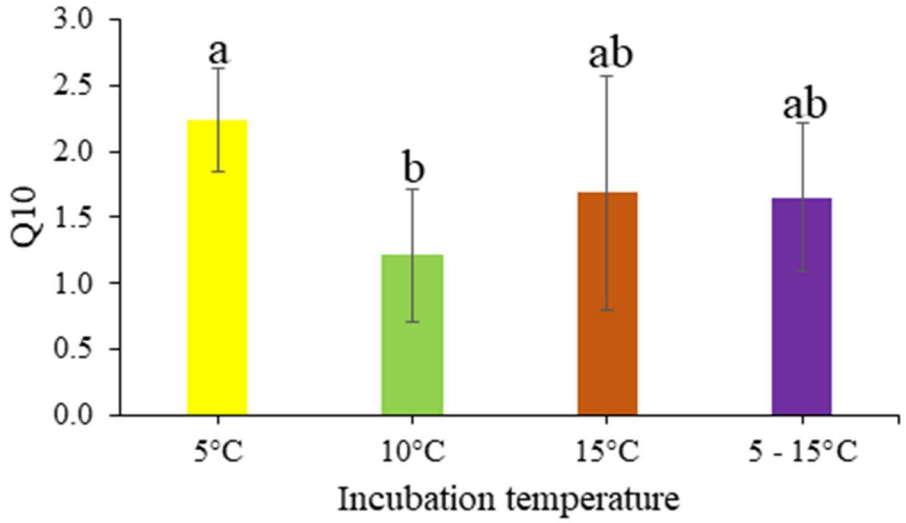

Figure 4.3: Temperature sensitivity (Q10) of soil respiration as affected by incubation temperature. Bars and error bars are the means and standard error of data recorded weekly for 17 weeks. Bars that share the same letter within a graph are not significantly different from each other (*P* > 0.05).

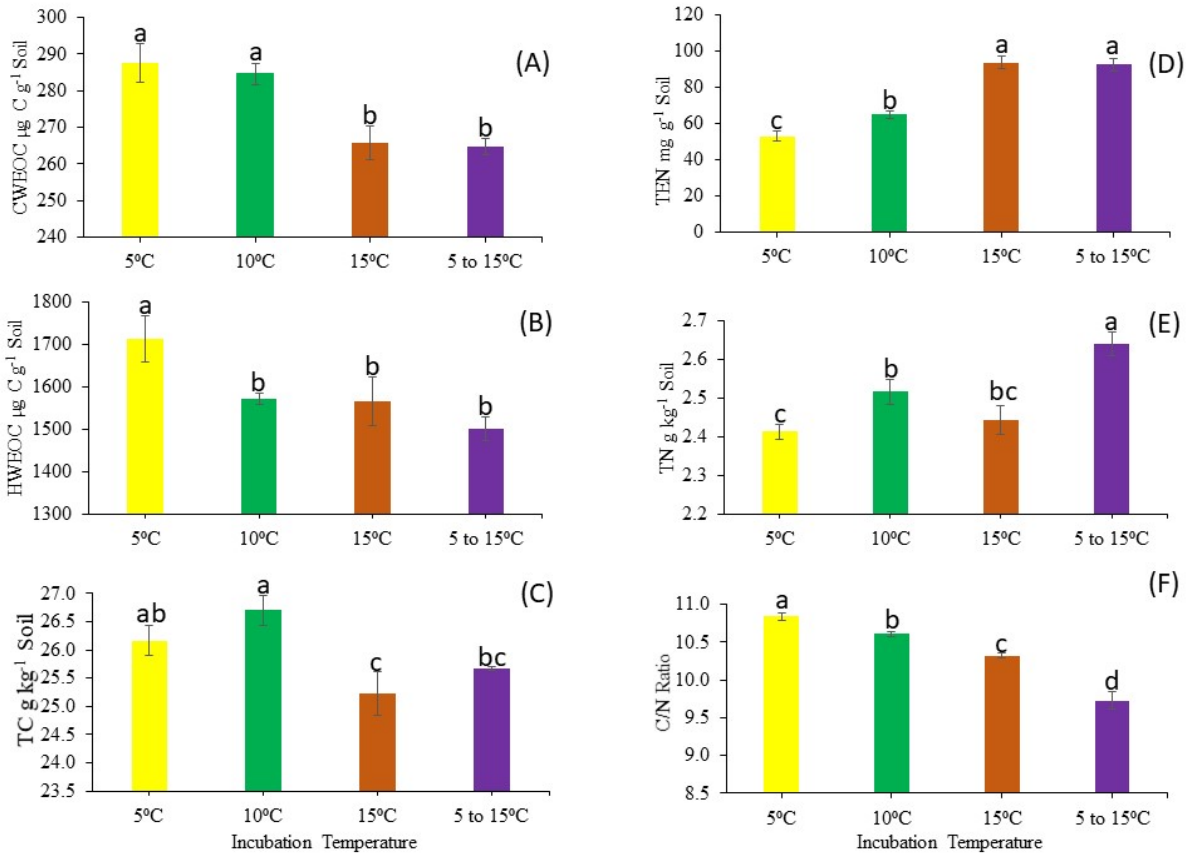

Figure 4.4: Influence of incubation temperature on cold (CWEOC = A), and hot (HWEOC = B) water extractable carbon, Total Carbon (TC = C), Total extractable N (NH_4^+ and NO_3^-) (TEN = D), Total Nitrogen (TN = E), and C/N Ratio (F). Bars and error bars represent mean and standard error of the mean (n = 4). Bars that share the same letter within a graph are not significantly different from each other ($P > 0.05$).

4.4.4 Impacts of incubation temperature on soil microbial community composition

The structure of the soil microbial community, as measured using PLFA biomarkers, was affected by soil incubation temperature, as shown in Figure 4.5. PERMANOVA analysis revealed that temperature had a significant ($P = 0.002$) effect on microbial community distance (Bray Curtis distance between the PLFA profiles). One-way ANOVA of NMDS score 1 (NMDS1) showed that the soil microbial community structure in soils incubated at 5 °C and 10 °C were not statistically different to one another, but distinct ($P = 0.0001$) from those in soils incubated at 15 °C or oscillated between 5 °C and 15 °C (Figure 4.6, Table 4.5). There was a slightly (non-significant; $P > 0.05$) lower abundance of bacteria, and total PLFA (i.e. total microbial biomass) in soils incubated at 15 °C, or oscillated between 5 °C and 15 °C,

compared to soils incubated at 5 ℃ or 10 ℃ (Figure 4.6A and 4.6B). The abundance of fungal biomarkers and the fungal/bacterial ratio was significantly ($P < 0.05$) lower in soils incubated at 15 ℃ or oscillated between 5 ℃ and 15 ℃, compared to soils incubated at 5 ℃ or 10 ℃ (Figure 4.6D and 4.6E). Furthermore, the ratio of Gram-negative/Gram-positive bacteria was significantly ($P < 0.05$) greater in soils incubated at 5 or 10 ℃ compared to soils incubated at 15 ℃ or oscillating between 5 ℃ and 15 ℃ (Figure 4.6C; Table 4.5). Likewise, the ratios of (i) cis to trans isomers, (ii) iso to anteiso branching, and (iii) cyclopropyl fatty acids to their monoenoic precursors were all significantly ($P < 0.05$) higher in soils incubated at 15 ℃ or oscillating between 5 ℃ and 15 ℃, compared to those incubated at 5 ℃ or 10 ℃ (Figure 4.6F, 4.6G and 4.6H; Table 4.5).

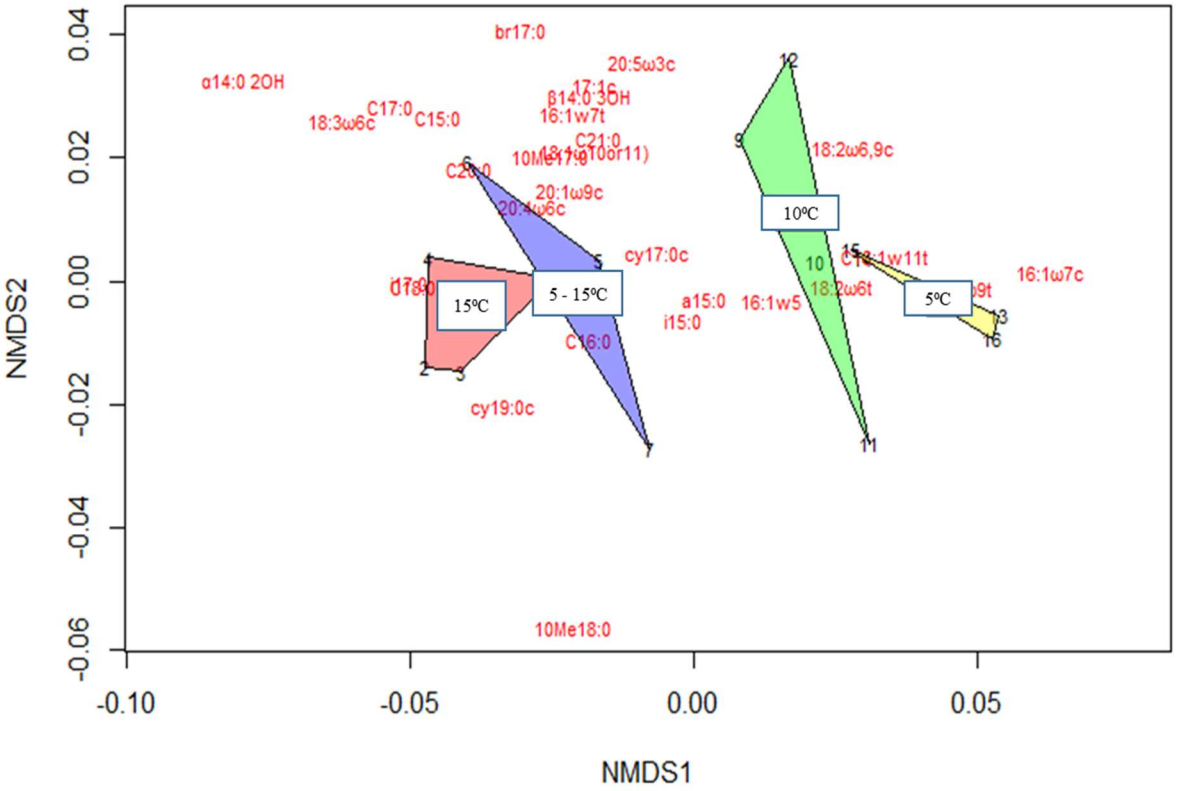

Figure 4.5: NMDS plot showing the distribution of lipid biomarkers as influenced by soil temperature on the soil microbial community structure measured using Phospholipid Fatty Acid Analysis. The distance is Bray Curtis, performed in 2 dimensions, with stress factor of 0.05370305. Dots of the same colours represent replicates of the same treatment (n = 4).

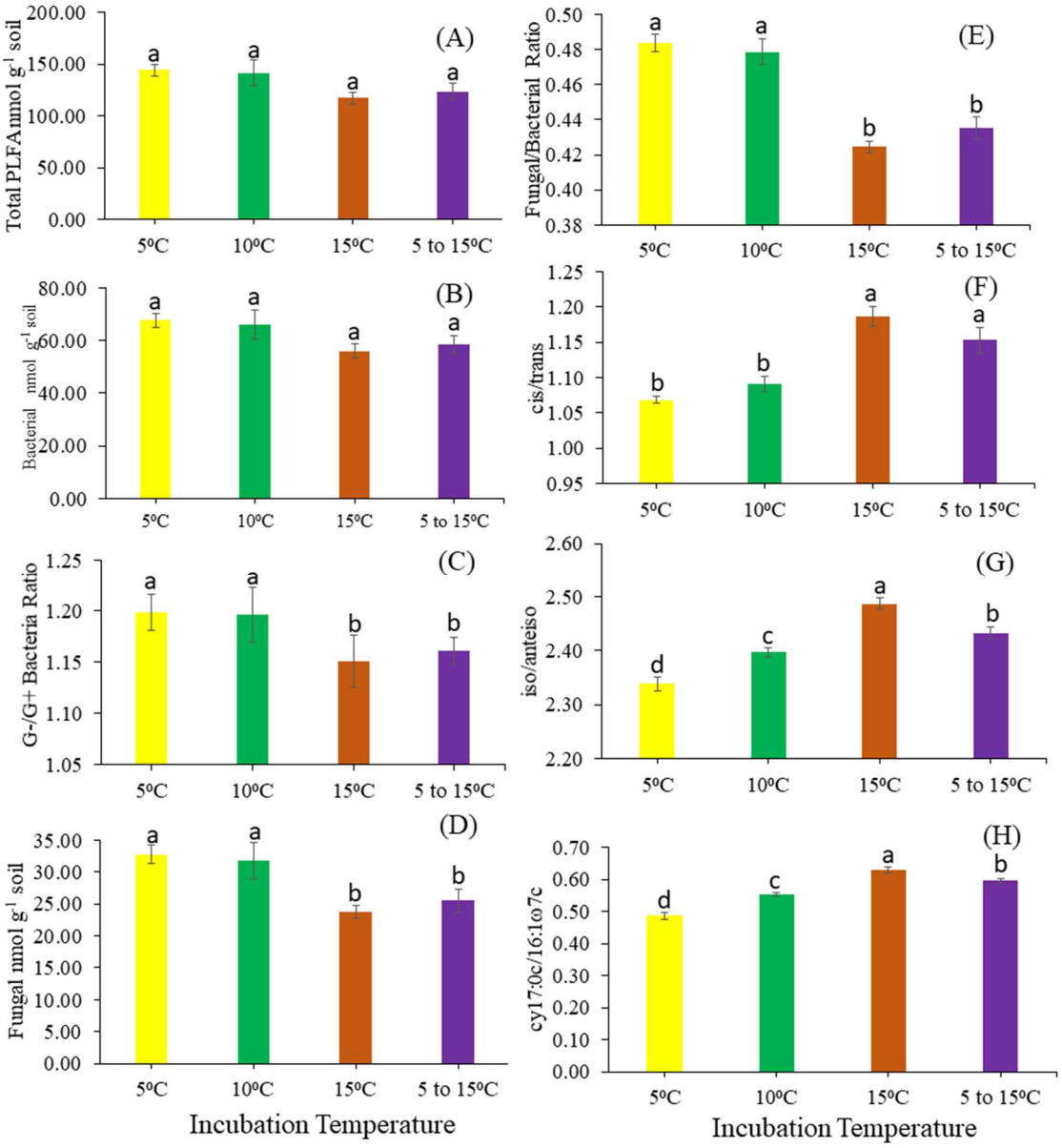

Figure 4.6 : Influence of incubation temperature on total PLFA (A), bacterial abundance (B), gram-negative/gram-positive bacterial ratio (G-/G+) (C), fungal abundance (D), fungal/bacterial ratio (E) cis/trans isomer ratio (F), iso/anteiso braching ratio (G) and cyclpropyl to monoenoic precursor (cy17:0c/16:1ω7c) ratio (H). Bars and error bars represent mean and standard error of the mean (n = 4). Bars that share a letter are not significantly different from one another ($P < 0.05$).

Table 4.5: Summary ANOVA table for a one-way ANOVA on the impact of incubation temperature on the soil microbial community composition assessed using PLFA biomarkers

Sources	df	F Values	P Values
Total PLFA (nmol g-1 soil)	3	2.42	0.117
Bacterial (nmol g-1 soil)	3	2.38	**0.121**
G-/G+ Bacteria Ratio	3	5.11	**0.017**
Fungal (nmol g-1 soil)	3	5.51	**0.013**
Fungal/Bacterial Ratio	3	27.39	**0.0001**
cis/trans Ratio	3	18.36	**0.0001**
iso/anteiso ratio	3	31.87	**0.0001**
cy17:0c/16:1ω7c	3	58.32	**0.0001**
PERMANOVA	3	9.14	0.002
NMDS1	3	36.85	0.0001
NMDS2	3	0.52	0.675

Values in bold letters are statistically significant at $P < 0.05$

4.5 Discussion

4.5.1 The legacy of previous incubation temperature on soil respiration

It is well established in multiple soil warming experiments undertaken in the field and laboratory that soil respiration increases in response to temperature rises (Chen et al. 2000; von Lützow and Kögel-Knabner 2009; Bell et al. 2010; Karhu et al. 2014; Carey et al. 2016; Melillo et al. 2017). Faster metabolism of microbially available organic carbon is the major reason suggested for the increases in soil CO_2 flux observed (Zogg et al. 1997; Melillo et al. 2017; Walker et al. 2018). Counter to expectations, the incubation temperatures had a larger impact on CO_2 flux than the temperature at which CO_2 flux was measured (Figure 4.2 and Table 4.3) whereby CO_2 flux was greater from soils that had been incubated at higher incubation

temperatures, regardless of measurement temperature. Our observation implies that the temperature that a soil has previously been exposed to can exert a considerable legacy effect on the future soil respiration rate.

A possible explanation for this observation may be related to the knowledge that extracellular depolymerisation of macromolecular organic carbon is considered the rate-limiting step in the mineralisation of soil organic matter and that soil microorganisms invest more into extracellular enzyme excretion when placed under resource limited conditions (Allison, 2014). Jan et al. (2009) demonstrated that protein mineralisation to CO_2 is 20 times slower than amino acid mineralisation to CO_2 and is highly temperature sensitive. In soils incubated at 15 ℃, or oscillating between 5 ℃ and 15 ℃, the soil microbial community may have thermally adapted to produce a greater quantity of extracellular enzymes that depolymerise recalcitrant substrates (Meng et al., 2020), due to labile substrate depletion. The abundance of these extracellular enzymes in the soil environment during the measurement of respiration may have been adequate to depolymerise sufficient macromolecules to prevent the availability of low molecular weight compounds from being the rate limiting factor mediating respiration at any CO_2 flux measurement temperature. Extracellular enzymes could continue to depolymerise even at low measurement temperatures when microbial uptake of the produced monomers ceases due to temporary reductions in membrane fluidity (Nedwell, 1999). Concurrently, in soils incubated at 5 ℃ or 10 ℃, lower extracellular enzyme activity may have resulted in lower availability of low molecular weight compounds and thus a legacy of previous incubation temperature regime on soil respiration. This finding has important implications for soil scientists who pre-incubate soils prior to making respiration measurements. Broadly speaking, pre-incubation at high temperatures may result in substrate depletion and greater production of C-acquiring extracellular enzymes, increasing the probability that intracellular respiration becomes the rate limiting step during the measurement of respiration. Conversely, pre-incubation at low temperature may result in extracellular depolymerisation being the rate limiting step in respiration. Unfortunately, neither of these circumstances reflect the real diurnal oscillations that soils experience in the field.

We observed similar CO_2 flux from soils that were incubated constantly at 15 ℃ and soils incubated at diurnally oscillating soil temperature between 5 ℃ and 15 ℃ (Figure 4.2A). We

thus conclude that the time spent at 15 ℃ in the diurnally oscillating treatment was sufficient for soil microbial communities to produce extracellular enzymes to depolymerise enough macromolecules to prevent the availability of low molecular weight compounds from being the factor limiting the rate of intracellular respiration. This result also implies that maximum daily temperature is an important factor influencing the transformation of soil organic carbon to CO_2; perhaps more important than daily average temperature. This assertion has important implications for our predictions of the effect that future environmental change may have on the global carbon cycle. The last half of the 20th century saw daily minimum temperatures increased by 0.9 ℃ while daily maximum temperatures increased by only 0.6 ℃ (Braganza et al., 2004). Therefore, while the climate warms, we are experiencing a reduction in the diurnal temperature range (Alexander et al., 2006) due to increased cloud cover and sulphate aerosol emission (Hansen et al., 1995). It is thus imperative to ensure that the next generation of land-surface models adequately simulate the impact of this asymmetric warming on the production and activity of extracellular enzymes and the subsequent impacts on soil heterotrophic respiration.

We also observed that the temperature a soil had been previously incubated at influenced the Q10 calculated from the measurement of CO_2 flux at 5 °C and 15 °C (Figure 4.3). Soil previously incubated at 5 °C resulted in the highest Q10 and soil previously incubated at 10°C resulted in the lowest Q10. It is commonplace to refrigerate soils after field collection to supress microbial activity prior to making respiration measurements used to generate Q10 values(Gritsch et al., 2015; Li et al., 2015; Meyer et al., 2019). This activity may result in an overestimation of the Q10 because pre-existing extracellular enzymes may still be able to depolymerise macromolecules and generate a pool of labile carbon (Figure 4.4) that is not mineralised by microorganisms due to low membrane fluidity at low temperature (Nedwell, 1999) but provides an unrealistically high abundance of labile substrate to microorganisms when respiration is measured at a higher temperature. Soil microbial communities incubated at 10 °C may have become thermally adapted and able to maintain similar levels of metabolism at both 5 °C and 15 °C. There was no significant difference between the Q10 of soils incubated at an oscillating temperature between 5 °C and 15 °C and soils incubated at any of the constant temperatures (Figure 4.3). It seems that soils that had previously experienced time at 15 °C (constantly or oscillating between 5 °C and 15 °C) may have experienced the optimum temperature for microbial activity in the grassland soil where samples were collected and

prevented any build-up of labile carbon, or thermal adaptation to colder temperatures. The temperature optimum of soil respiration is a reflection of long term physiological adaptation of soil microbial community to climate and environment (Rinnan et al., 2009; Liu et al., 2018).

4.5.2 Shifts in the soil microbial community structure in response to temperature regimes

An explanation for our observations regarding the legacy of prior incubation temperature on soil respiration is that the soil microbial community could have shifted in response to the temperatures that they were incubated at, as observed by Bradford et al., (2010). It is clear from global datasets of soil microbial communities that lower soil respiration at lower temperatures is indicative of the development of soil microbial communities with slower metabolic activities, such as fungi (Crowther et al., 2019), leading to the accumulation of organic carbon in fungal dominated ecosystems in colder climates.

Along with higher rates of soil respiration, we observed a shift away from a fungal dominated microbial community to one dominated more by gram-positive bacteria in soils incubated at a higher (or diurnally oscillating) temperature (Figure 4.6). This shift is consistent with observations made in the literature from experiments undertaken under warming conditions in both field and laboratory incubation experiments (Frey et al. 2008; Salazar et al. 2019). Our results therefore lend support to the general hypothesis that soils with a lower fungal-to-bacterial ratio have a lower potential to accumulate soil organic matter due to lower carbon use efficiency (Malik et al. 2016; Bonner et al. 2018). Greater dominance of fungi and gram negative bacteria have also been observed in soils with more total and labile carbon (Whitaker et al., 2014; Fanin et al., 2019). Therefore, the presence of greater total, and hot and cold water extractable organic carbon that we observed in soils incubated at lower temperatures (Figure 4.4) may have caused, or been the result of, shifts in the soil microbial community that raised the fungal-to-bacterial ratio and gram negative-to-gram positive bacteria ratio and may constitute an indirect mechanism by which soils respond to changes in temperature.

4.5.3 Depletion of soil organic matter at higher or oscillating incubation temperatures

Walker et al. (2018) identified a role for both substrate depletion and a permanent acceleration in microbial physiology that leads to faster respiration, growth, and turnover in warmed soils. Like Zogg et al. (1997), we observed differences in soil microbial community composition (Figure 4.6) between soils incubated at different temperatures that correspond with substrate depletion and soil respiration. Our laboratory incubation experiment revealed, at the higher incubation temperatures, elevated soil CO_2 flux between week 5 and week 11, after which CO_2 flux then decreased over time. This observation can most plausibly be explained by depletion of the labile soil organic carbon supply after 11 weeks. This explanation is consistent with the results from You et al. (2019), who observed a decrease in soil CO_2 flux due to increasing temperature towards the end of a 35 day soil incubation study.

The lower concentration of CWEOC (Figure 4A) measured in soils incubated constantly at 15 ℃ or diurnally oscillated between 5 ℃ and 15 ℃ reveals that these soils have been depleted of dissolved organic carbon (DOC). This observation supports the results of Bertolet et al. (2018) who reported lower DOC under warmer temperatures in a 28 day incubation study. In another short term experiment, it was reported that increasing temperature reduced the DOC and microbial biomass carbon without any significant changes in soil organic matter or total C (You et al., 2019). In this study, we found higher DOC in soils incubated at 5 ℃ and 10 ℃, compared to those incubated at 15 ℃ or oscillated between 5 ℃ and 15 ℃. This observation indicates that a similar rate of substrate depletion occurred in soil incubated constantly at a high temperature and soils which oscillated between high and low temperatures. Our findings therefore imply that daily maximum temperature plays a more important role in soil organic matter (de)stabilisation than daily mean temperatures, complementing our interpretation of the CO_2 flux measurements. The notion that the availability of low molecular weight compounds limits microbial intracellular respiration in our soils, and not the availability of a stoichiometric supply of nutrients, is supported by our results, which reveal greater N mineralisation in soils incubated at 15 ℃ or oscillated between 5 ℃ and 15 ℃, compared with those incubated at 10 ℃ or 5 ℃.

The lower C/N ratio in soils incubated at higher temperatures (Figure 4.4F) lends support to our interpretation that, at higher temperatures, readily available C is being depleted and N being

mineralised. This evidence, coupled with the lack of leaching or plant uptake allowed in the experiment, indicates that the C left in the soil is more microbially processed (Bach et al., 2018) and more stable. The chemical fractionations of C and N in the soils incubated under oscillating temperature are most similar to the constant 15 °C incubation treatment, indicating that changes to C and N are dictated by maximum daily temperature rather than average daily temperature or minimum daily temperature. Soil microorganisms adapt to these changes in C and N availability to fulfil their energy and nutrient demands, thus causing shift in microbial community composition and physiology (Wan et al., 2014; Schnecker et al., 2015). However, shifts in community composition may also occur due to different groups of organisms outcompeting others for resources at the given temperature (Crowther et al., 2014).

4.5.4 Adaptation of the soil microbial community

In response to the stress associated with higher temperatures and substrate depletion, microorganisms are able to alter the composition of their cell walls to increase membrane stability (acclimatisation), but it is not possible, using fatty acid biomarkers, to distinguish between this phenomenon and a shift in the composition of the microbial community to one that comprises organisms with inherently more stable membranes (Frostegård et al., 2011). Commonly used microbial stress indicators include changes to the ratio of cyclopropyl fatty acids to their cis mono-unsaturated precursors, the ratio of gram-negative/gram-positive bacteria, cis/trans ratio, and iso/anteiso branching ratio (Kaur et al. 2005; Feng and Simpson 2009; Ruess and Chamberlain 2010; Sizmur et al. 2011; Willers et al. 2015; Bai et al. 2017). In our study, we found that higher incubation temperatures resulted in (i) a lower ratio of gram-negative/gram-positive bacteria biomarkers, (ii) a higher ratio of cy17:0c/16:1ω7c, (iii) a higher ratio of cis/trans ratio isomerization, and (iv) a higher iso/anteiso branching.

Both gram-negative/gram-positive ratio and the ratio of cyclopropyl fatty acids to their cis mono-unsaturated fatty acids precursors (cy17:0c/16:1ω7c in this study) are known indicators of temperature-induced nutrient depletions (Bai et al., 2017). Changes in cis/trans ratio isomerisation, and iso/anteiso branching have also been used to explain bacterial physiological adaptations (Ruess and Chamberlain, 2010) under stress conditions. The combination of these indicators could also represent microbial adaptation to sub-optimal temperature changes (Siliakus et al., 2017). Stress indicators were similar, although slightly lower, in soils oscillated

between 5 °C and 15 °C, compared to soils incubated constantly at 15 °C, indicating that the changes to the phospholipid bilayer may be more associated with temperature than substrate depletion. Microbial adaptation, in the form of changing membrane composition, helps the community to combat environmental change (Feng and Simpson, 2009; De Maayer et al., 2014; Siliakus et al., 2017). Significantly lower ratios of cyclopropyl fatty acids to their cis mono-unsaturated fatty acid precursors (cy17:0c/16:1ω7c) and the iso/anteiso ratio in soils incubated under oscillating temperature, compared to those soils incubated constantly at 15 °C, reveals that temperature effects on the microbial community are lower in diurnally oscillating soils, compared to soils incubated at constant temperature. This observation could be because the oscillating treatments makes the best use of the diversity of the microbial community in the oscillating treatment, since different species may be capable of occupying different 'temperature niches' in a fluctuating environment (Upton et al., 1990). Our results thus imply that soil microbial communities incubated in fluctuating environments are less sensitive to change, compared to those incubated under constant conditions (Hawkes and Keitt, 2015).

4.6 Conclusions

We demonstrate that the daily maximum temperature a soil is exposed to has an important impact on soil microbial community composition, the rate and temperature sensitivity of soil respiration, and the depletion of soil organic matter in a temperate grassland soil. Our findings suggest that the daily maximum temperature mediates the production of extracellular enzymes which are capable of depolymerising macromolecules at lower temperatures overnight in sufficient quantity to maintain intracellular respiration. Microbial communities undergo changes to their composition and physiology when incubated under different temperature regimes. Lower temperatures shift the population towards a fungal dominated soil microbial community. Higher temperatures shift the population towards a bacterial dominated community with more gram-negative bacteria and greater membrane stability, due to thermal adaptation and in response to the stress associated with substrate depletion. The microbial communities of soils oscillated diurnally between 5 °C and daily 15 °C were similar to those maintained constantly at 15 °C. However, incubating soils at oscillating temperatures allows communities to exploit several different 'temperature niches'. This knowledge is critical to advance new soil biogeochemical models that predict the impact of environmental change on soil respiration because asymmetric warming and a dampening of the diurnal temperature range

is known to be occurring. It is recommended that the short-term impact of daily maximum and daily minimum temperatures on extracellular and intracellular enzyme activities, and the long-term impact of climate shifts on microbial community composition and physiology are incorporated into the next generation of soil carbon models. Soil respiration assays performed on soils pre-incubated at realistic temperatures that are representative of the daily maximum, daily average, and daily and minimum temperatures of the site from which the soils are samples would generate useful assessments of the likely temperature sensitivity (Q10) of soil respiration to future environmental change.

Chapter 5

This chapter is formatted as a paper to be submitted to *Soil Biology and Biochemistry*

Adekanmbi, A.A., Dale, L., Shaw, L.J. and Sizmur, T., (In prep). Temperature Sensitivity of Intracellular and Extracellular Enzyme Activities is Affected by Previous Soil Temperature. *Soil Biology and Biochemistry*

5.1 Abstract

Predicting the pattern of soil organic matter (SOM) decomposition as a feedback to climate change, via release of CO_2, is extremely complex and has received much attention. However, investigations often do not differentiate between the extracellular and intracellular processes involved and work is needed to identify their relative temperature sensitivities, especially when there is possible acclimation of microbial communities to different thermal regimes. Samples were collected from a grassland soil at Sonning, UK with average daily maximum and minimum soil temperature of 15 °C and 5 °C. We measured potential activities of β-glucosidase (BG) and chitinase (NAG) (extracellular enzymes) and glucose-induced CO_2 respiration (intracellular enzymes) at a range of assay temperatures (5 °C, 15 °C, 26 °C, 37 °C, and 45 °C). Samples were pre-incubated at various pre-incubation temperatures (5 °C, 15 °C and 26 °C) to acclimatise the microbial communities to different thermal regimes for 60 days prior to enzyme assays. Q10 and Ea were calculated to assess the temperature sensitivity of intracellular and extracellular enzymes activities. Between 5 °C and 15 °C intracellular and extracellular enzyme activities show equal temperature sensitivity, but between 15 °C and 2 6 °C intracellular enzyme activity was more temperature sensitive than extracellular enzyme activity and between 26 °C and 37 °C extracellular enzyme activity was more temperature sensitive than intracellular enzyme activity. This result implies that depolymerisation of higher molecular weight carbon is more sensitive to temperature changes at higher temperatures (e.g. changes to daily maximum summer temperature) but the respiration of the generated monomers is more sensitive to temperature changes at moderate temperatures (e.g. changes to daily mean summer temperature). We conclude that, extracellular and intracellular steps are not equally sensitive to changes in soil temperature and that the previous temperature a soil is exposed to may influence the activity and temperature sensitivity of extracellular and intracellular enzymes. A higher pre-incubation temperature can remove extracellular depolymerisation as the rate limiting step to soil respiration. Therefore, global warming may reduce the importance of

extracellular depolymerisation and increase the importance of intracellular catalytic activities as the rate limiting step of SOM decomposition.

5.2 Introduction

Obtaining a better understanding of organic matter decomposition and subsequent CO_2 release will help in the prediction of how carbon cycling will respond to climate change. There are two major enzymatically mediated steps in the multi-stage decomposition of organic matter and the subsequent release of CO_2 (Bárta et al., 2013; Maire et al., 2013; Blagodatskaya et al., 2016). The first step, extracellular depolymerisation, requires microbes to secrete extracellular enzymes into the soil to depolymerize macromolecular constituents of soil organic matter and produce soluble low molecular weight microbial assimilates (Maire et al., 2013). The second step, intracellular metabolism, results in the release of CO_2 after the soluble substrates are absorbed and utilised by microbial cells. Intracellular metabolism is an energy yielding process carried out by myriads of endo-enzymes within the microbial cell, leading to release of CO_2 through respiration. Although these two separate processes are both critical for soil organic matter decomposition to occur, it is unclear which of these is most sensitive to temperature changes and which will be responsible for the way in which soil organic matter will respond to future global temperature changes (Blagodatskaya et al., 2016).

It is generally expected that decomposition of organic matter in soil will increase with increasing temperature, and understanding the nature of this increase is critical to understanding how ecosystems respond to global change (Conant et al., 2011; Blagodatskaya et al., 2016). Model predictions of the response of ecosystems to warming largely agree with kinetic theory that describes chemical reactions. This kinetic theory states that the rate of decomposition increases with temperature only when substrate is available and enzyme activity does not limit the decomposition rate, and that increases in the decomposition rate with an increase in temperature should be greatest at colder temperatures (Conant et al., 2011). The temperature sensitivity of soil organic matter decomposition is one of the factors controlling the magnitude of carbon cycle-climate feedbacks (Davidson and Janssens, 2006; Zhu and Cheng, 2011) because the extent of this sensitivity determines how much CO_2 is released to the atmosphere from soil as a result of warming. Understanding the temperature sensitivity of both extracellular and intracellular enzymatic reactions involved in organic matter decomposition will therefore advance our understanding of carbon-cycle climate feedbacks.

Many ecological studies have examined temperature sensitivity of organic matter decomposition in soils, but most of them measure the end product as respired CO_2 (e.g. Wang et al., 2013) or mass loss of C substrate (e.g. Kirwan et al., 2014), which does not differentiate between the contribution of extracellular and intracellular enzymes. Therefore, obtaining information regarding the distribution and comparative importance of different microbially-mediated reactions from such assessments is impossible (Nannipieri et al., 2003). Extracellular, rather than intracellular, enzyme activity is widely thought to be rate-limiting step to respiration of organic matter in soils (Bradford, 2013) but very few studies have explicitly compared the temperature sensitivity of extracellular and intracellular enzymes to understand how each step might respond to increases in temperature and whether intracellular processes are always rate-limited by extracellular enzyme activity.

The temperature sensitivities of extracellular enzymes in soil have been well reported in the literature whereby assays are performed in the laboratory at different temperatures (Allison et al., 2018). Generally, the effect of temperature on the rate of physiological or biochemical processes (including enzyme activities) are represented by temperature response curves (TRCs), as shown in Figure 5.1. These curves are unimodal and have three regions; (i) a rising phase where temperature increases lead to increasing reaction rate, (ii) a plateau which represents the optimal temperature (Topt) and (iii) a steep falling phase where rate declines beyond the optimum temperature (Schulte, 2015). Differences in the shape of TRCs can be used partly to describe the differences in the adaptive capacity of community taxa, plasticity, acclimation, or methodological challenges while choosing assay temperatures. Classical models like the Arrhenius equation have being used to describe the response rate and the derivatives like activation energy (Ea) used to quantify the temperature sensitivity (Muñoz et al., 2016). However, the Arrhenius equation can only predict the rising (exponential) phase of the TRC (Postmus et al., 2008; Schulte, 2015), and also assumes that the steep falling phase is a result of protein denaturation; a process that is not commonly observed in temperature-induced soil biochemical processes (Schipper et al., 2014). Macromolecular rate theory (MMRT) proposed by Hobbs et al., (2013) is known to capture all three phases of the TRC, without invoking denaturation (Schulte, 2015; Schipper et al., 2014) and has since being used to model soil respiration and various extracellular enzyme activities in soils and other media (Schipper et al., 2014; Alster et al., 2016; Robinson et al., 2017; Alster et al., 2018). Model parameters including temperature optimum (T_{opt}) and the point of maximum temperature sensitivity (TS_{max}), derived from fitting the MRRT model can then be used as a measure of

temperature sensitivity. However, many authors have expressed the need to adopt more than a single metric to properly characterize the temperature sensitivities of biochemical reactions in soil because, soil is a complex system whereby, under certain conditions, the mechanisms of temperature effects on soil respiration could switch from biochemical to physical chemistry processes such as sorption/desorption or diffusion (Schipper et al., 2019; Alster et al., 2020). In such cases the response of enzymes to increases in temperature may switch from a MMRT (with T_{opt}) style response to an Arrhenius (exponential rate) style response (Schipper et al., 2019).

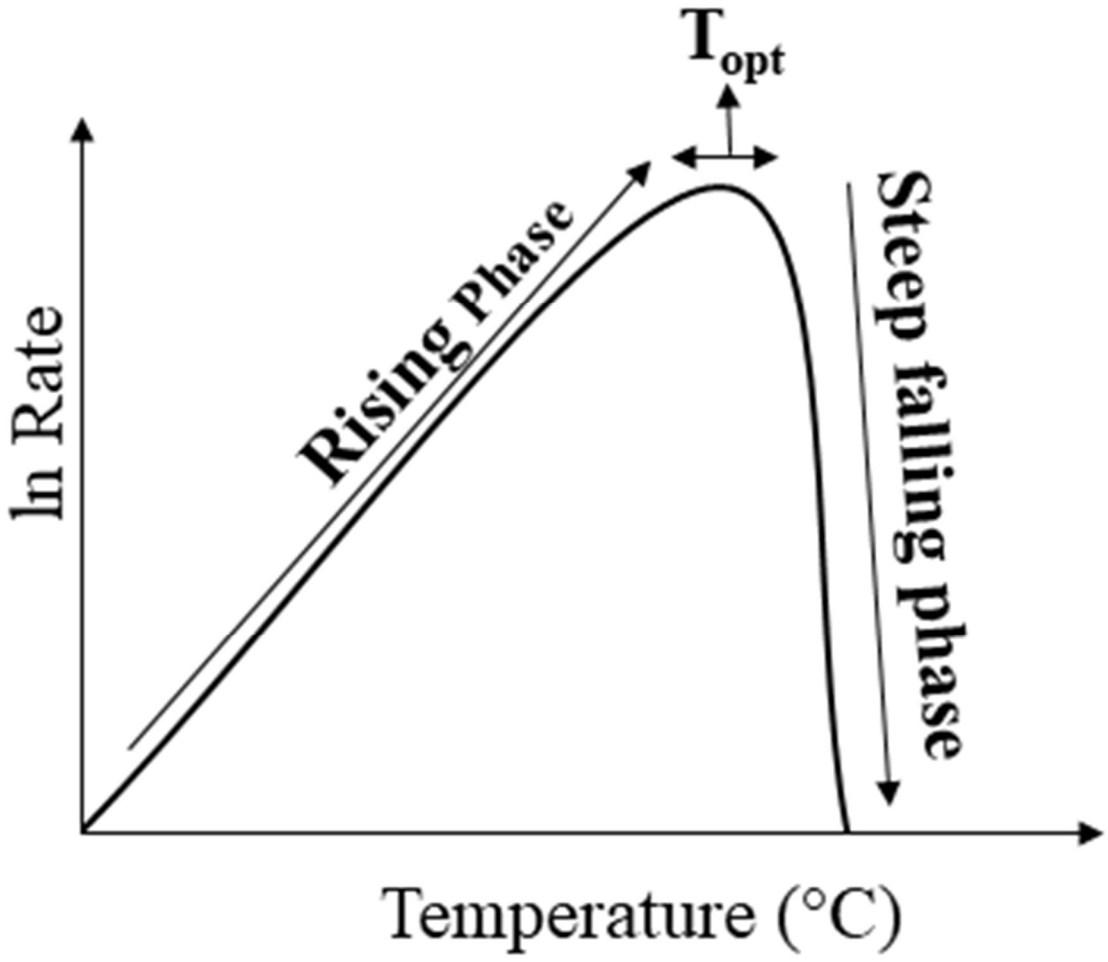

Figure 5.1 Temperature response curve (TRC) showing the three distinct regions (a rising phase, a plateau (Topt), and a steep falling phase)

It has been observed that extracellular enzyme activity remains at temperatures apparently too high for culturing microorganisms (Nannipieri et al., 2018). These observations imply that hydrolytic (extracellular) enzyme activity and intracellular enzyme activity are differently

sensitive to temperature changes. The activity of intracellular enzymes depends on the physiological properties of the cell, various co-factors, and the capability of microorganisms to position themselves adjacent to other enzymes (Maire et al., 2013). Temperature effects at the cellular level could be (i) physical whereby membrane fluidity, protein folding and diffusion rate are hampered, or (ii) chemically where rate of reaction in the cell is affected (Postmus et al., 2008). Microbial adaptation to temperature, involving alteration to lipid-composition or membrane-fluidity, could influence the activity of intracellular enzymes (Schulte, 2015). Thermal adaptation (the increase or decrease in heterotrophic microbial activity per unit mass of microbial biomass due to sustained decrease or increase in temperature, respectively) can limit the response and temperature sensitivity of both intracellular and the extracellular activities (Bradford et al., 2008; Blagodatskaya et al., 2016). Therefore, if the ambient temperature of soil shifts for a long period of time, there could be a shift in the optimum temperature of enzymes produced by microorganisms in soils due to adaptation of microbial organisms to the new temperature regime. Therefore, we propose that the ambient temperature that a soil has previously been exposed to influences the optimum temperature and temperature sensitivity of soil enzymes and that these optima and sensitivity differ between intracellular and extracellular enzymes. Since extracellular enzymes catalyse what is believed to be the rate limiting step in soil organic matter (SOM) decomposition (Duly and Nannipieri, 1998; Alvarez et al., 2018), thermal adaptation of extracellular enzymes will then determine how much substrate is available for subsequent uptake and respiration. Such adaptation exerts an important control on the response of ecosystems to warming (Bradford, 2013). Understanding whether historical temperature mediates the activity or production of extracellular enzymes and subsequent CO_2 release (intracellular step) will therefore help us to better predict the effects of temperature change on soil respiration.

In this study, we measured potential extracellular and intracellular enzyme activities at 5 assay temperatures (5 °C, 15 °C , 26 °C, 37 °C and 45 °C) following pre-incubation for 60 days at 5 °C, 15 °C, or 26 °C to compare the relative importance and temperature sensitivity of extra- and intracellular processes related to organic matter decomposition in soils thermally adapted to different temperature regimes, alongside measurements of key soil properties that we consider may lead to changes in enzyme kinetics. The pre-incubation temperatures were selected to acclimatize the microbial community to temperatures which are realistic for the site where the soil was sampled. We hypothesised that the rate limiting step of basal respiration, at least in soils exposed to the higher pre-incubation temperature, comes not from extracellular

enzyme activity but subsequent intracellular steps, mediated by substrate uptake and/or carbon use efficiency (Allison et al., 2018). We therefore hypothesise that (i) extracellular depolymerase potential and intracellular catabolic enzyme activities are not equally sensitive in their response to increasing temperature, and that (ii) the relative sensitivity of extracellular and intracellular activities to temperature depends on pre-incubation soil temperature

5.3 Methodology

5.3.1 Soil sampling and Pre-incubation

Soils samples were collected from a permanent grassland field at Sonning UK (latitude 51° 28.564′, longitude 000° 54.198′), sieved with a 4 mm sieve, mixed, and homogenised before randomly allocating to replicates. Four 'field moist' replicate sub-samples (750 g) were pre-incubated at 5 °C, 15 °C (daily minimum and maximum temperatures of the area) and 26 °C (typical of hot summer days in the area) for a period of 60 days in rectangular plastic containers. To maintain constant soil moisture, the weight of each container and the soil were recorded as initial (field weight – approximately 12.7% moisture content). The cover of each container was loosely closed to prevent the soil from becoming anaerobic. Moisture content was adjusted every two weeks for soils incubated at 5 ºC and 15 ºC and weekly for soils incubated at 26 ºC back to their initial field weight. The sampled soil was a slightly acidic loamy soil, classified as Chromic Endoskeletic Luvisol (Adekanmbi et al., 2020). A detailed description of the site is provided by Adekanmbi et al., (2020) in Chapter 3 .

5.3.2 Experimental Design

The experiment was a two factorial experiment involving 3 pre-incubation temperatures (5 °C, 15 °C, and 26 °C), and 5 assay temperatures (5 °C, 15 °C , 26 °C, 37 °C and 45 °C). This design resulted in 15 treatments replicated 4 times, resulting in 60 experimental units. Basal respiration, substrate induced respiration using glucose as the substrate (intracellular enzyme activity), and the potential activity of two extracellular enzymes (β-glucosidase and chitinase) were the assays undertaken. Assays were performed on all experimental units within the same week to avoid variability due to time of assay. Incubation temperatures were randomised to prevent systematic bias in the results. The two extracellular enzymes selected were β-1,4-glucosidase (BG), which catalyses cellulose degradation to release CO_2 from the end of cellulose chain, and N-acetyl β – D - glycosaminidase (NAG) which is involved in the degradation of chitin and peptidoglycan polymers of bacterial and fungal origin (Blagodatskaya et al., 2016). A third assay was carried out where soil CO_2 flux was measured during 1 hour of

incubation in the presence of glucose, to represent intracellular enzyme activity, and in the absence of glucose, to represent basal respiration (the combination of both extracellular and intracellular processes in the degradation of SOM). A portion of the soil from each replicate sample was also analysed for Total carbon (TC), total nitrogen (TN), pH, and Microbial Biomass Carbon (MBC).

5.3.3 Extracellular enzyme assays

Extracellular enzyme assay methods were based on Eivazi and Tabatabai, (1988) and Parham and Deng, (2000) for β-glucosidase and β-glucosaminidase (chitinase hereafter), respectively. For each experimental replicate, 1 g of soil was weighed into a 50 ml centrifuge tube and mixed with 4ml MUB buffer (pH 6) and either 1ml 25mM *p*-nitrophenyl-β-D-glucopyranoside (β-glucosidase) or 10 mM *p*-nitrophenyl-N-acetyl-b-D-glucosaminide (chitinase) solution, to assess β- glucosidase and chitinase activity, respectively. Samples were incubated at 5 °C, 15 °C, 26 °C, 37 °C, or 4 5°C for 30 minutes, after which 1 ml 0.5 M $CaCl_2$ and 4 ml Tris buffer (pH 12) was added to stop the reaction. Samples were mixed by swirling, then filtered with Whatman No. 2 filter paper.

Additionally, 2 blanks (for each run) were created by adding substrate to tubes containing the mixture after the reaction had stopped. Colour intensity of the filtrate - directly proportional to the level of reaction product p-nitrophenol (pNP), and hence level of enzyme activity - was measured using a spectrophotometer at 400 nm. Working *p*-nitrophenol standard solutions including 0, 10, 20, 30, 40 and 50 μg *p*-nitrophenol were used and the mass of *p*-nitrophenol in each reaction (0-50 μg) plotted against the OD_{400nm} reading. The level of absorbance was converted to potential enzyme activity by dividing the measured concentration by dry weight equivalent of soil.

The duration of 30 minutes assay time was determined after a preliminary trial experiment where samples were incubated for intervals of 15, 30, 45 and 60 minutes to ensure that samples were within the range where product was accumulating linearly with time, confirming that substrate limitation was not influencing the reaction, and to ensure reliable readings while minimising run times. Please see the supplementary document for details and the results of these preliminary trial runs (Appendix 2).

5.3.4 Soil CO_2 respiration assay

For each replicate sample, 15 g (13.31 g dry weight equivalent) of soil was weighed into a 50 ml centrifuge tube. Glucose solution (2 ml) was added at concentrations of 0 (deionized water only) or 10 mg g^{-1} soil (i.e. running the experiment with and without a metabolic substrate), thus bringing the soil to 58 % of its water holding capacity. The soil was then mixed to distribute the solution throughout. The glucose concentration of 10 mg/g was determined by a preliminary trial where samples were run with concentrations of 0, 0.1, 1 and 10 mg g^{-1}. 10 mg g^{-1} was chosen as the curve of CO_2 concentration vs. glucose concentration levelled off at that concentration, suggesting saturation, therefore avoiding concerns of substrate limitation. Please see the supplementary document for details and the results of these preliminary trial runs (Appendix 2).

Following soil-substrate mixing, the tube was ventilated by blowing in lab air with a 20 ml syringe, ensuring air away from the user was extracted to avoid contamination with human-generated CO_2. The tubes were sealed with septum stoppers and 15 ml of lab air was injected. The headspace was flushed by moving the syringe plunger up and down several times before sampling 15 ml of head space gas and injecting into a 12 ml exetainer vial (T0), creating overpressure, using a tap and needle attached to the syringe. The samples were incubated for one hour at the same five temperatures as for the extracellular enzyme assays, at the end of which the process of injecting air, flushing and sampling was repeated (T1). Headspace gas samples were stored at 20 °C prior to analysis by an Agilent 7890B gas chromatograph. After calibrating with CO_2 gas standards, the concentration of CO_2 in mg L^{-1} was converted to C-CO_2 mg C g^{-1} h as described by (Salazar-Villegas et al., 2016); see the formula as follows:

$$CO_2\ (\text{mg C g}^{-1}\ \text{h}^{-1}) = \frac{V\ (T1 - T0)}{Wt}$$

Where V = volume of headspace in the centrifuge tube; T1 is CO_2 concentration after a 1 hour incubation in mg L^{-1}; T0 is CO_2 concentration before 1 hour incubation in mg L^{-1}, W is the dry weight of the soil, and t is the time between T0 and T1 measurements in hours.

5.3.5 Measurement of TC, TN, pH and MBC in the pre-incubated Soil

MBC was measured using the fumigation/extraction method described by Vance et al. (1987). Four replicates from each pre-incubation temperature weighed to the moist mass equivalent to 50 g oven-dried soil in beakers and placed in a vacuum desiccator lined with damp paper towel

to ensure high humidity, along with a beaker containing about 50 ml ethanol-free chloroform and several anti- bumping granules. The desiccator was evacuated, and the chloroform allowed to boil for two minutes before the valve was closed and the desiccator kept in the dark for 24 hours. Before extraction, the chloroform was removed, the desiccator evacuated three times and the samples left to vent to ensure no chloroform remained in the soil.

Extraction was carried out on both fumigated soil and non-fumigated duplicates. Samples of both were placed into 350 ml polypropylene bottles, to which 200 ml 0.5 M K_2SO_4 was added, before being placed on an oscillating shaker for 30 minutes. The suspension was then filtered into polypropylene universal tubes before being stored in a freezer prior to analysis. On removal from the freezer samples were diluted by a factor of 10, and filtered to remove $CaSO_4$ that had precipitated, before analysis for total organic carbon (TOC) using a Shimadzu TOC 5000. Also analysed were method blanks consisting of K_2SO_4 that had not been used to extract soil, to correct for any part of the reading not due to organic carbon content. TOC extracted from fumigated and non-fumigated samples was converted to a biomass carbon value by multiplying the difference (E_c) by 2.64, as in (Vance et al., 1987). The TOC of the non-fumigated soil before conversion represents the K_2SO_4 extractable carbon.

TC and TN were determined using the dry combustion method. 2 mm sieved soil samples were ground for three minutes in an agate ball mill. From the residue, 10 mg duplicates were weighed out using a five-point balance and placed in tin foil capsules for measurement. Total N and C concentrations were analysed using a C/N Elemental Analyser (Thermo Flash 2000 EA). The C/N ratio was calculated from total C and N.

pH was determined in water (1:2.5 ratio) using the potentiometric method. Four replicates of 10 g air-dried and 2 mm sieved soil from each pre-incubation temperature were weighed into 50 ml centrifuge tubes and 25ml of deionised water was added to each tube. Samples were placed in an end-over-end shaker running at 30 rpm for 15 minutes. The pH meter was calibrated with pH 4.0 and 7.0 buffer solutions before use, and re-calibrated after half the samples were measured.

5.3.6 Temperature sensitivity

Model fit of MMRT (Hobbs et al., 2013), and Arrhenius equations were performed to describe the temperature response. Ea was calculated from the slope of the relationship $-1/R_0T$ and the natural logarithm of rate of enzyme activity (R_0 = the gas universal constant : 8.314 J mol^{-1} ; temperature is Kelvin), as described by Li et al., (2015). Arrhenius fit and Ea derivation were

performed for two ranges of temperature (5 °C – 26 °C and 5 °C – 45 °C) with the wider range believed to include temperatures which extend beyond the rising phase identified in Figure 5.1. R code published by Alster et al., (2020) was used to generate MMRT derivatives, including change in heat capacity ($\Delta C_p^{\ddagger}$), temperature optimum (T_{opt}) and point of maximum temperature sensitivity (TS_{max}), which were used to assess temperature sensitivity. Temperature sensitivity (Q10) of both the intra (CO_2) and extra-cellular (chitinase and β-glucosidase) enzyme activities was calculated using the equal time measurement, as described by Karhu et al., (2014).

5.3.7 Statistical analysis

Two-way Analysis of Variance (ANOVA) was carried out to assess the effects of pre-incubation temperature and assay temperature on basal respiration, intracellular, and extracellular enzyme activities. We also assessed whether intracellular and extracellular enzymes were equally sensitive to temperature, and whether this was influenced by pre-incubation temperature, by performing a two-way ANOVA on the Ea and Q10 values using enzyme type, and pre-incubation temperature as factors. One way ANOVA was carried out to assess the effect of pre-incubation temperature on MBC, TC, TN, C/N ratio and pH of soil. ANOVA was performed in Minitab version 18. Tukey pairwise comparison was used to assess the significance of differences between individual treatment means. We did not perform any statistical analysis of the MMRT derivatives since a single model was fit to average data, yielding one value for each derivative per treatment (i.e. no statistical replicates).

5.4 Results

5.4.1 Impact of incubation temperature on selected soil properties

The effects of soil pre-incubation temperature on soil TC, TN, C/N ratio, pH and MBC is presented in Figure 5.2. While TC was slightly lower in soils incubated at 26 °C, compared to 15 °C and 5 °C, and TN slightly lower in soil pre-incubated at 5 °C and 15 °C, compared with 26 °C, pre-incubation temperature did not have a statistically significant impact on TC (P = 0.641) or TN (P = 0.439). However, soil C/N ratio was significantly (P < 0.0001) higher in soil pre-incubated at 15 °C and 5 °C, compared to soil pre-incubated at 26 °C. Also, soil pH was significantly (P < 0.0001) higher in soils pre-incubated at 5 °C, compared to 15 °C and 26 °C. Although, MBC was marginally lower in soil pre-incubated at 26 °C, compared to 5 °C or 15 °C, there was no statistically significant effect of soil pre-incubation temperature on MBC (P = 0.206).

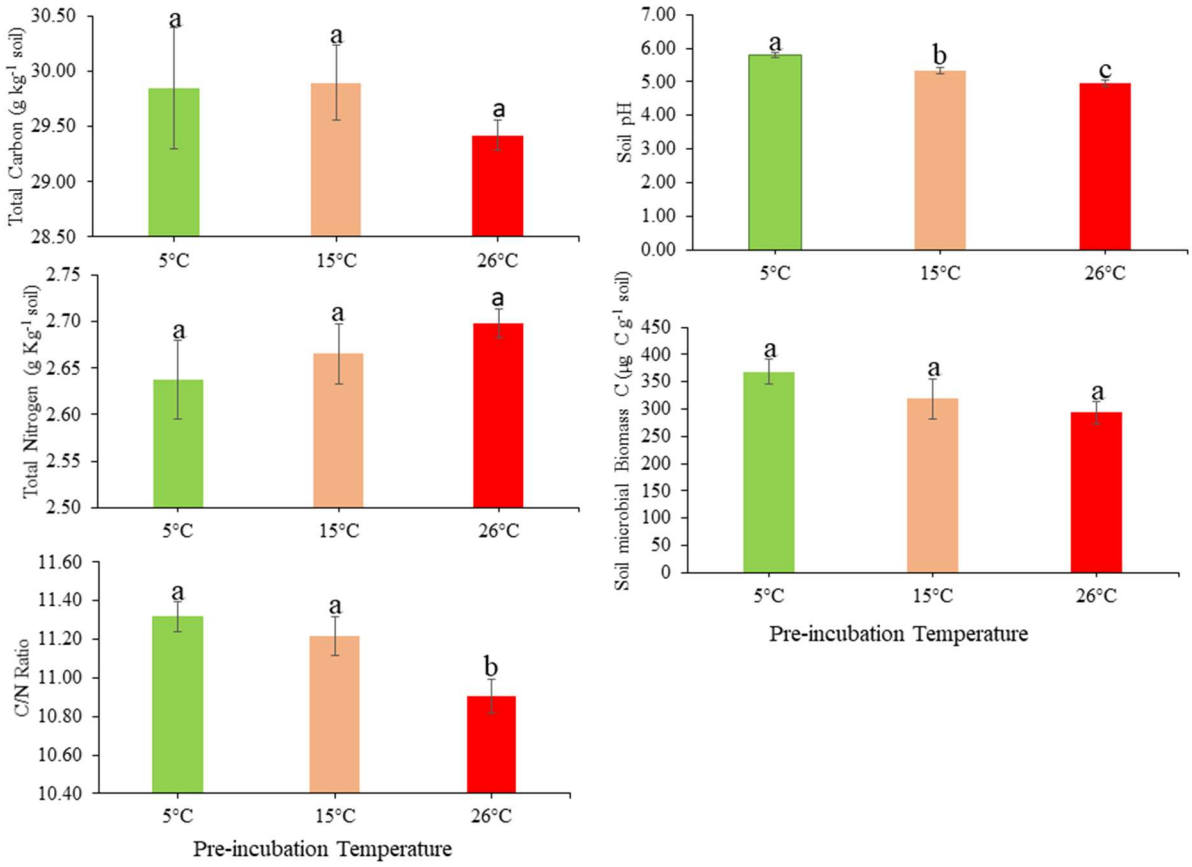

Figure 5.2 Effects of pre-incubation temperature on soil Total Carbon (TC), Total Nitrogen (TN), Carbon-to-Nitrogen (C/N) ratio, pH and Microbial Biomass Carbon (MBC). Each bar and error bar represents mean and standard error of 4 replicate samples at each incubation temperature. Means with the same letter are not significantly different ($P > 0.05$).

5.4.2 Responses of intracellular and extracellular enzyme activities to pre-incubation temperature and assay temperature.

The influence of pre-incubation temperature on the potential activities of extracellular enzymes (Figure 5.3A and 5.3B), the rate of intracellular enzymes activity (Figure 5.3C) and the basal respiration rate (Figure 5.3D) within the full range of assay temperatures (5 °C to 45 °C) are presented in Figure 5.3. Pre-incubation temperature ($P < 0.0001$) significantly influenced β-glucosidase activity in soil and its response to assay temperature (Figure 5.3A). Soils pre-incubated at 15 °C had significantly ($P < 0.0001$) greater β-glucosidase activity when assayed at temperatures ranging 5 °C to 45 °C compared to sols pre-incubated at 26 °C. Increasing assay temperature increased β-glucosidase activity up to the maximum assay temperature of

45 °C. Furthermore, β-glucosidase activity in soils pre-incubated at 15 °C was only slightly higher than soils pre-incubated at 5 °C. Chitinase activity increased with increasing assay temperature, reaching maximum around 37 °C, but was then lower when assayed at 45 °C (Figure 5.3B). Pre-incubating soil at 26 °C and assaying at 37 °C resulted in a significantly ($P < 0.001$) greater chitinase activity than pre-incubating at 5 °C. With ($P < 0.0001$) or without ($P < 0.001$) glucose addition, pre-incubating soil at 26 °C resulted in lower soil respiration compared to pre-incubating soil at 5 °C or 15 °C (Figure 5.3C and 5.3D). Basal respiration increased ($P < 0.0001$) with increasing assay temperature up to 26 °C then declined slightly. Glucose-induced respiration increased ($P < 0.0001$) with increasing assay temperature, reaching maximum between 26 °C and 37 °C, but was significantly lower at 45 °C. The addition of 10 mg g^{-1} soil of glucose led to about a 4 fold increase in CO_2 respired, compared to no addition of glucose substrate.

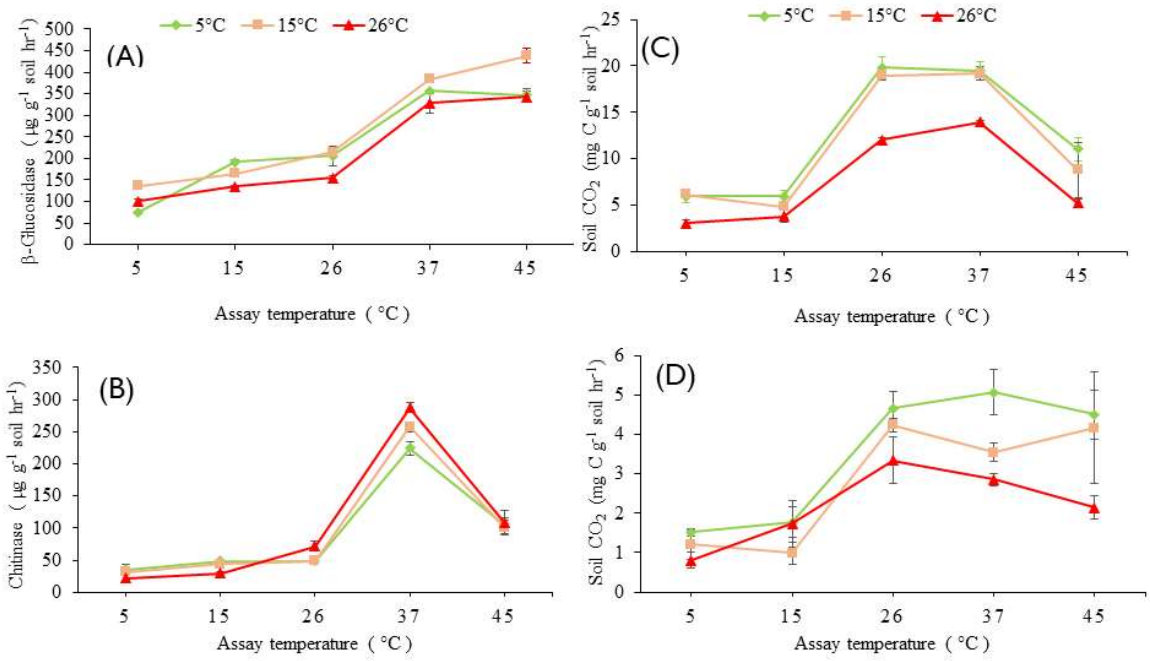

Figure 5.3 Response of enzyme activity (A and B) and respiration rate (C and D) at various pre-incubation and assay temperatures. C and D represent CO_2 released from soil with added glucose at 10 mg g^{-1} soil and 0 mg g^{-1} soil, respectively. Each symbol and error bar represent mean and standard error of 4 replicate samples.

5.4.3 Temperature sensitivity of intra and extracellular soil enzyme activity

5.4.3.1 Temperature coefficient (Q10)

The results of the effects of pre-incubation temperature and enzyme type on $Q10_{5\text{-}15^{o}C}$, $Q10_{15\text{-}26^{o}C}$, $Q10_{26\text{-}37^{o}C}$, and Ea (kJ Mol^{-1}) are presented in Figures 5.6 and 5.7 respectively. There was no significant difference in $Q10_{5\text{-}15^{o}C}$ between the three pre-incubation temperatures (P = 0.162), enzyme types (P = 0.393), or their interaction (P = 0.700). However, $Q10_{15\text{-}26^{o}C}$ significantly differed with enzyme type ($P < 0.0001$), but not pre-incubation temperature (P = 0.162), or their interaction (P = 0.160). The $Q10_{15\text{-}26^{o}C}$ for intracellular enzyme activity was significantly higher than both extracellular enzymes (chitinase and β-glucosidase), irrespective of pre-incubation temperature. This result indicates that intracellular enzymes are more temperature sensitive than extracellular enzymes in this soil between 15 °C and 26 °C. Also, $Q10_{26\text{-}37^{o}C}$ was significantly affected by enzyme type ($P <0.0001$) and there was a significant interaction between enzyme type and pre-incubation temperature (P = 0.018). The $Q10_{26\text{-}37^{o}C}$ for chitinase activity was considerably greater than the $Q10_{26\text{-}37^{o}C}$ of β-glucosidase activity which, in turn, was significantly ($P < 0.0001$) greater than the $Q10_{26\text{-}37^{o}C}$ of intracellular enzyme activity. In all cases, basal respiration showed a similar sensitivity to temperature as intracellular enzyme activities. This finding indicates that extracellular enzymes are more temperature sensitive than intracellular enzymes in this soil between 26 °C and 37 °C.

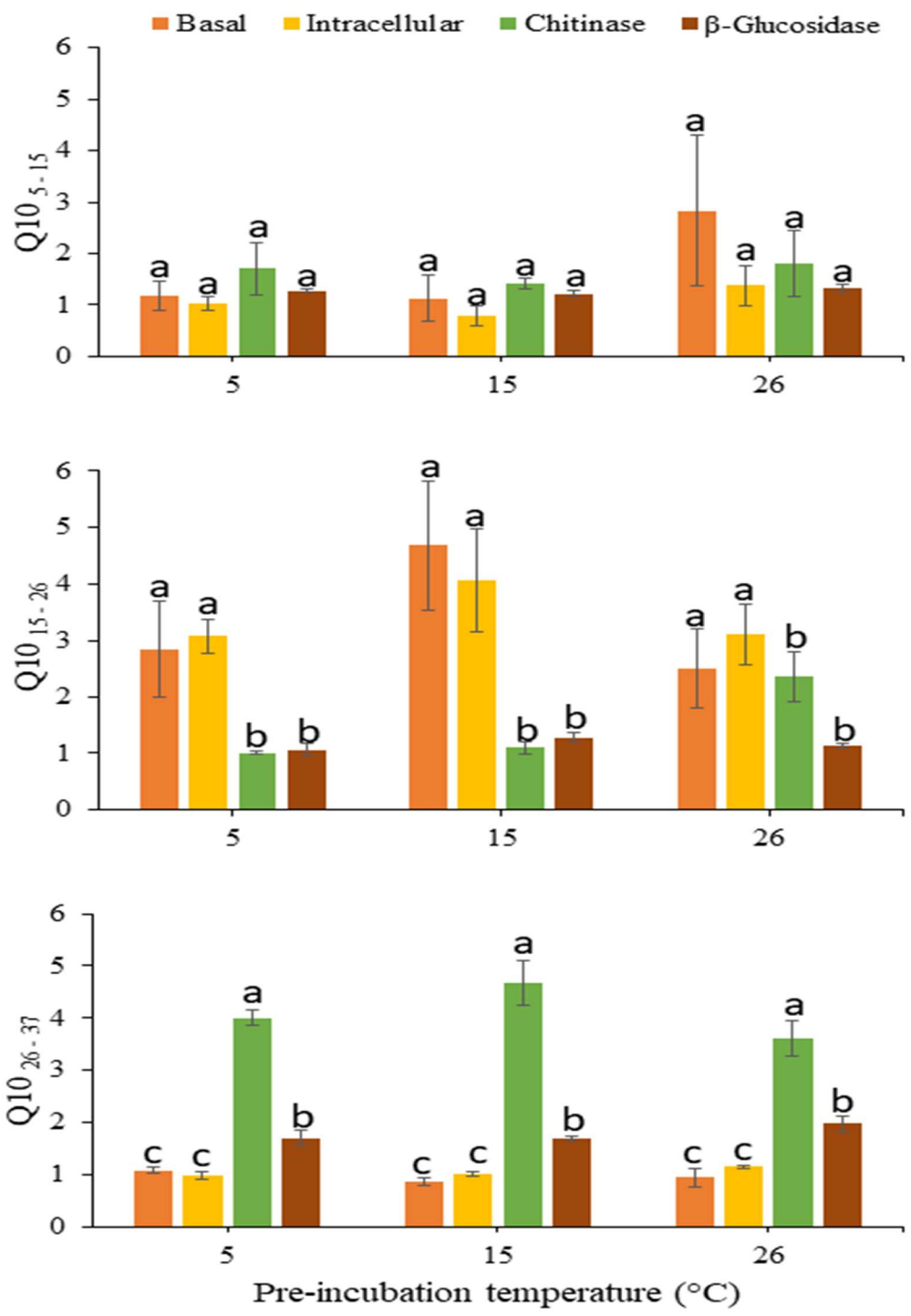

Figure 5.4 Effects of pre-incubation temperature on temperature sensitivities (Q10) of intracellular (glucose substrate induced respiration) and extracellular (chitinase and β-glucosidase) enzyme activities. Each bar and error bar represent mean and standard error of 4 replicate samples at each pre-incubation temperature. Bars with the same letters above them represent enzyme types that are not significantly different from one another ($P > 0.05$).

5.4.3.2 Arrhenius vs MMRT models

The model fit showing both the rising (exponential) phase, T_{opt} and the steep falling phase (Figure 5.1) of the temperature response curve using Arrhenius and MMRT models are presented in Figure 5.4 (Soil respiration) and Figure 5.5 (Extracellular enzymes). Arrhenius fits were performed for two temperature ranges, (5°C – 26°C and 5°C – 45°C) to exclude and include the T_{opt} and steep falling phase, respectively. MMRT predicted both the exponential phase, the optimum and steep falling phase for soil CO_2 respiration with and without the addition of glucose. Addition of 10 mg^{-1} glucose g^{-1} soil influenced the curvature of MMRT fit, showing a steeper curvature compared to basal respiration when soils were pre-incubated either at 5 °C or 15 °C. The MMRT fit showed similar curvature (determined by $\Delta C_p^{\ddagger}$ in table 5.1) for soils pre-incubated at 26 °C, with or without glucose addition. The Arrhenius fit using soils assayed in the temperature range of 5 °C to 26 °C showed a similar exponential phase to the MMRT model fit, whereas the Arrhenius fit using soils assayed in the temperature range of 5 °C to 45 °C typically generated a curve with a lower gradient. Both the MMRT and Arrhenius predictions showed similar shape of TRC (exponential increase in rate) for the extracellular enzymes within the range of assay temperatures when soils were pre-incubated at 5 °C or 15 °C, with the MRRT failing to predict T_{opt} within the range of temperatures assayed especially for β-glucosidase enzyme. In soils pre-incubated at 26 °C, the MMRT fit predicted a T_{opt}, for chitinase enzyme.

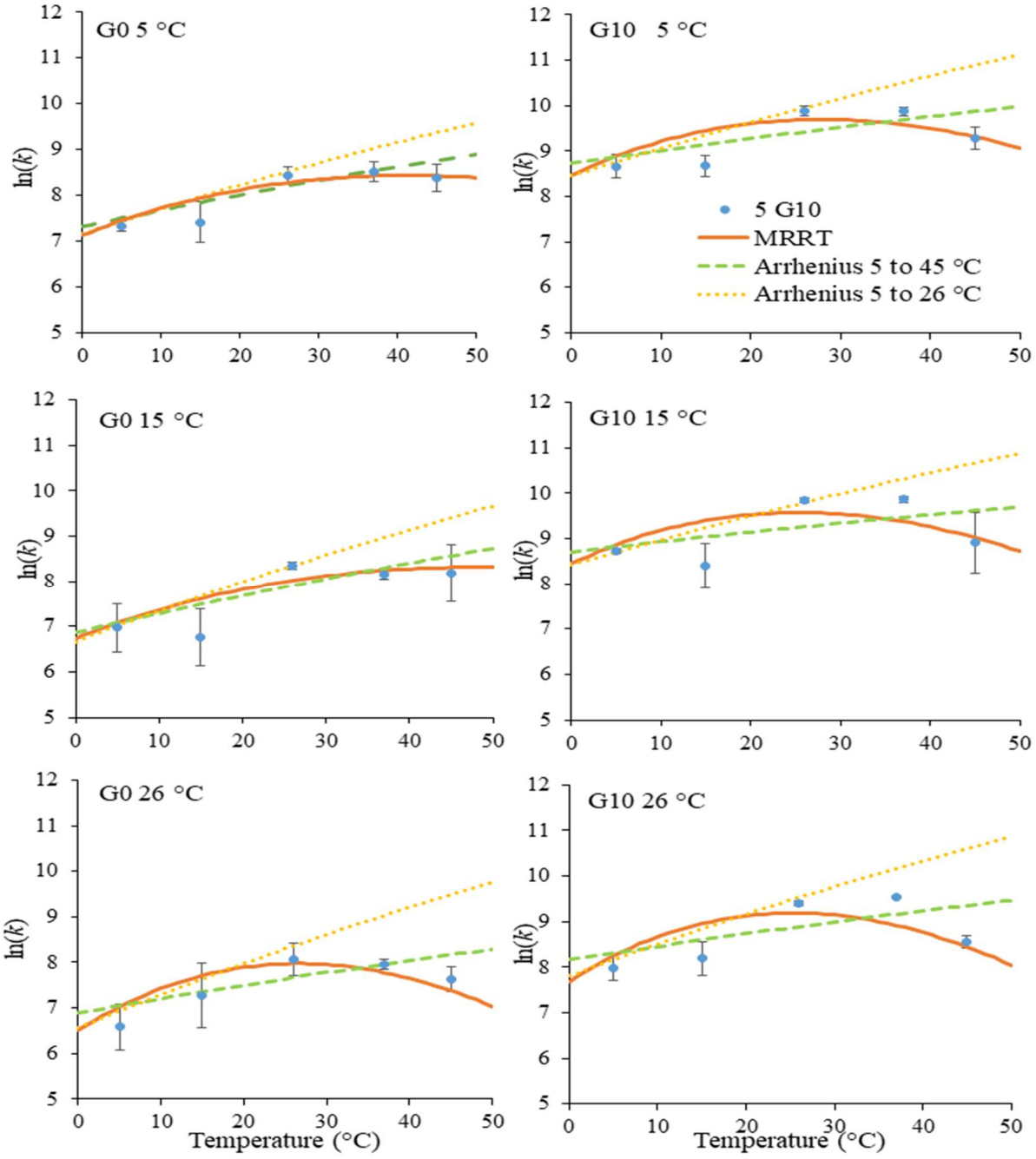

Figure 5.5 Model fit of intracellular enzyme activity (G10) and basal respiration (G0) to MMRT (orange line) and Arrhenius (yellow dotted lines = model fit using soils assayed in the temperature range 5 °C to 26 °C; and green dash lines = model fit using soils assayed in the temperature range 5 °C to 45 °C) equations.

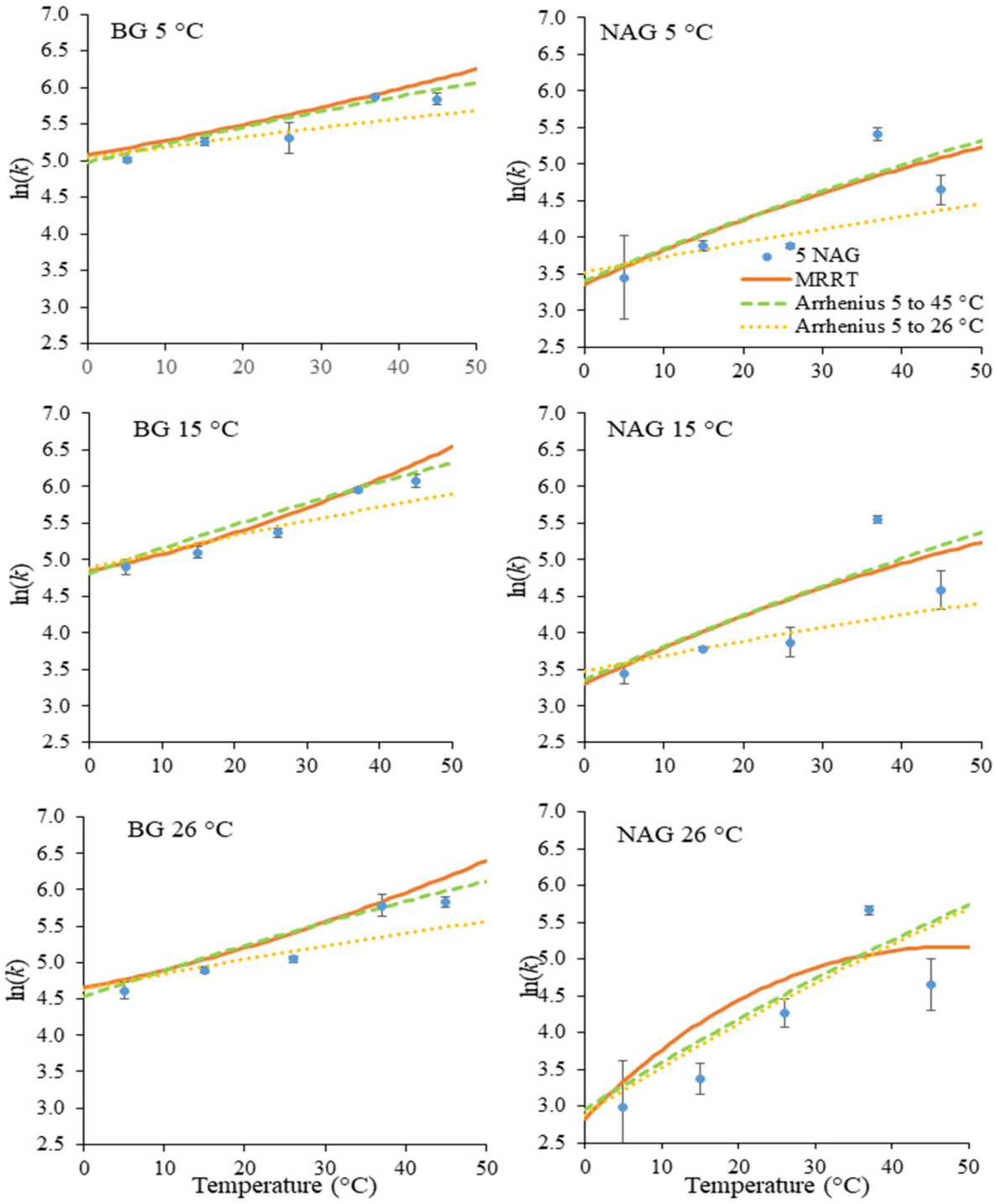

Figure 5.6 Model fit of potential activity of Chitinase (NAG) and β-glucosidase (BG) enzymes (blue dots) to MMRT (orange line) and Arrhenius (yellow dotted lines = model fit using soils assayed in the temperature range 5 °C to 26 °C; and green dash lines = model fit using soils assayed in the temperature range 5 °C to 45 °C) equations.

Table 5.1: Model parameters of MMRT fits of soil respiration rates from soils pre-incubated at three different temperatures and two levels of substrate (glucose) addition. Parameters are: change in heat capacity ($\Delta C_p^{\ddagger}$), temperature optimum (T_{opt}) and point of maximum temperature sensitivity (TS_{max}). n = 4

Substrate	Pre-incubation temperature (°C)	$\Delta C_p^{\ddagger}$ (kJ mol^{-1} $°C^{-1}$ K^{-1})	T_{opt} (°C)	TS_{max} (°C)
None (basal)	5	-1.1	46	20.5
None (basal)	15	-0.981	53.1	25.6
None (basal)	26	-2.87	31.5	15.9
Glucose	5	-2.18	32.9	15.1
Glucose	15	-2.39	30.4	13.5
Glucose	26	-3.19	30.3	15.6

5.4.3.3 Effects of pre-incubation temperature and enzyme type on Arrhenius activation energy

The activation energy (Ea), derived from the fit of the Arrhenius equation (Figure 5.7) to assays performed between 5 °C and 26 °C (the rising phase), differs significantly with enzyme type ($P < 0.0001$) and pre-incubation temperature ($P = 0.003$) and there was a significant interaction between enzyme type and pre-incubation temperature ($P = 0.022$). A pre-incubation temperature of 26 °C resulted in chitinase requiring a higher activation energy, whereas a lower $Ea_{5\text{-}26}$ resulted from pre-incubating soil at 15 °C or 5 °C. Intracellular catalytic activities had a higher $Ea_{5\text{-}26}$ compared to chitinase which, in turn, was significantly greater than β-glucosidase. Also, pre-incubating soil at 26 °C yielded similar Ea for basal respiration and intracellular activity for soils pre-incubated at 5 °C, 1 5°C or 25 °C. Basal respiration showed a similar Ea to intracellular activity. When the Ea was derived from the fit of the Arrhenius equation to assays using the whole range of assay temperatures (between 5°C and 45°C), chitinase activity had a significantly ($P < 0.0001$) higher $Ea_{5\text{-}45}$ compared to the intracellular activity, basal respiration, or β-glucosidase activity.

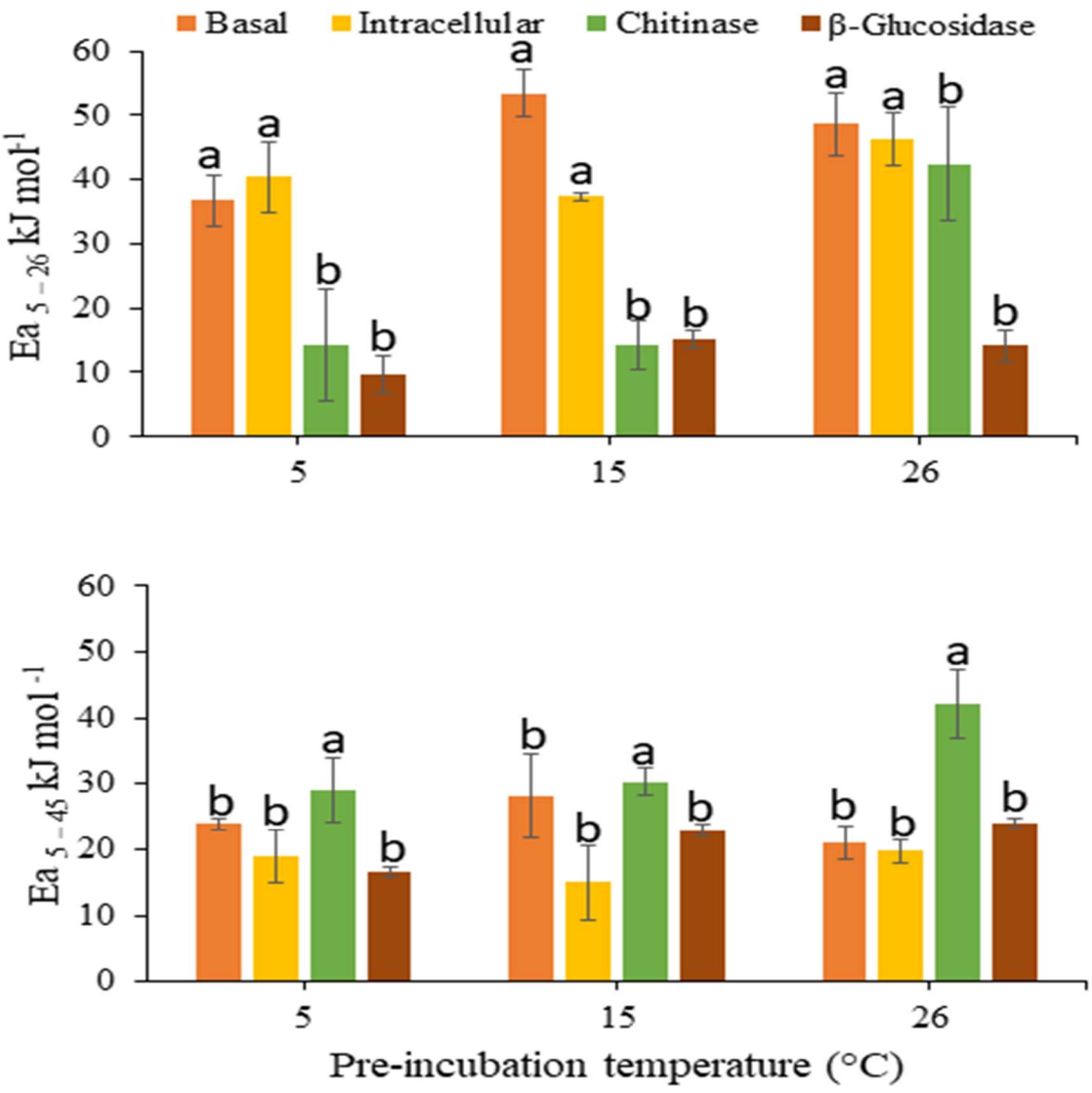

Figure 5.7 Effects of pre-incubation temperature on Activation energy (Ea) of intracellular (glucose substrate induced respiration) and extracellular (chitinase and β-glucosidase) enzyme activities and basal respiration. Ea_{5-26} = Activation energy calculated using an Arrhenius model fit using soils assayed in the temperature range 5 °C to 26 °C. Ea_{5-45} = Activation energy calculated using an Arrhenius model fit using soils assayed in the temperature range 5 °C to 45 °C. Each bar and error bar represent mean and standard error of 4 replicates samples at each pre-incubation temperature. Bars with the same letters above them represent treatments that are not significantly different from one another ($P > 0.05$).

5.4.3.4 Effects of pre-incubation temperature on MMRT derivatives

Derivatives of the MMRT model, which include change in heat capacity ($\Delta C_p^{\ddagger}$), temperature optimum (T_{opt}) and point of maximum temperature sensitivity (TS_{max}) of soil respiration, are presented in Table 5.1. Without adding glucose, increasing pre-incubation temperature from 5 °C to 15 °C resulted in less negative $\Delta C_p^{\ddagger}$, but with the addition of glucose increasing pre-incubation temperature from 5 °C to 15 °C led to more negative $\Delta C_p^{\ddagger}$. Also, increasing pre-incubation temperature from 5 °C to 15 °C increased T_{opt} and TS_{max} (by 7.1 °C and 5.1 °C, respectively), but a further increase to 26 °C led to a decline in T_{opt} and TS_{max} (by 21.6 °C and 9.7 °C respectively) without glucose addition. The effect of changes in pre-incubation temperature were marginal on intracellular enzyme activity. Pre-incubating soil at 26°C yielded similar $\Delta C_p^{\ddagger}$, T_{opt}, and TS_{max} for both the basal respiration and intracellular enzyme activity. MMRT could not generate the parameters for β-glucosidase because the model fit is concave (Figure 5.6) and $\Delta C_p^{\ddagger}$ was positive. The T_{opt} and TS_{max} for chitinase were 52.9 °C and 30.1 °C when soils were pre-incubated at 26°C, but unrealistically high in soil pre-incubated at 5 °C (221 °C and 128 °C) or 15 °C (168 °C and 96.3 °C) and for these reasons, the data is not shown. These parameters showed decreasing trends for chitinase as pre-incubation temperature increased from 5°C to 26°C.

5.5 Discussion

Understanding whether soil intracellular and extracellular enzyme activities, which each play a distinct role in soil organic matter decomposition processes, are equally sensitive to temperature changes was the major motivation for this study. We know that extracellular enzymes initiate heterotrophic carbon cycling, and that the rate of subsequent intracellular processes depends on the amount of substrate available after extracellular depolymerisation. We also expect that temperature-induced changes in soil properties may contribute to the thermal adaption of the soil microbial community and result in altered sensitivities of these processes to increasing temperature. Therefore, we pre-incubated soil samples at three different temperatures to acclimatise the soil microbial community to a particular thermal regime. We then assayed intracellular and extracellular enzyme activity in the presence of excess substrate to ensure there is no substrate limitation. Alongside intracellular and extracellular enzyme activity we measured basal respiration to determine the extent to which the response of basal respiration to temperature is similar to the response observed for intracellular or extracellular enzymes.

Evidence of thermal acclimation of microbial activity may be expressed as change in the shape of TRC or change in the position of the curve on the y axis(Carey et al., 2016). Extracellular enzymes (Figure 5.3A and 5.3B) differed in their optimum temperature, with β-glucosidase steadily increasing in activity between the lowest (5 °C) and highest (45 °C) assay temperatures, indicating an optimum temperature greater than 45 °C (Figure 3). This result was supported by a good fit to the Arrhenius model when all the assay temperatures were included (Figure 5.5). Our result is consistent with the reported exponential increase in β-glucosidase activity from assay temperatures as low as 2 °C to a maximum (optimum) of 70 °C in other studies (Trasar-Cepeda et al., 2007; Bárta et al., 2013; Steinweg et al., 2018). In contrast to the temperature response of β-glucosidase activity, chitinase activity appears to have a narrower temperature optimum, between 26 °C and 45 °C, since the greatest activity was observed at 37 °C in our study. The chitinase activity between 5 °C and 26 °C fit the Arrhenius equation well (Figure 5.5), indicating an exponential increase in chitinase activity within this temperature range. A T_{opt} of 52.9 °C (in soils pre-incubated at 26 °C) derived from the MMRT fit implies that β-glucosidase has a higher temperature optimum than chitinase activity. A T_{opt} of 50 °C has been reported for chitinase activity elsewhere (Rodriguez-Kabana et al., 1983), which supports our results. Failure to derive a T_{opt} within the range of assay temperatures used in this study when β-glucosidase activity and chitinase activity was fit using the MMRT model (for soils pre-incubated at 5 °C and 15 °C) resulted in no clear differences between fits based on MMRT or Arrhenius (Schipper et al., 2014) for the two extracellular enzymes.

Potential intracellular activity increased with increasing assay temperature, reaching a maximum between 26 °C and 37 °C, and then declined at higher temperatures (Figure 5.3). The MMRT derived T_{opt} (i.e. 30.3 °C, 30.4 °C and 32.9 °C for soils pre-incubated at 5 °C, 15 °C and 26 °C, respectively) are well within the range of assay temperatures (Table 5.1) for intracellular activity, but are only slightly affected by pre-incubation temperature. In contrast to intracellular activity, the MMRT derived T_{opt} for basal respiration was greater by 7.1 °C when pre-incubation temperature increased from 5 to 15 °C, but decreased by 21.6 °C when soils were pre-incubated at 26 °C (Table 5.1). This observation suggests faster depletion of carbon at higher pre-temperature. Depolymerisation of the available substrate is then being carried out at higher temperature than the previous pre-incubation temperature, indicating microbial adaptation to higher temperature. This result is consistent with the notion that intracellular processes are less sensitive to short term stresses, such as those imposed by differences in assay temperature, compared to the extracellular processes, since intracellular

processes are being carried out by greater diversity of soil microorganisms involving multiple distinct physiological processes (Mooshammer et al., 2017). Our findings imply that, in soils thermally adapted to 26 °C, intracellular activity will be rate limiting due to lower substrate availability, whereas extracellular enzyme activity may be the rate limiting step in soils pre-incubated at 5 °C or 15 °C.

We also found that temperature sensitivity, calculated using the temperature coefficient (Q10), differs with the range of assay temperatures used to calculate the co-efficient. Intracellular catalytic activity was more sensitive to temperature changes within a moderate range of temperatures (15 °C and 26 °C) than extracellular enzymes. Conversely, extracellular enzymes were more sensitive to temperature changes within a higher range of temperature (26 °C and 37 °C). This result implies that extracellular depolymerase activities are more sensitive to temperature changes at higher temperatures than intracellular catalytic enzymes. So extracellular enzymes might be more sensitive to increases in the daily maximum temperature and intracellular enzymes might be more sensitive to increases in daily average temperatures. This finding supports our first hypothesis that extracellular depolymerase potential and intracellular catabolic enzyme activities are not equally sensitive in their response to increasing temperature. The Q10 of C mineralization has previously been reported to change with incubation temperature intervals (5–15 °C, 15–25 °C, and 25– 35 °C) and elevation gradient (Wang et al., 2013). The authors found that $Q10_{5\text{-}15}$ for labile carbon was lower than $Q10_{15–25}$, but $Q10_{15–25}$ had similar sensitivity to $Q10_{25–35}$. Also, the $Q10_{15–25}$ of recalcitrant carbon was higher than $Q10_{25–35}$. Temperature sensitivity for C mineralisation is generally believed to decrease with temperature (Niklińska and Klimek, 2007; Wang et al., 2013). However, these two studies did not separate the sensitivity of extracellular from intracellular activities as we did in this study. In agreement with our findings, Carey et al., (2016) reported that the temperature sensitivities of soil respiration decreased at temperatures above 25°C. Below 25°C, a synthesis of soil respiration measurements from laboratory studies revealed that Q10 correlates negatively with range of temperatures used to generate the Q10 value (Hamdi et al., 2013). Hamdi et al., (2013) also found that Q10 was negatively correlated with total organic carbon of the soil, especially in forest and grassland soils.

The higher Ea observed in soils pre-incubated at 26 °C compared to 5 °C, irrespective of enzyme type, could be due to changes in the biochemical properties of enzymes as a result of the temperature regime. Temperature regime can influence the ability of enzymes to change their structural conformation and this can alter the Ea of soil enzyme reactions, resulting in

differences in the substrate affinities of enzymes from different climates (Steinweg et al., 2013b). Our result conforms to the physiological prediction that enzyme activity in warmer environments require a higher Ea. However, this is not always the case because environmental factors like soil characteristics, microbial community composition, and vegetation type can influence enzymatic expression as well as temperature (Allison et al., 2018). Changes in other environmental variables could therefore obscure the impact of temperature on the biochemical properties of individual enzymes or groups of enzymes.

We observed that the Ea of intracellular activity was significantly higher than those of extracellular activities within the range of 5 to 26 °C. This observation is contrary to Arrhenius theory, which dictates that the depolymerisation reaction is more temperature sensitive compared to oxidation of more labile substrate due to the higher activation energy (Blagodatskaya et al., 2016). Similarities between the Ea of intracellular enzyme activity and basal respiration in our study could reflect the importance of intracellular activity in mediating soil organic matter mineralisation rate at moderate temperatures. Blagodatskaya et al., (2016) observed higher temperature sensitivity of intracellular enzyme activity than extracellular depolymerisation. These authors concluded that the observation could be due to changes in enzyme mechanisms due to warming. The differences in Ea observed between the different extracellular enzymes could also be due to differences in their individual structural conformation or substrate affinity (Steinweg et al., 2013b). For example, the differences in Ea between β-glucosidase and chitinase activities may reflect thermal adaptation of β-glucosidase to a greater extent than chitinase. The lower Ea of β-glucosidase, compared to chitinase or intracellular enzymes, could benefit both the intracellular enzyme activity and overall soil respiration because enzymes catalyse the rate of biochemical reaction by lowering their Ea (Razavi et al., 2017).

The finding that Q10 and Ea were similar in both the basal respiration and intracellular enzyme assays could mean that extracellular processes were not the rate limiting step for organic matter decomposition in the soils pre-incubated at any of the temperatures in this study. This result could mean that the temperature sensitivity of intracellular catalytic activity reflects the overall sensitivity of soil respiration at moderate temperature range and when substrate is not limiting. The previous temperature experienced by microbial community could modulate the environmental factors influencing both their activity and temperature sensitivity thereby confounding current temperature sensitivity. Birge et al., (2015) reported that after a long period of incubation-depletion at 30 ºC, the extracellular enzyme pool did not respond to the

addition of substrate and did not limit soil respiration. The authors then concluded that available substrate, rather than lack of extracellular enzymes or microbial biomass, controlled the rate of soil respiration. Therefore temperature-induced changes in available substrate at various pre-incubation temperatures might have led to the similar Ea and Q10 observed for basal respiration and intracellular enzyme activity.

The T_{opt} and TS_{max} derived from the MMRT model could be used to infer changes in community composition whereby higher T_{opt} and TS_{max} implies higher abundance of bacteria compared to fungi and higher abundance of Gram positive bacteria compared to gram negative bacteria (Alster et al., 2018). When glucose was added in excess, only slight differences were observed in terms of TS_{max} and T_{opt} between the pre-incubation temperatures, whereas major differences in TS_{max} and T_{opt} were observed between soils pre-incubated at different temperatures without addition of glucose (Table 5.1), and these differences could be the result of changes in microbial community size and composition. Higher T_{opt} and TS_{max} in soils pre-incubated at either 5 °C or 15 °C, compared to 26 °C, could suggest a shift from bacteria dominated community to a fungal dominated community and a shift from Gram positive to Gram negative bacteria. A similar TS_{max} was observed for intracellular (15.6 °C) and basal respiration (15.9 °C) when soils were pre-incubated at 26 °C, implying that a similar community composition was responsible for both intracellular and extracellular processes at this pre-incubation temperature. However, we did not measure the community composition in this study.

The pre-incubation of soils at 5 °C, 15 °C and 26 °C was undertaken to allow thermal adaptation of the soil microbial community, and their extracellular and intracellular enzymes, prior to measurement of their temperature sensitivity. The observation that pre-incubation at 26 °C resulted in significantly lower activity of β-glucosidase and intracellular catalytic enzymes (as well as basal respiration), compared to pre-incubation at 5 °C or 15 °C, reflects the depletion of more labile carbon during the pre-incubation at 26 °C. It is well agreed in previous studies that β-glucosidase catalyses the degradation of the labile carbon pool and its potential activity is high when there is abundant substrate (Ferraz De Almeida et al., 2015). This observation may also imply that the potential activity of β-glucosidase may benefit intracellular catalytic enzymes in nature by supplying the degraded monomers to enhance CO_2 release. Possibly because β-glucosidase is the rate limiting step in the microbial degradation of cellulose to glucose (Tang et al., 2014).

It was evident that pre-incubating soils at 26 °C reduced the C/N ratio to a greater extent than pre-incubation at 5 °C or 15 °C (Figure 5.2). Pre-incubation at 5 °C or 15 °C resulted in greater potential β-glucosidase activity and greater intracellular catalytic enzyme activity. Soil pre-incubated at 26 °C had a higher chitinase activity especially at 37 °C than soil pre incubated at 5 °C or 15 °C, possibly due to a change in soil microbial community composition. Perhaps the microbial community at 26 °C was smaller (slightly lower MBC compared to 15 °C or 5 °C), but more active and it was releasing more chitinase into the soil to acquire the mineralised nitrogen. The differences observed highlight the relative importance of β-glucosidase and intracellular catalytic enzymes to C acquisition and the relative importance of chitinase to N acquisition. This finding is consistent with the report that the activity of N acquiring enzymes (chitinase and leucine amino peptidase) positively correlates with N content and β-glucosidase activity positively correlates with C content in a permanent grassland (Cenini et al., 2016). Similar to our result,(Steinweg et al., 2013a) observed a seasonal shift in enzyme C/N acquisition activity ratio with high C acquisition and low N acquisition in the winter season in a field warming experiment. This result coincided with increased mineralisation relative to N mineralisation, leading to higher microbial C utilisation compared to N transformation during winter. The authors attributed the result to temperature-induced increase in maintenance costs, whereby winter temperature resulted in continuous demand of C substrates without a corresponding need for N. The fact that activities of β-glucosidase and intracellular catalytic enzyme were higher in soils pre-incubated at 5 °C and 15 °C, compared to soils pre-incubated at 26 °C may also imply that microbial communities do not differ with respect to the production of these enzymes or that they are both controlled by the same factors.

We found that pre-incubation of soils at 26 °C resulted in a lower pH compared to pre-incubation at 15 °C or 5 °C. The lower pH due to increasing pre-incubation temperature could result from the production of carbonic acid and protons or nitrification process during decomposition of organic matter, which is mostly favoured by higher temperature and greater microbial activities (Adeli et al., 2005). Sinsabaugh et al., (2008), reported evidence that the potential for enzymes to hydrolyse labile or oxidising recalcitrant components of SOM is controlled by soil pH, alongside a suite of other factors. Their results showed that chitinase activity negatively correlates with soil pH. Although our method of assaying extracellular enzymes used a buffered pH (at pH 6), evidence of pH adapted soil enzyme production by the soil microbial community, irrespective of pH buffering, was reported by Puissant et al., (2019). The optimal pH of chitinase activity was reported to be between 5.0 and 5.5 (Rodriguez-

Kabana, R. et al., 1983), similar to the pH of the soil pre-incubated at 26 °C in our study (4.96). The pH optimal for β-glucosidase is within the range of 5.0 – 6.8 (Eivazi and Tabatabai, 1988), similar to pH of the soil pre-incubated at 5 °C. Enzyme-substrate interaction such as inhibition, adsorption, stabilisation and humification due to warming could result in changes in pH optima, and activation energy (Sinsabaugh, 1994). When soil conditions diverge away from the internal cell pH, which should ordinarily be around 6.0, it may reduce MBC and increase maintenance cost (Neina, 2019). This divergence may be responsible for lower intracellular activity and higher Ea and temperature sensitivity of intracellular activity reported in soil pre-incubated at 26 °C compared to those pre-incubated at 5 °C or 15 °C. Respiration in low pH soils is often dominated by fungal respiration rather bacterial respiration since fungi are more adapted to acidic conditions (Neina, 2019). Similar Ea observed between basal respiration, intracellular and chitinase activities in soil pre-incubated at 26 °C could mean that microbes secreting chitinase enzymes contributed more to the depolymerisation of polymers resulting into the subsequent intracellular and basal respiration. We found higher activities of intracellular and β-glucosidase enzymes in soils pre-incubated at lower temperatures with correspondingly higher pH. We then infer that the lower pH observed in soils pre-incubated at 26 °C favoured chitinase production to a greater extent than soils pre-incubated at lower temperatures where less soil organic matter degradation occurred during the incubation.

Our results advance our understanding of SOM decomposition under future global warming conditions. We have demonstrated that, extracellular enzymes (especially β-glucosidase) are more temperature sensitive than intracellular catalytic enzymes in warm soils (26 °C to 37 °C) This finding implies that the rate of extracellular depolymerase activities will increase with warming more than the rate of intracellular catalytic enzymes. It also suggests that excess release of dissolved organic matter (monomers) could cause CO_2 release with warming in the absence of enzyme producing microbes. This phenomenon is referred to as soil respiration through the 'Exomet pathway' where intracellular oxidative enzymes bypass the normal processes of assimilation and respiration and directly oxidise SOM (Maire et al., 2013; Birge et al., 2015; Bore et al., 2017). The sensitivity of extracellular enzymes to higher temperature is also consistent with the recent evidence that extracellular enzymes (especially from thermophilic microorganisms) at high temperature are now accumulating in soil, and this has implications for understanding soil functioning in order to better predict feedbacks to future warming (Gonzalez et al., 2015; Gómez et al., 2020). The extracellular enzymes assessed in our study showed increasing thermophilic nature within the MMRT framework whereby

enzymes approach Arrhenius behaviour at near zero $\Delta C_p^{\ddagger}$ (Arcus et al., 2016). This is a situation where enzyme reactions conform only to Arrhenius rate predictions, showing exponential increase to increasing temperature.

We observed temperature-induced shifts in pH and C/N ratio following pre-incubation at different temperatures, which resulted in changes in Ea and C to N acquisition potential between warm and cold adapted enzymes. We focused only on enzyme potential, but what will happen soil organic matter in field soils exposed to warming conditions will depend on substrate availability as well as intrinsic catalytic properties of the enzymes and possible interactions between the microbial biomass and other members of the soil food web at higher trophic levels. We acknowledge that relationships may be different from one biome or climatic region to another, and from one soil type to another. We recognise that substrate availability in field soils will be influenced by several factors including temperature and its impact on soil moisture, which may influence plant production, carbon allocation and quality.

5.6 Conclusion

We show that the rates of different steps involved in SOM decomposition are not equally sensitive to changes in temperature and that individual extracellular enzymes each have a different temperature sensitivity. The previous temperature experienced by the soil microbial community can influence the TSmax, Topt, Ea and Q10 of the intracellular and extracellular enzymes involved in SOM metabolism and the reasons for this apparent thermal adaptation is most likely temperature-driven changes in C/N ratio (stoichiometry) of soil organic matter and soil chemistry (pH). Measurements of CO_2 alone as a response variable while studying the effect of warming may obscure our understanding of the temperature sensitivity of the various stages of organic matter decomposition. Soil properties that may be affected by previous temperature or soil condition should be considered in future studies and during model development.

Chapter 6

The use of Open Top Chambers to experimentally warm soils over winter on a field-plot experiment**.**

6.1 Abstract

The soil organic matter of arable soils may be increased by the incorporation of cover crop residues and provide benefits to the proceeding cash crop. Cover crops can be grown in species mixtures and thus may also enhance functional diversity of soils and increase the resilience of the soil microbial community. However, soils in the UK are expected to be warmer during the winter due to climate change, and this may reduce the resilience of the microbial community. This study aimed to examine the resilience of soil microbial community function, focusing on the interaction between cover crops and exposure to winter warming. In the winter of 2019/2020 we introduced Open Top Chambers (OTCs) to warm the soil surface of a field plot experiment in which cover crops (single species monocultures and 4-species polycultures) were grown over the summer in between autumn sown cash crops in a cereal rotation. The aim of this experiment was to assess the legacy effects of winter warming and cover crop incorporation on soil microbial community composition, soil properties and the resilience and resistance of soil respiration to drying and rewetting cycles. The soil temperature under the OTCs were then monitored to ascertain warming effects. However, the sudden incidence of the COVID-19 pandemic prevented the subsequent soil sampling and laboratory analysis. Therefore, only the temperature data collected under the OTCs, and on adjacent ambient plots, are presented in this chapter. We observed that the use of OTCs to invoke warming is effective at increasing soil temperature above the ambient condition. OTCs warmed soils by up to a maximum of 2.31 °C in a control plot, compared to 1.72 °C in a plot with previous history of cover crop mixture incorporation. However, cloudiness and soil saturation during the winter reduced the effectiveness of OTCs. The observed inconsistency or ineffectiveness is because OTCs are a passive method that rely on solar radiation. An enhanced OTC coupled with a heating cable (OTC-Cable systems) may help mitigate this problem. We recommend that this experiment be repeated/continued in the future to elucidate the mechanisms by which winter warming and cover crops interact to impact on the resistance and resilience of soil microbial community function.

6.2 Introduction

The earth is warming at an increasing rate and warming of up to 6.4 °C is expected during the 21st century if mitigation methods are not in place (Carey et al. 2018). Warming, which is often assessed through soil experimental warming, could increase soil respiration and increase CO_2 flux from soils to the atmosphere, thereby yielding a positive feedbacks (Rustad et al. 2000; Bardgett et al. 2008; Dutta and Dutta 2016). Soil warming experiments have the potential to show the impact of climate warming on soil respiration as they can be performed both in the laboratory and in the field. However, field warming, which have been used to elevate soil temperatures by 0.3 to 6 °C in various ecosystems, have consistently shown increases in soil respiration (Rustad et al. 2001; Kuffner et al. 2012). Active and passive warming methods have been used effectively to invoke warming of ecosystems in experimental treatments, but the use of passive techniques are known to be less expensive to construct and maintain (Aronson & McNulty, 2009). Open Top Chambers (OTCs) are one of the most commonly used passive warming methods and have been demonstrated to be effective in many terrestrial biomes (Carey et al. 2018).

It is generally expected that the soil microbial response to warming can be altered during winter, especially as a result of reduced inputs of plant C residue, soil freezing and reduced microbial biomass; thus influencing microbial substrate availability and demands (Bell et al., 2010) and lowering soil microbial activity. Winter warming has been projected to outpace summer warming by 2ºC (Kreyling et al., 2019) and this can have serious ecological impact that is not often studied. A rise in winter temperatures of between 0.5 and 2.0 have resulted in increases in soil pH and available phosphorus, but lower phosphatase, catalase, and urease activities (Guoju et al., 2012). Kreyling et al., (2019) showed that winter warming of soil by up to 1.7 ºC from October to March increased winter ecological processes, including plant performance, soil respiration and soil biological and chemical properties. Understanding the response of the soil microbial community to winter warming may enhance our ability to predict the impact of climate change on soil respiration (Kreyling 2010; Kreyling et al., 2019) and ecological processes generally.

One important strategy introduced to manage soil in arable systems is to incorporate agro-ecological service crops such as cover crops into crop rotations to enhance soil quality, encourage soil biodiversity, and reduce CO_2 emissions (Papp et al., 2018; Radicetti et al., 2019). Such cover crops are planted as a subsidiary crop to a cash crop to enhance the overall

conditions of the soil. For instance, they can help enhance soil microbial biomass through their root exudates that release a uniform supply of organic carbon, a major source of energy to soil microbes (Gyssels et al. 2005; Paterson et al. 2007; Calderón et al., 2016; Papp et al., 2018). The effect of the supply of carbon from cover crop root exudates on the soil microbial community functions before crop termination can be greater than the decomposing roots and crop residues after crop termination, and this legacy effect can influence the subsequent cash crop (Calderón et al., 2016; Papp et al., 2018). Growing mixtures of cover crops, rather than the single species monocultures, can diversify the release of exudates through roots and thereby potentially increase the diversity of rhizosphere microbial community. The growing of cover crop mixtures is now commonly practiced. However, when their above ground biomass in incorporated and their roots decompose they also promote nutrient cycling; thus enhancing the soil fertility and crop growth.

Measuring soil microbial activities, as affected by farm management practices, is a means of assessing the ecological stability of the system (Tang et al., 2014) in form of resistance or resilience. Warming-induced changes may alter the ecological stability of soils in managed agricultural system under predicted climate change. While there could be short term effects of temperature on heterotrophic soil respiration, we expect thermal adaptation of microbial respiration and fast depletion of soil organic matter to result from long term warming (Bradford et al., 2008). It has been noted that winter warming may have a similar or greater magnitude than summer warming and that changes in soil temperature can impact soil enzyme activities (Fraser et al., 2013). It is well agreed that soil enzymes catalyse the rate of organic matter degradation thus influence soil respiration. Also, the magnitude of temperature effects on heterotrophic soil respiration is known to relate more with metabolically active soil microbes rather than abundance of different microbial groups (Salazar et al., 2019a). Shu et al., (2019) reported that maize residue incorporation increased the resistance and resilience of soil microbial processes to heat and metal stresses due to its effects in increasing microbial biomass, and extractable carbon and nitrogen. However, we do not yet know how winter warming and cover crops (grown and incorporated) will affect resilience and resistance of soil respiration to drying and rewetting cycles.

In this study we introduced Open Top Chambers (OTCs) over the 2019/20 winter to warm the soil surface of an ongoing (third year) field plot experiment in which cover crops (single species monocultures and 4-species polycultures) are grown over the summer in between autumn sown cash crops in a cereal rotation. We intended on collecting soils samples in spring 2020 to assess

the resistance and resilience of the soil microbial community in warmed and un-warmed plots where cover crops had been grown in monoculture, polyculture, or absent. However, due to the COVID-19 pandemic, soil sampling and laboratory analysis was not possible. Our original aim and hypotheses were as follows:

Aim: To assess the legacy effects of winter warming using OTC and cover crop incorporation on microbial community composition, soil properties, and resilience and resistance of soil respiration to drying and rewetting cycles.

Hypotheses:

- **Warmed plots are less resilient than ambient (un-warmed) plots**, since there is the likelihood of greater DOC loss in warmed plots, compared with ambient soils, due to increased soil microbial activity. Winter warming may also reduce soil moisture in the warmed plots, as observed by Compant et al. (2010).

- **Plots where cover crops residues were incorporated are more resilient than control plots** because organic matter incorporation has the capacity to conserve soil moisture, thereby enhancing the resilience of the soil microbial community to drying and rewetting (Yang et al. 2018; Kong et al. 2019).

- **The soil microbial communities in cover crop polyculture plots are more resilient than single species monoculture plots** since it is expected that a more diverse plant residues will provide more diverse carbon substrates that enhance the functional diversity of the soil microbial community.

- **There is a significant interaction between cover crops and warming treatments on the resilience of the soil microbial community.** We expect a synergistic effect whereby microbes adapt to a more favourable condition due to available substrate and warmed soil during the winter season.

- **Resilience of soil microbial functions relate to the size and composition of the soil microbial community and key soil properties.** We expect that soil respiration and soil microbial community composition and function will change under the different cover crop and warming treatments. We think that these differences will be a result of changes in soil properties under each treatment combination. This is because a correlation exists between soil respiration following carbon addition or a wetting and drying stress and specific soil biological and chemical properties (Fraser et al. 2016;

Todman et al. 2018)**.** We expect that, such relationships will enable us to identify what combination of treatments influences the resilience of the soil microbial community respiration.

6.3 Materials and Methods

6.3.1 Experimental design:

The field experiment is located on Broadmoor field of Sonning Farm, in Berkshire, UK. The plots were on their third crop rotation schedule involving autumn sown cereals (cash crop) and summer grown cover crops. The cereal annual rotation includes; **1.** 2017/18 Winter Wheat; **2.** 2018/19 Winter Barley; **3.** 2019/20 Winter Oats; **4**. 2020/21 Winter Wheat. The cover crops used are: Buckwheat, Clover, Oil Radish, Sunflower or a Quaternary mixture of these four. The experiment was a two factorial experiment comprising of two warming levels (i.e. Warmed and Ambient); 6 Cover crop treatments (i.e. Quaternary mixture, Buckwheat, Clover, Oil Radish, Sunflower and No residue), and four replicates arranged in a randomized block design (circled positions shown in Figure 6.1). The field plot experiment also included treatments where the aboveground residues were removed from some plots and added to others but these plots were not included in this study. The aim was to concentrate on the plots where the cover crop was grown and the residue incorporated into the soil to have the dual benefit of root exudates, root residues, and shoot residues. The selected plots involving these treatments (i.e. mulched plots) are the circled positions shown in Figure 6.1.

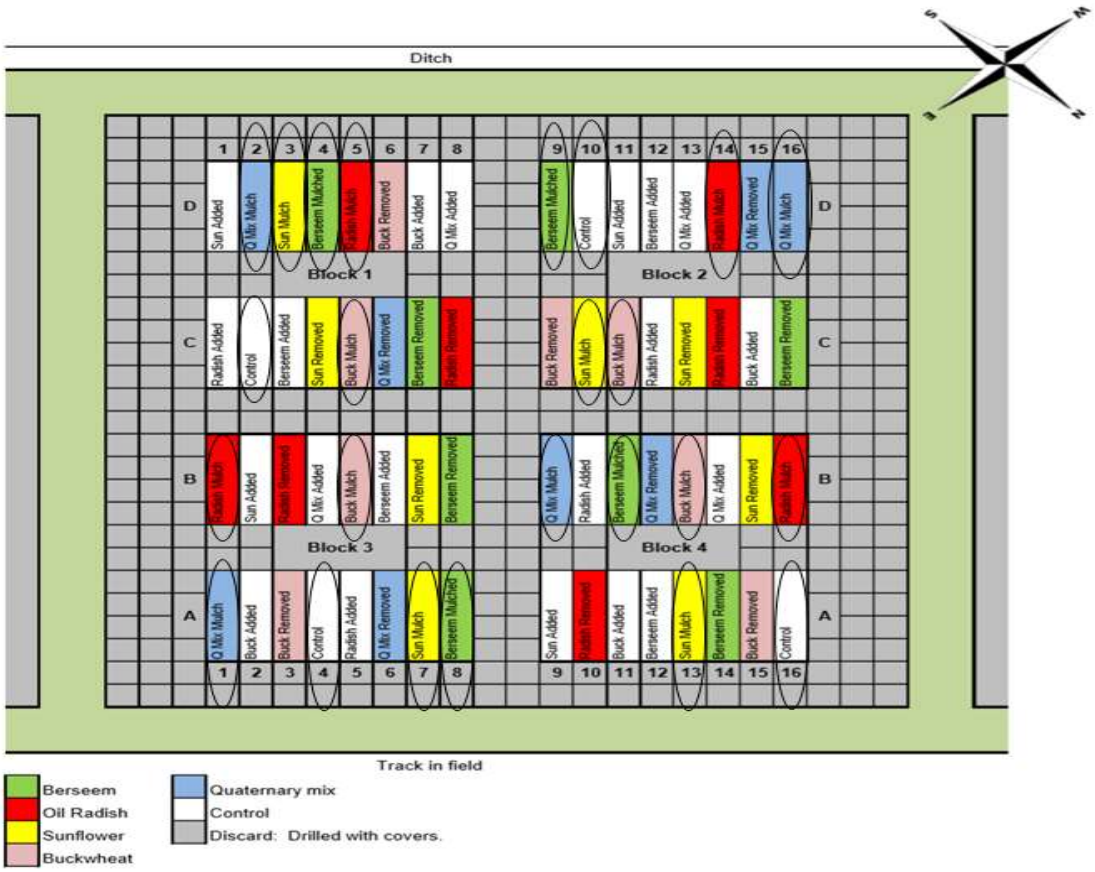

Figure 6.1 Experimental design of the field experiment located at Sonning Farm, in Berkshire, UK. Circled plots indicate those upon which OTCs were placed on the southwest end of the plot.

6.3.2 Soil Warming:

Open Top warming Chambers (See Figures 6.2 and 6.3) were installed after crop establishment on 18th December 2019 to create warm and ambient areas of plots in a split-plot design. The chambers were installed at oat seedling stage when about 5 leaves unfolded. Each chamber was placed in a 2 m area on the southwest end of each plot to allow for yield assessment within rest of the plot. The OTC chamber was a six sided hexagon made from a clear extruded Perspex acrylic plates with the following dimensions: 5 mm thickness with a 100 cm base, 57.74 cm top, 62 cm side cut at an angle 71.16° and each side is 50 cm high. Our design for the OTC chamber conforms to the characteristics of the International Tundra Experiment (ITEX), with similar shape and reinforcement as the hexagon chamber described by Marion et al., (1997). Clear Perspex acrylic sheets can transmit light in excess of 92 % of visible light and have higher light transmission capacity than glass. We monitored the temperature within the top 10 cm of soil by installing a temperature probe coupled with a data logger using Plus 2 Tinytag loggers

(Gemini data loggers, UK) in both the ambient and warmed areas of a Control plot (A4; Figure 6.1) and Quaternary mixture cover crop plot (A1; Figure 6.1). The loggers were set to log temperature every 15 minutes and we collected data from December to late March. There were disruptions in the data collected between 25th January and 20th March due to animals chewing through the cables of the data loggers, wind storms, and periodic flooding of some plots. The data from 18th Dec 2019 and 24th January, 2020 (Period 1) and from 20th March to 30th March, 2020 (Period 2), for which we obtained uninterrupted measurements for all four temperature probes, are presented here. We calculated the temperature differences (delta T) between warmed and ambient areas of each plot during the two monitoring periods. We also present, alongside the temperature data, the daily solar radiation and rainfall data collected from a nearby meteorological station (about 500 m away from the plot) established by the Crops Research Unit of the University of Reading.

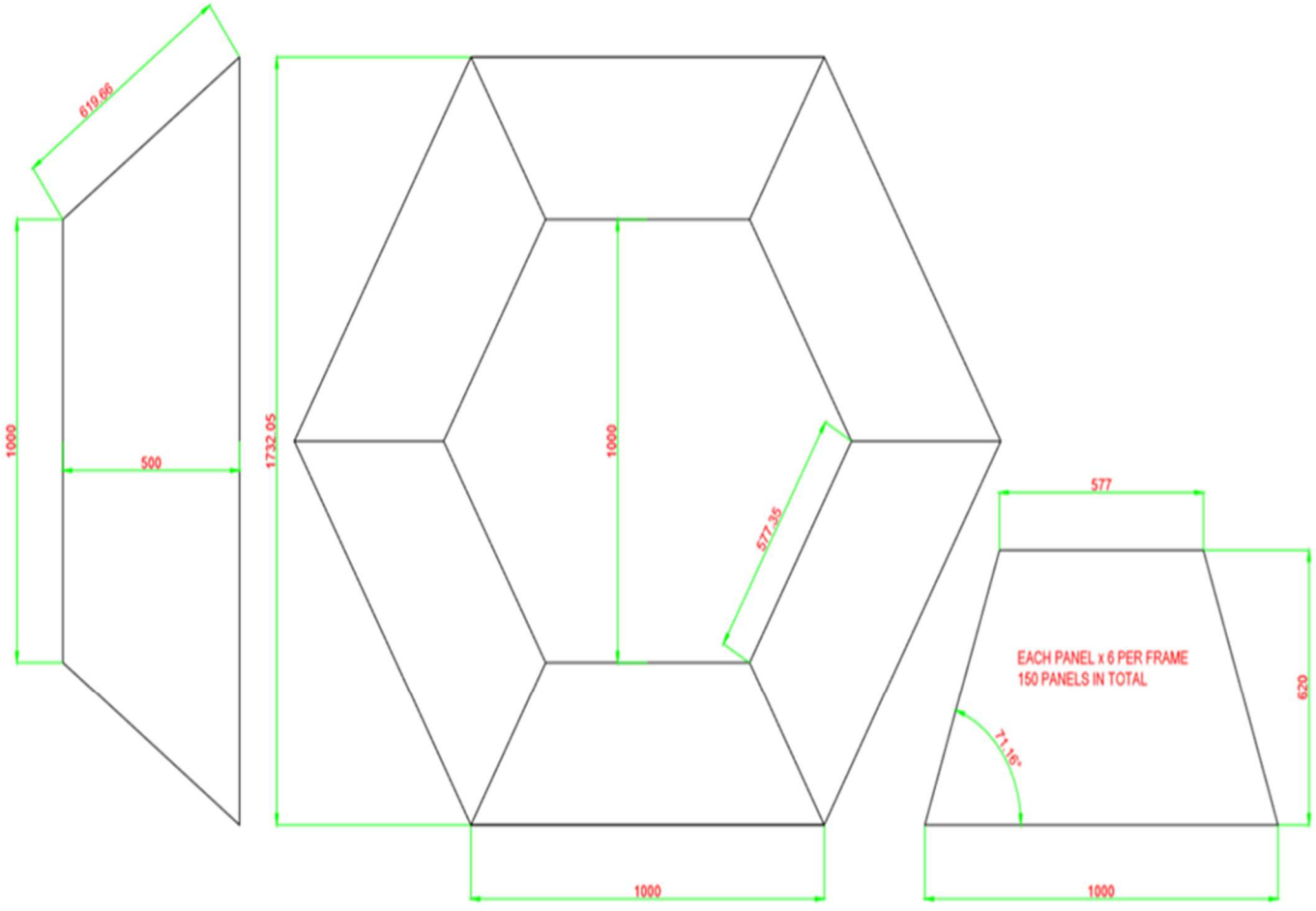

Figure 6.2: The design of the OTC warming chambers deployed to warming plots (unit of measurement on the drawing is millimetre (mm). Credit for drawing: Mike Charij.

Figure 6.3: Image showing the installed chambers (right pane) and temperature monitoring session (left pane)

6.3.4 Intended resilience and resistance assay: Dry/rewetting cycles

We intended to sample soils from underneath the OTCs, and ambient soil adjacent to OTCs on each of the plots circled in Figure 6.1. The soil samples collected would have been sieved to 4 mm and divided into three subsamples. The first subsample would have been refrigerated at 4 °C for soil biochemical analysis. The second subsample would have been freeze-dried for downstream analysis of the soil microbial community by phospholipid fatty acid analysis. The third subsample would have been used to assess the resistance and resilience of the soil microbial community. First, the water holding capacity of soil from each sample would have been determined. Then, a pre-incubation carried out at 26 °C prior to the resistance and resilience assay using a method modified from Todman et al. (2018) and Fraser et al. (2016), which involves repeated cycles of drying and rewetting the pre-incubated soil samples.

To undertake the resistance and resilience assay, six subsamples (10 g) of fresh soil would have been weighed out. To one subsample (0 cycles of drying and rewetting), 100 mg of Barley grass powder would have been added and mixed. Following mixing, CO_2 evolution would be measured consistently for five days at various time interval using an automated multichannel respirometer. Samples that had not received substrate would then be exposed to 1, 2, 4, and 8 drying and rewetting cycles; where each cycle consists of 3 days drying (enclosed in a sealed chamber with silica gel desiccant) followed by rewetting to 45 % WHC for a further 4 days

following (Todman et al., 2018). Thereafter, substrate will be added after each cycle of drying and rewetting and CO_2 analysis would have been undertaken as in cycle 0.

Had the resistance and resilience assay gone ahead, a descriptive model would have been fitted to the respiratory response from each of the soil samples as described by Todman et al. (2018) and Fraser et al. (2016). Respiratory type would have been identified using a cluster analysis, and the respiratory type then grouped into resilience types (resilient or not). Naïve, Bayesian belief will be used to relate resilience measures with soil characteristics and management types similar to the methods in (Fraser et al., 2016). Naïve Bayesian belief network shows variables that influence response of interest graphically and describe the relationships that exist between explanatory variables (e.g. management types and soil properties) and responses that they influence as a set of conditional probability (Taalab et al., 2015; Fraser et al., 2016).

6.4 Results.

The temperature data collected was divided into two periods. The soil temperature for Period 1 (between 18th December and 25th January) and Period 2 (between 14th March and 30th March), weather data, and differences in soil temperature between OTC and ambient temperatures (delta T) from the cover crop mixture plot and control plot are presented in Figures 6.4, 6.5, 6.6 and 6.7, respectively. We observed that, during Period 1, soil temperature under the cover crop mixture was higher under ambient conditions compared to soils warmed by the OTC, especially when the solar radiation was below 4 MJ m^{-1} (Figure 6.4 and 6.6). When solar radiation increased to around 5 MJ m^{-1} (i.e. between 19th and 21st December), and there was little or no rainfall, we observed that soil in the cover crop mixture plot under the OTC had a higher temperature compared to the soil under ambient conditions. However, soils from the control plot a had consistently higher temperature under the OTC compared to the ambient conditions, except when the soil was saturated (rainfall between 6 and 12 mm or 18 mm) between 19 and 22nd December 2019, and between 15th and 17th January. The magnitude of warming by the OTCs was higher in control plot than the cover crop mixture plot during Period 1 where soil was 1.72 °C and 2.31 °C warmer within the OTC compared to the ambient condition (Figures 6.6 and 6.7). Also, soil was 4.40 °C and 1.78 °C cooler underneath the OTC in cover crop mixture plot and control plot, respectively compared to the ambient conditions across all times and days during period 1. The cooling was recorded on 19th December, 2019.

During Period 2 the OTC consistently warmed soil, compared to the ambient conditions, in both the cover crop mixture plot and the control plot. However, the magnitude of warming was

higher in the control plot compared to the cover crop mixture plot (Figure 6.5). The only exception was the slight cooling observed under the OTC of the cover crop mixture plot on 20th March. Soil was, on average, 1.62 °C and 1.77 °C warmer underneath the OTC across all times and days of Period 2 compared to the ambient condition in cover crop mixture plot and control plot, respectively.

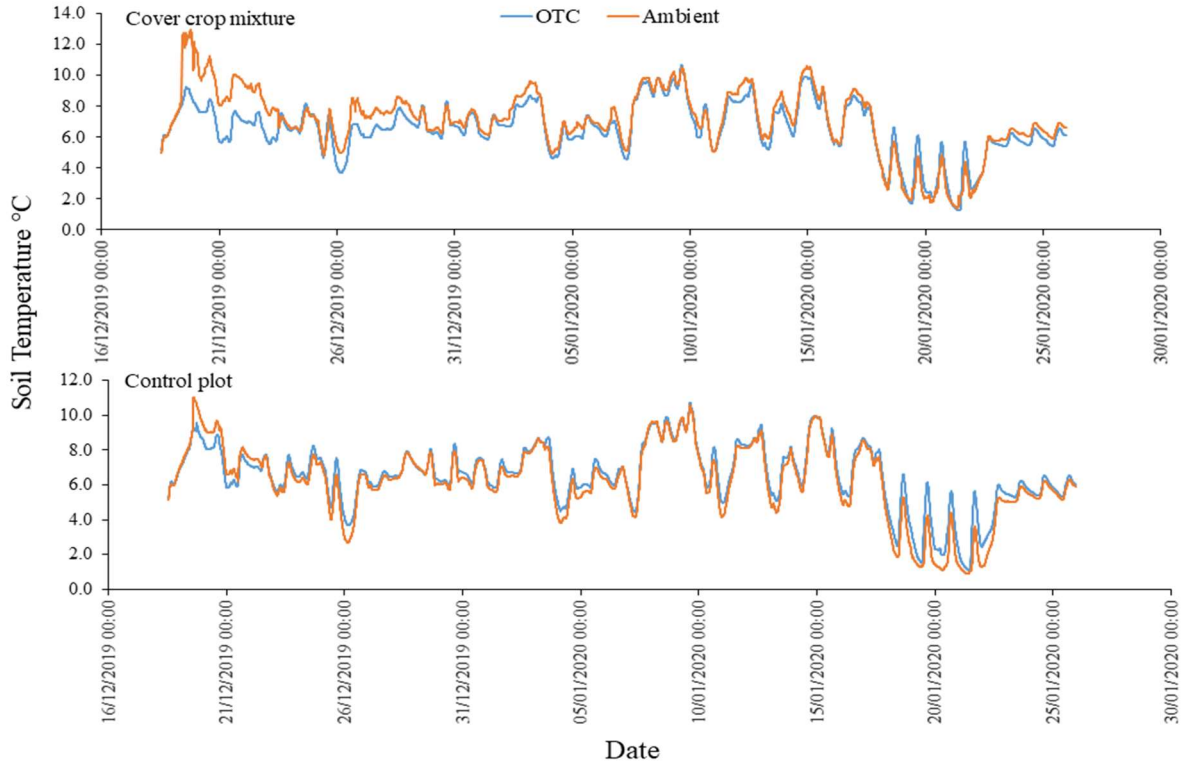

Figure 6.4: Warmed (OTC) and ambient soil temperature over time during Period 1 between 18th December 2019 and 25th January 2020 in a cover crop mixture plot (A1) and a control plot (A4).

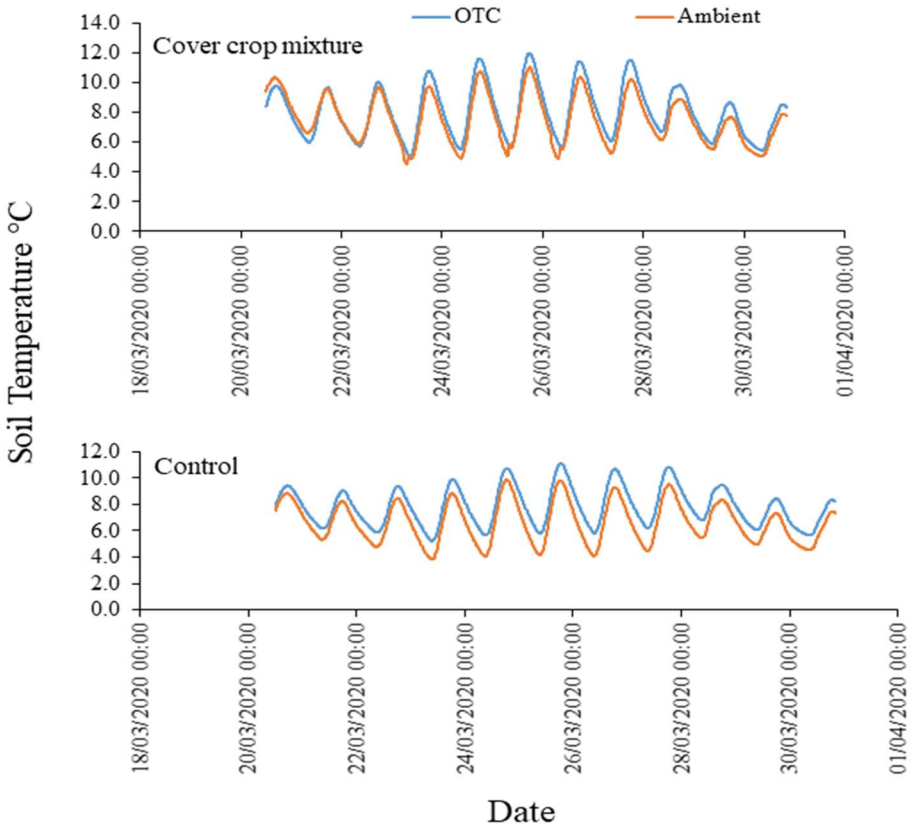

Figure 6.5: Warmed (OTC) and ambient soil temperature over time during Period 2 between 14th March and 30th March in a cover crop mixture plot (A1) and a control plot (A4).

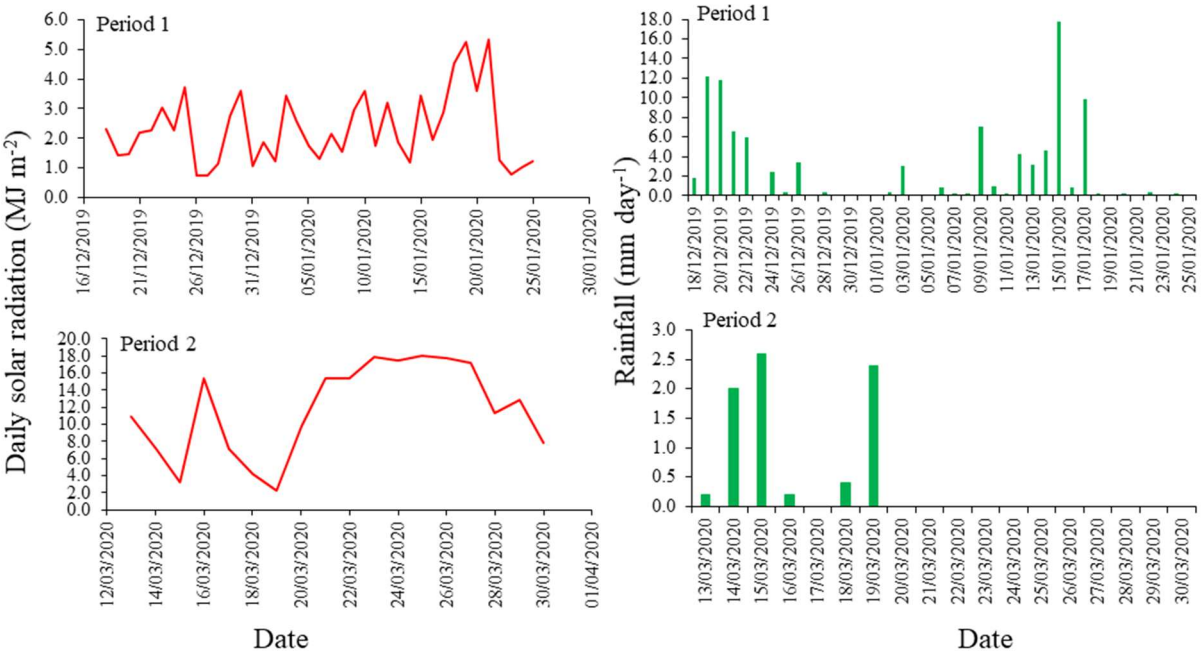

Figure 6.6: Daily solar radiation and rainfall at Sonning farm during Period 1 (18th December 2019 to 25th January 2020) and Period 2 (14th to 30th March).

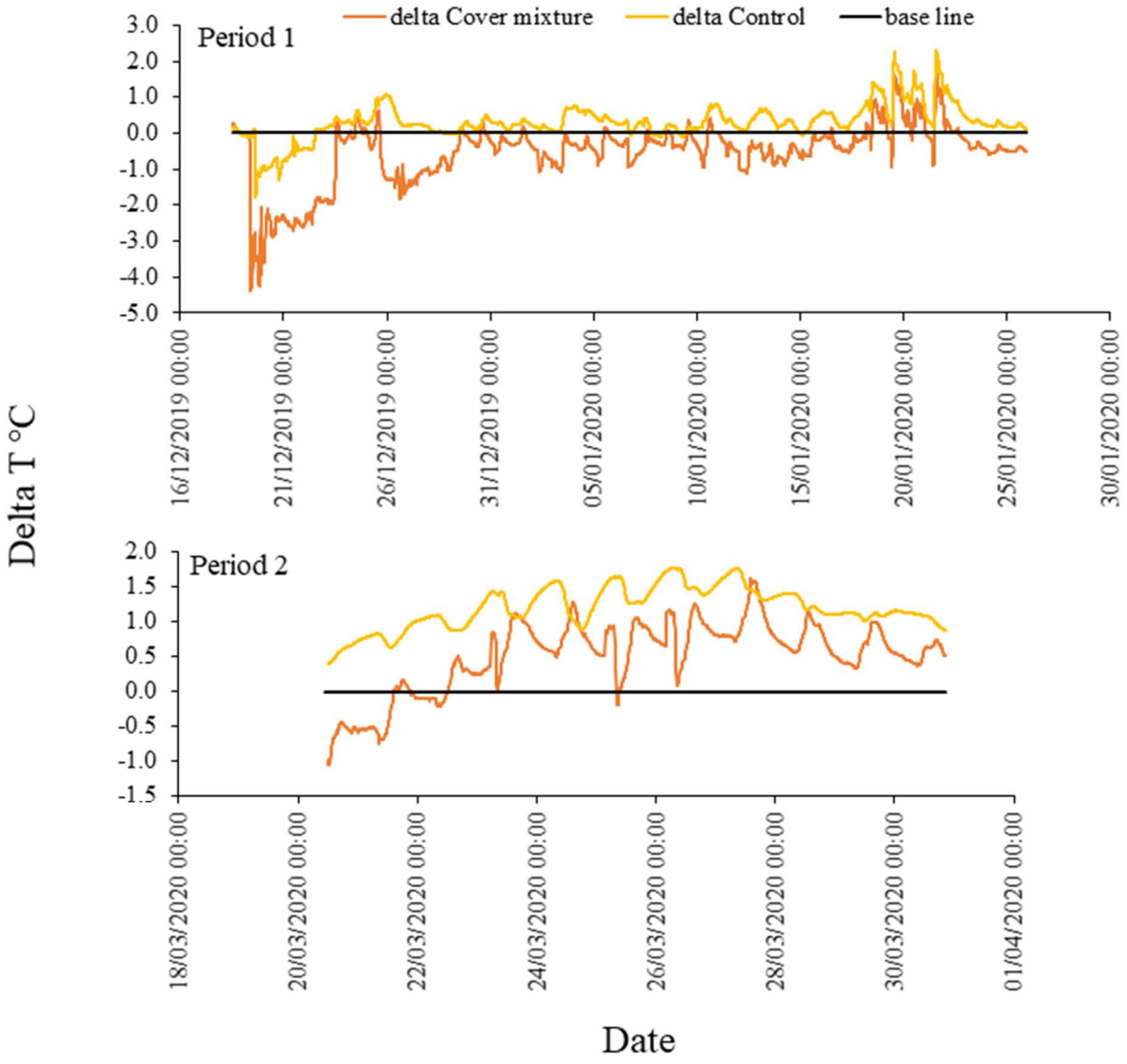

Figure 6.7: Difference in soil temperature (Delta T) between soil underneath the OTC, compared to the ambient temperature of the same plot, between 18th December 2019 and 25th January 2020 (Period 1) and 14 and 31st March (Period 2) in plots previously planted with a cover crop mixture (A1) and a control plot (A4). When the Delta T is above the black line, this indicates the OTC warms the soil and when the Delta T is below the black line this indicates that the OTC cools the soil.

6.5 Discussion

Passive warming systems like OTCs have recorded great success in field warming experiments, in both terrestrial and aquatic conditions (Carey et al., 2018), and our results further support their efficacy. Use of OTCs indeed increased soil temperature compared ambient conditions. We reported, on average, that OTCs warmed the soil by 2.31 °C (A4) in a plot where no cover

crop was planted within the last 3 years and 1.72 °C (A1) in a plot where soil was planted with a quaternary mixture of cover crops and the residues incorporated prior to drilling winter oats. The warming achieved here was well above the < 1 °C reported in the Antarctic during three years of warming using a similar method (Rinnan et al., 2009b). However, due to excess rainfall leading to soil saturation (Appendix 3) and cloudy days with low solar radiation (Figure 6.6), there were instances where OTCs resulted in soil cooling rather than warming. Such an effect is not uncommon when using OTC chambers to warm the soil since it is a passive warming method (Sun et al., 2013; Carey et al., 2018). The cooling observed underneath the OTC on 19th December corresponded with high rainfall (12 mm) and low solar radiation. In contrast, the soil warming observed under the OTC on 21st January 2020 occurred on a day with no rainfall and higher solar radiation than 19th December 2019 (Figure 6.6 and 6.7). We also observed fewer rainy days and generally higher solar radiation in March 2020 than December/January 2019/20 (Figure 6.6) and attribute the more consistent warming by the OTC in March compared to December/January period to this.

Our results are similar to OTC warming patterns reported in other studies (Marion et al., 1997; Pold and DeAngelis, 2013). Our observations that low solar radiation reduced the effectiveness of the OTC at warming soils, which could be because OTC is a passive technique that depends on natural heat/light to invoke warming. In this case it is likely that the OTCs accelerated evaporation which led to drier soils underneath OTCs, compared to the ambient conditions. Drier soils are less able to retain heat and are thus cooler, particularly overnight. The observation that wetter soil resulted in cooling rather than warming also suggests that climate variables interact to influence soil temperature. It has been reported that climatic conditions including high solar radiation, low cloud cover, lack of vegetation, and dry soil are characteristics of environments where passive OTC warming can be more effective (Marion et al., 1997; Carey et al., 2018).

Differences in warming by OTCs were observed between the control and the cover crop mixture plots. The OTCs showed greater warming effectiveness where there were no cover crops. Cover crop residues (roots and shoots) incorporated into the soil may have helped to build the soil organic matter and help the soil hold on to more water, as reported in other studies (Rawls et al., 2003; Yang et al., 2014). It has been reported that organic residues applied as a mulch resulted in cooling and also kept the soil wet and cold longer than bare soil (Horton et al., 1996). Also, Hatfield and Prueger, (1996), noted that soil profiles covered with organic residue showed decreasing soil water evaporation and increasing water storage and this can

result in the reduction of summer time soil drying at the seed zone due to a reduction in soil temperature. We therefore conclude that cover crop residue incorporation alters the temperature regime underneath OTCs, thus reduces the warming effect. Of course, we should acknowledge that we only have one replicate and cannot statistically demonstrate an impact of cover crops on the effectiveness of OTCs at soil warming.

Use of OTC-cable where a standard open-top chamber (OTC) is coupled with a heating cable can be an upgraded methodology to remove the effect due to wet and cloudy days since it combines both the passive and active warming method (Sun et al., 2013). Sun et al., (2013) reported effective warming, and a decrease in soil moisture content compared to the ambient condition using this method. Also, the fact that temperature can be adjusted to a desired level is a benefit of this enhanced OTC system.

6.6 Conclusion

Use of OTCs to invoke warming is effective, on average, at increasing soil temperature above the ambient condition. However, clouds and soil saturation during the season could reduce their effectiveness. This is because OTCs are a passive method that depend on solar radiation. An enhanced open-top chamber (OTC) coupled with a heating cable (OTC-Cable systems) can help alleviate this problem. Addition of soil organic matter through cover crop residue incorporation may have reduced the magnitude of the soil warming by increasing the soil water holding capacity. We recommend that this experiment be repeated in the future to elucidate the mechanisms by which cover crops and winter warming will interact and impact the resilience of soil community functions to climate extremes.

Chapter 7

General Discussion and Conclusions

7.1 General discussion of results

Insufficient knowledge about the temperature sensitivity of soil microbial processes is a major source of uncertainties in understanding the magnitude and direction of global carbon cycle feedbacks under climate change (Allison et al., 2018). To further explore the mechanisms by which soil carbon is lost into the atmosphere, or the feedbacks under climate change scenarios, 3 laboratory and 1 field experiment were conducted. The major findings are discussed below.

7.1.1 Effect of soil sieving disturbance on basal respiration of soils from different land uses

In Chapter 3 we found that, regardless of the land use type, sieving (4 mm sieve) disturbance did not significantly influence soil basal respiration. We concluded that soil sieved to 4 mm at field moisture content can be a suitable methodology while performing laboratory measurements of *ex situ* soil respiration. This result helps to resolve the problem of heterogeneity between intact soil cores that leads to the requirement for large sample sizes or large replicate numbers while using undisturbed soil cores, as highlighted by Oertel et al., (2016). Also, using soil sampled at field moisture and larger sieve sizes (i.e. 4 mm) helps to obtain the needed homogenized sample and prevents the need to air dry soil prior to sieving, while soil micro aggregates (bearing an important fraction of C) are preserved.

We found that soils collected from grassland soil released more CO_2 compared to those collected from woodland and arable soils, irrespective of sieving treatments. This finding strengthens the evidence in other studies that changes in land use are one of the major factors controlling the release of CO_2 into the atmosphere from soil. Grassland soils (as well as arable soils) being the largest type of land use globally (covering 31.5 % land surface area) could offer an opportunity for climate change mitigation if properly managed (Oertel et al., 2016), though grassland soils were ranked third (after wetlands and woodlands) in term of their ability to emit CO_2 into the atmosphere.

Although we know that land use change can alter soil temperature due to changes in vegetation cover (Lal, 2020), soils from different land uses also differ in their physicochemical properties

and microbial community composition (Shi et al., 2020). Higher soil respiration reported from grassland soil was attributed to more favourable levels of water holding capacity and quantity and stoichiometry of organic matter, compared to the woodland and arable soils.

7.1.2 Legacy effect of constant and diurnal oscillating temperature on soil respiration

In Chapter 4 we used the same grassland soil used in Chapter 3 and incubated this under several different temperature regimes, after sieving field moist soil to 4 mm.

The observation that soil constantly exposed to 15 °C, which was the average maximum daily temperature at the site from which the soil was collected, released similar soil CO_2 flux as soil diurnally oscillated between 5 °C and 15 °C (i.e. between daily minimum and maximum), but more than 10 °C (daily average) or 5 °C (daily minimum), may point to the fact that soil microbial activity is greatest at the daily maximum temperature of 15 °C. This observation may be because the daily maximum temperature is close to the optimum temperature condition for the activity of heterotrophic soil microbes in the grassland soil. This finding could mean that the soil microbial community responsible for the process may have acclimatised to the daily maximum temperature, thereby saving the cost of adaptation to fluctuating temperature. This observation opposes the assumption that microbial communities thermally adapt based on their average daily temperature regime to minimise adaptation cost, as reported by Uvarov et al., (2006).

We also observed that incubation at higher or oscillating temperatures caused a corresponding shift in soil microbial community composition and this resulted in depletion of readily available organic carbon and increased the mineralisation of inorganic N. We know that a positive relationship exists between soil respiration and changes to the bacterial and fungal abundances (Chen et al., 2015) and such a relationship was attributed to substrate quality and availability and other soil properties (Luo et al., 2001; Chen et al., 2015). A fungal dominated community and higher extractable carbon in soils incubated at lower temperatures was observed in our study. Fungal dominated communities have been linked with greater carbon storage potential at lower temperature regimes or cold regions (Whitaker et al., 2014; Malik et al., 2016; Crowther et al., 2019). Our result also revealed evidence of physiological adaptation to temperature stress in soils incubated at 15 °C and diurnal oscillating temperature between 5 and 15 °C, but the microbial community under temperature oscillation was stressed less than those maintained constantly at 15 °C. Soil microbial adaptation to higher temperature has been reported earlier (Bradford et al., 2019; Dacal et al., 2019). Evolutionary trade-offs in the

structure and function of enzymes under various temperature regimes could be an alternative explanation to such adaptation (Dacal et al., 2019).

The abovementioned shift in microbial community composition, depletion of available and easily degradable carbon, and the consequent reduction in soil respiration observed in soils incubated at 15 °C or diurnally oscillating between 5 and 15 °C in our study is evidence of soil respiratory acclimatisation, as described by Luo et al., (2001). This observation implies that, as temperature oscillates diurnally between the daily minimum and maximum temperatures, soil microbial community function close to their critical maximum temperature. Such effect of diurnal temperature effect have been demonstrated in tropical ectotherms (Zeh et al., 2014). However, respiratory acclimatisation, which is often stronger at higher temperature, can weaken the positive feedback into the atmosphere (Luo et al., 2001; Blagodatskaya et al., 2016). The temperature at which full acclimatisation occurs differs between autotrophic and heterotrophic respiration in Luo et al., (2001), with heterotrophic respiration attaining full acclimatisation at higher temperature (about 20.8 °C) than the autotrophic respiration (13.9 °C). The temperature at full acclimation reported by Luo et al., (2001) was higher than those used in this study, suggesting full acclimatisation may not have being reached in our study. Also, our laboratory conditions help quantify heterotrophic respiration without confounding factors common to field measurements, as experienced by Luo et al., (2001).

It is expected that climate change will lead to a substantial shift in temperature extremes and maximum daily temperature may exceed the critical thermal optima during summer days (Ma et al., 2015). Predicted increases in the frequency of hot days due to climate change (Ye et al., 2018; Jia et al., 2019) may lead to an overall higher average daily maximum temperature and this may lead to further release of CO_2 into the atmosphere as climate changes as a result of thermal adaptation of the soil microbial community. It is thus imperative to ensure that the next generation of land-surface models adequately simulate the impact of asymmetric warming, including daily temperature extremes, on the activities of extracellular enzymes and soil heterotrophic respiration.

7.1.3 Legacy of previous temperature on consequent enzyme activity and temperature sensitivity.

The idea developed in the discussion of Chapter 4 that the daily maximum temperature may be the most important parameter controlling extracellular depolymerisation, and thus mediating soil respiration was further investigated in the third experimental chapter (Chapter 5) which

considered how a previous soil temperature condition influences the temperature response of two extracellular depolymerase enzymes and intracellular catalytic activity. Soil respiration is a product of organic matter decomposition and it requires the input of extracellular enzymes to depolymerize higher molecular weight cabon polymers before the intracellular metabolism that yields soil microbial respiration (CO_2 evolution). By depleting soil organic matter at various pre-incubation temperatures (5 °C, 15 °C and 26 °C), we were able to create soil microbial communities that were pre-exposed to three different levels of temperature acclimation. These three temperatures represent the average daily minimum and maximmum temperatures used in Chapter 4 and a typical hot day in summer respectively. Our result showed that the previous temperature experienced by the soil microbial community can influence the temperature sensitivity (e.g. TS_{max}, T_{opt}, Ea and Q10) of the intracellular and extracellular enzymes involved in their metabolisms. This observation provides evidence to challenge the assumptions in conventional SOM models that past conditions do not influence future responses of biological systems (Bradford, 2013) The reasons for this apparent thermal adaptation is most likely temperature-driven changes in C/N ratio (stoichiometry) of soil organic matter and soil chemistry (pH). This explanation is consistent with the fact that, climate legacy exerts control on decomposition of organic matter in soil under warming and varying stoichiometry (Li et al., 2013). Li et al., (2013) examined the interaction between substrate stoichiometry and warming-induced patterns of microbial carbon mineralization. Similar to our findings, they showed that soil containing higher C/N substrate enhanced warming-induced soil respiration. Alternatively, interaction between temperature and soil C/N stoichiometry (e.g. higher N availability relative to C) can influence the activity of specific microbial groups and the subsequent production of extracellular enzymes (Li et al., 2013; Blagodatskaya et al., 2016). This interaction could result in variable response of microbial carbon use efficiency (CUE) with warming. Also, enzyme-substrate interactions, such as humification process that change the pH optima of enzymes, could result in changes in temperature sensitivity (through changes in activation energy) and overall enzyme kinetics (Sinsabaugh et al., 1991; Sinsabaugh, 1994). Temperature sensitivity of enzymes depends largely on the activation energy of the enzyme-substrate reactions (Sinsabaugh et al., 1991).

We also demonstrated that rates of extracellular depolymerization and intracellular catalytic activity that yield soil respiration are not equally sensitive to temperature. Intracellular catalytic activity showed higher sensitivity to temperature increases between 15 °C and 26 °C, which may indicate that it may be sensitive to increases in daily maximum temperature during the

winter months or daily mean temperature during the summer months. However, extracellular depolymerization showed greater sensitivity to increases in temperature between 26 °C and 37 °C, indicating that it may be sensitive to increases in daily maximum temperatures and extreme events during the summer. The point of maximum temperature sensitivity (TS_{max}) for soil respiration was modelled (MMRT) for soil respiration and the range was between 13.5 °C and 25.6 °C. The range of temperatures at which the highest temperature sensitivity of extracellular enzymes were predicted to occur are not commonly experienced by the soil microbial community in the study area. The high temperature sensitivity of intracellular catalytic enzymes between 15 °C and 26 °C could be responsible for the observed optimum soil respiration reported at 15 °C or oscillation between 5 and 15 °C in Chapter 4.

7.1.4 Legacy effect of cover crop management and winter warming on soil

In the final data chapter (Chapter 6) an attempt was made to observe the impact of soil warming on the thermal adaptation of the soil microbial community under field conditions. We set out to examine whether the addition of soil organic matter through cover crop residue incorporation mitigates the effect of simulated climate change (winter warming) on the resilience of the soil microbial community to wet and dry cycles. The field warming component revealed that our custom designed Open Top Chambers (OTCs) invoke warming effectively, but inconsistently, above the ambient temperature. Due to the fact that OTCs are a passive technique, we found that wet days and days with low solar radiation (on cloudy days) reduce their effectiveness at increasing the soil temperature. The plot where no organic matter was incorporated (control) within the last three years was generally warmer than the plot where a cover crop mixture was grown and incorporated into the soil. After rainfall events, and when there was low solar radiation, the plot which received cover crop mixture incorporation also become cooler (and probably wetter) under the OTC compared to control plot. A recent study suggested that wet meadow, heath, or organic soil layers could protect soil from losing nitrogen, carbon and moisture during long term warming (Alatalo et al., 2017). We weren't able to provide evidence of such in our current study due to the COVID 19 pandemic preventing us from executing our planned laboratory measurements. However, studies looking at the legacy of winter warming and cover crop residue incorporation on the resilience of soil microbial community are very rare, despite the prediction that winter season warming up to 2 °C is expected in the future. Such a study could help explore the effectiveness of regenerative agricultural practices in mitigating the impact of the predicted climate change.

7.1.5 Evidence for thermal adaptation

Overall, there was evidence of soil microbial adaptations in this study. While it is well evidenced in the literature that soil microorganisms adapt to temperature, the effects of temperature-induced changes to soil chemical and physical properties and the subsequent adaptation of the soil microbial communities to their altered physiochemical environment (i.e. an indirect effect of temperature) was supported by our findings. For instance, we observed that soil previously exposed to higher temperatures had significantly lower pH and altered stoichiometry of C and N, compared to soil exposed to lower temperatures (especially in Chapters 4 and 5). A unit change (either reduction or increase) in environmental pH can inhibit microbial metabolic activity by half (Jin and Kirk, 2018).

Depending on the microbial groups involved, microbially-mediated processes in soil have specific pH optimum above which microbes cease to function (Jin and Kirk, 2018; Neina, 2019), thereby influencing changes in microbial community composition and function. When the internal pH of a microbial cell diverges from the surrounding pH (i.e. soil pH), there will be a reduction in microbial biomass and this will in turn influence the microbial metabolic quotient and raise the maintenance energy requirement (Anderson, 2003; Neina, 2019). Our result in chapter 5 where soil pre-incubated at 26 °C showed a lower pH, slightly lower microbial biomass and higher activation energy compared to those pre-incubated at 5 °C supports these claims. The low pH observed in soils incubated at a higher temperature in this study may be the result of faster SOM decomposition that releases more carbonic gas, liberates more protons, and accelerates the nitrification process (Adeli et al., 2005).

We observed higher extractable N (about 90 % NO_3-N) and lower extractable C in soils pre-incubated at higher temperatures in Chapter 4, consistent with temperature-induced substrate availability. This may be due to temperature-induced changes in C/N ratio, also observed in Chapter 5. Prior research has reported warming-induced decomposition of organic matter, but has also identified warming-induced increases in inorganic N in the soil solution as the reason behind increased plant growth that is well documented due to global warming (Melillo et al., 2011). However, the warming-induced shift in substrate C/N stoichiometry influences both the shift in soil microbial community and temperature sensitivities of both the intracellular and extracellular steps involved in SOM mineralisation to subsequent warming events, as we have found in this study.

7.2 Implications for modelling the impacts of warming on soil respiration

Our findings have great implications for how soil respiration responds to global warming and can help enhance our predictions of how climate change will impact terrestrial carbon losses into the atmosphere. This thesis uncovered warming-induced changes (including indirect effects of temperature) in soil systems that occur due to the influence of both the soil microorganisms and their enzyme functions that have direct implications on how much carbon is released into the atmosphere. This claim is based on the mechanisms of adaptation uncovered in this study. Current projections that use only the size of the soil carbon stock and temperature to predict the response of carbon loss from soil from warming may not accurately represent the actual mechanisms and may have overestimated total C losses (e.g. Crowther et al., 2016). This overestimation is because soil organic matter decomposition, carbon stocks, and persistence are primarily the products of controls that occur locally and there is need for more robust soil biogeochemical models that better represent how historical condition and subsequent disturbances shape response of SOM to warming (Bradford et al., 2021).

Based on this study, we recommend that future projections and soil biogeochemical model parameterization should only adopt use of laboratory data to estimate temperature sensitivity when the laboratory data is collected at temperatures that represent the typical diurnal range within the region where predictions are being made. Also, daily maximum temperature (or optimum temperature for microbial activity) should be used as an input parameter, rather than daily mean temperature, for parameterising soil carbon models. Soil properties such as the C/N ratio that may be affected by a previous temperature regime or soil condition should be considered in future studies and during model development while predicting impact of climate change on soil respiration. We also recommend that identifying and modelling the thresholds of temperatures where each step of SOM mineralization are most sensitive to temperature should be considered in model structure.

7. 3 General Conclusions

To investigate the hypotheses highlighted in Chapter 1 (section 1.5), experiments were conducted and the following conclusions drawn:

1. I present evidence that soil sieving (freshly sieved to 4 mm mesh size) disturnace did not affect the short term basal soil respiration, but land use did. Differences in soil respiration rate were due to differences in the soil water holding capacity and quantity

and stoichiometry of organic matter of the various land uses studied. This evidence did not provide support for my first hypothesis, but did lend support to the understanding that land use changes may influence the impact of climate change on CO_2 emission.

2. I demonstrated that soil frequently exposed to diurnal oscillation between daily minimum and daily maximum temperatures results in similar basal soil respiration rate to soil constantly exposed to the daily maximum temperature. This evidence was supported by a shift in microbial community composition, physiological adjustment and depletion of extractable carbon, consistent with acclimatisation to higher temperature. This evidence provides support for my second hypothesis and thus challenges the justification that leads to researchers performing experiments by incubating samples at the daily mean temperature without oscillation.

3. I found that prior incubation temperature influenced the temperature sensitivity of extracellular depolymerising and intracellular catalytic enzyme activities, even though these two enzyme-mediated processes were not equally sensitive to temperature. This apparent thermal acclimation was attributed to temperature-induced changes in C/N ratio (stoichiometry) of soil organic matter and soil chemistry (pH). This evidence supported my third hypothesis.

4. I also found that OTCs are effective at warming soil in an agricultural system. However, incorporating cover crops as a means of soil carbon sequestration reduces the magnitude of the warming effect. Although this evidence was reported without replication, it points to the benefit of using cover crops to regulate the effect of temperature change on soil properties and soil microbial community functions.

5. Overall I conclude that soil microbial community and function (microbial respiration) thermally adapts to daily maximum temperature when measured using constant or diurnally oscillating temperatures representing average daily ambient (minimum , maximum, and mean temperatures) conditions of a site. The apparent thermal adaptation was consistent with higher sensitivity of extracellular enzymes to warmer conditions, compared to the intracellular activity. Possibly because previous temperature influenced changes in soil chemical properties (C/N and pH), which in turn shaped the enzyme-substrate interaction and the consequent structure and function of

enzyme. Extracellular enzyme activity is the rate limiting step in organic matter decomposition.

6. Use of cover crops as a means of organic matter sequestration can be used to conserve soil moisture thereby mitigating the effect of global warming on soil respiration.

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Appendices

Appendix 1: Supplementary material for Chapter 4

This supplementary material contains:

- A description of the laboratory procedures used for characterisation of soil samples prior to incubations
- Figure S-1: Effects incubation temperature on soil CO_2 released from soil when CO_2 flux was measured at 5 ℃, 10 ℃, and 15 ℃
- Figure S-2: Effects measurement temperature on soil CO_2 released from soil previously incubated at 5 ℃, 10 ℃, 15 ℃, or oscillating between 5 ℃ and 15 ℃
- Table S-1 The designation of individual PLFA biomarkers to microbial community groups

A description of the laboratory procedures used for characterisation of soil samples prior to incubations

Soil characteristics were measured using standard laboratory methods. The particle size distribution of soils was determined using a Malvern Mastersizer3000 Laser Granulometer after dispersing the soil in a solution containing 3.3 % sodium hexametaphosphate + 0.7 % sodium carbonate. The data was converted from % volume to % mass as described in Yang et al., 2015) . Soil pH was determined by shaking soil samples with deionised water (1:10 mass/volume ratio) for 30 min and leaving the mixture to stand for 2 min before pH was measured using a digital type DMP-2 mV/pH meter (Thermo Orion). Total N and C concentrations were determined using C/N Elemental Analyser (Thermo Flash 2000 EA). The C/N ratio was then calculated from total C and N. Nitrate and ammonia were extracted in 1M KCl and then analysed using a Continuous Flow Analyzer (San++ Automated Wet Chemistry Analyzer - SKALAR). Moisture content and loss on ignition were determined by weight loss at 105 °C and 500 °C, respectively. Soil water holding capacity (WHC) was determined using saturation and drain method by submerging a 30 g air-dried sample in a plastic cylinder with a mesh bottom in water for 12 h to ensure complete saturation and then allowing the water to drain for another 12 h. The drained soil was then oven-dried at 105 °C.

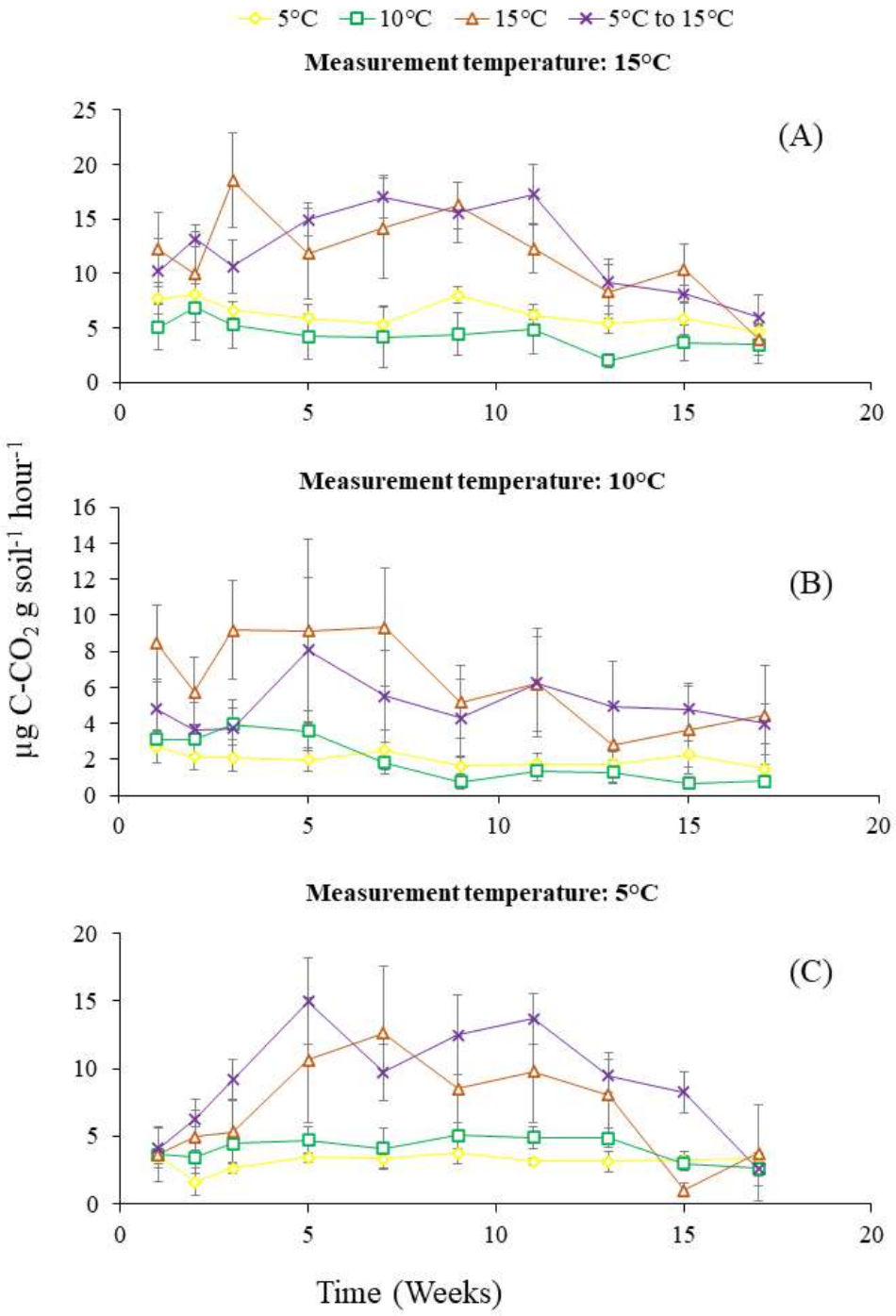

Figure S-1: Effects incubation temperature on soil CO_2 released from soil when CO_2 flux was measured at 5 °C, 10 °C, and 15 °C.

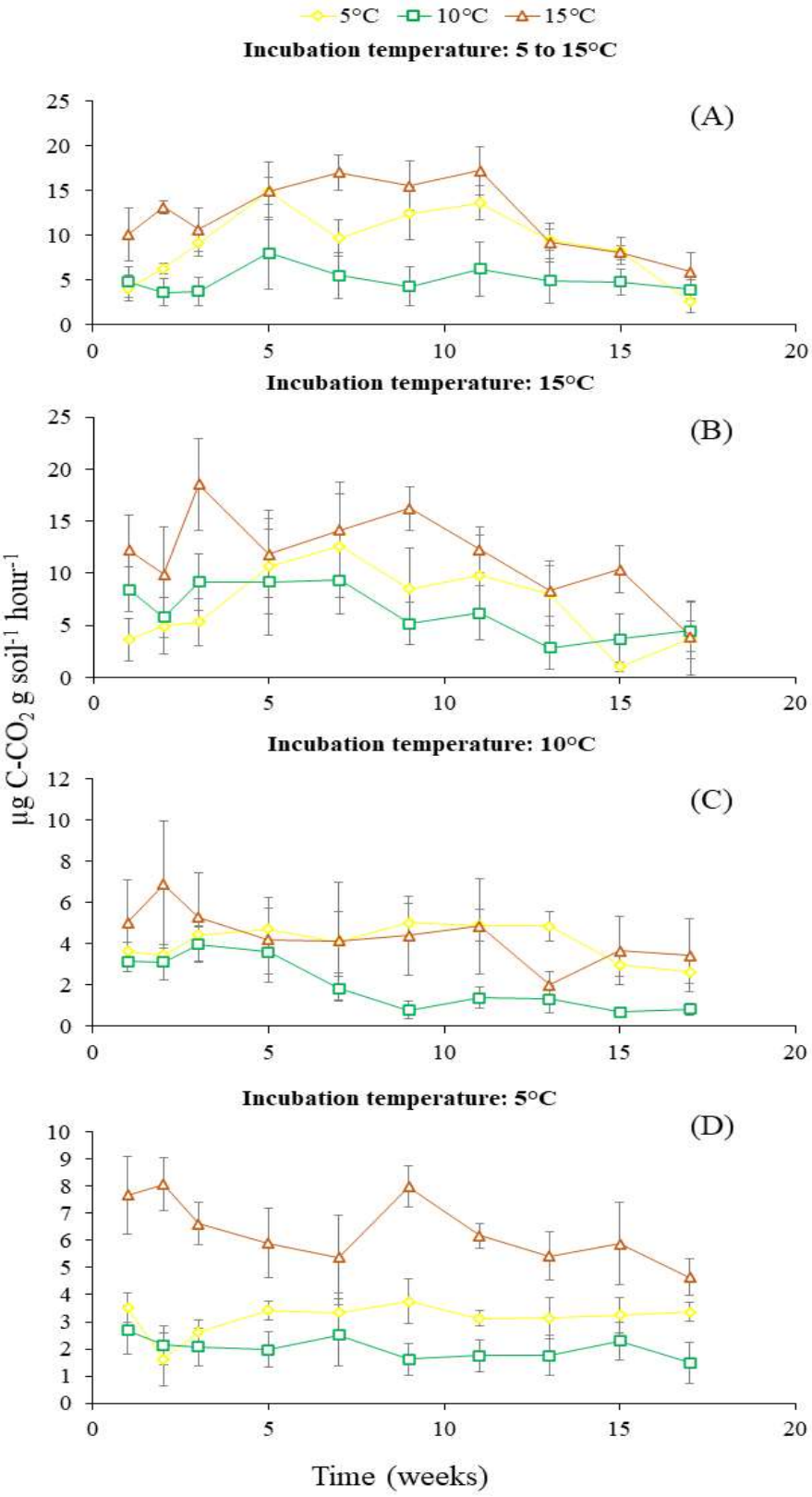

Figure S-2: Effects measurement temperature on soil CO_2 released from soil previously incubated at 5 °C, 10 °C, 15 °C, or oscillating between 5 °C and 15 °C.

Table S-1 The designation of individual PLFA biomarkers to microbial community groups

Group	Biomarker	Reference
Gram negative bacteria	α14:0 2OH, β14:0 3OH, cy17:0c, and cy19:0c	Kaur et al., (2005); Willers et al., (2015)
Gram positive bacteria	i15:0, a15:0, i16:0, br17:0, i17:0, 16:1ω7c, 16:1w7t, and 16:1w5,	Kaur et al., (2005); Willers et al., (2015)
Bacteria	C15:0, C17:0 and C20:0	Bossio and Scow, (1998); Willers et al., (2015)
Fungi	18:1ω9t, 18:2ω6t, and 18:2ω6,9c	Bossio and Scow, 1998); Kaur et al., (2005)
cis isomers	16:1ω7c, 17:1c, cy17:0c, 18:3ω6c, 18:2ω6,9c, cy19:0c, 20:4ω6c, 20:5ω3c, 20:1ω9c,	Quideau et al., (2016)
trans isomers	C16:1w11t, 16:1w7t, 18:2ω6t, and 18:1ω9t	Quideau et al., (2016)
iso	i15:0, i16:0, and i17:0	Quideau et al., (2016)
anteiso	a15:0	Quideau et al., (2016)

Supplementary References

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265–278. https://doi.org/10.1007/s002489900082

Kaur, A.A., Chaudhary, A., Choudhary, R., Kaushik, R., Kaur, A.A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid – A bioindicator of environment monitoring and assessment in soil ecosystem. October 89, 1103–1112. https://doi.org/10.2307/24110962

Quideau, S.A., McIntosh, A.C.S., Norris, C.E., Lloret, E., Swallow, M.J.B., Hannam, K., 2016. Extraction and analysis of microbial Phospholipid fatty acids in soils. Journal of Visualized Experiments 2016, 1–9. https://doi.org/10.3791/54360

Willers, C., Jansen van Rensburg, P.J., Claassens, S., 2015. Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. Journal of Applied Microbiology 119, 1207–1218. https://doi.org/10.1111/jam.12902

Appendix 2: Supplementary material for Chapter 5:

This supplementary material contains:

- A description of the laboratory procedures used for trial experiment for extracellular Enzyme
- Figure S-1: β-glucosidase measured at four different reaction time
- Figure S-2: Chitinase measure at four different reaction time
- A description of the laboratory procedures used for trial experiment for Substrate Induced Respiration
- Figure S-3: Soil CO_2 measured at various concentration Glucose

Trial experiment for extracellular Enzymes

The purpose of this experiment was to determine the optimal incubation time for the main investigation. 1 g of moist soil was weighed into a 50 ml centrifuge tube and mixed with 4 ml MUB buffer (pH 6) and either 1 ml 25 mM *p*-nitrophenyl-β-D-glucopyranoside (β-glucosidase) or 10 mM *p*-nitrophenyl-N-acetyl-b-D-glucosaminide (chitinase) solution, to assess β-glucosidase and chitinase activity, respectively. Samples were incubated at 45 °C for 15, 30, 45 or 60 minutes, after which 1 ml 0.5 M $CaCl_2$ and 4 ml Tris buffer (pH 12) was added to stop the reaction. Samples were mixed by swirling, then filtered with Whatman No. 2 filter paper.

Additionally, 1 blank (for each reaction time except for 45 minutes which was estimated from others) was created by adding substrate to tubes containing the mixture after the reaction had stopped. Colour intensity of the filtrate - directly proportional to the level of reaction product p-nitrophenol (pNP), and hence level of enzyme activity - was measured using a spectrophotometer at 400 nm. Working *p*-nitrophenol standard solutions including 0, 10, 20, 30, 40 and 50 μg *p*-nitrophenol were used and the mass of *p*-nitrophenol in each reaction (0-50 μg) plotted against the OD_{400nm} reading. The level of absorbance was converted to potential enzyme activity by dividing the measured concentration by dry weight equivalent of soil.

Results for β-glucosidase and chitinase are presented in Figure S-1 and S-2, respectively.

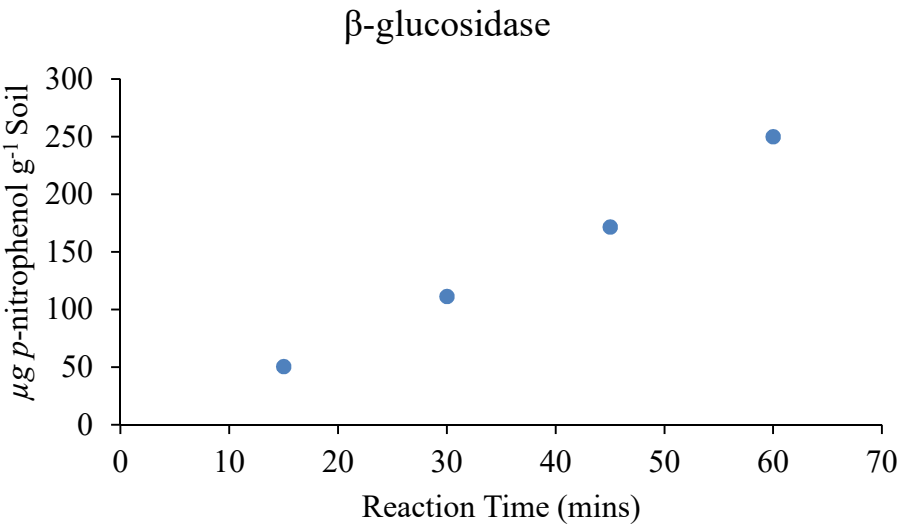

Figure S-1 β-glucosidase activity measured at four different reaction times

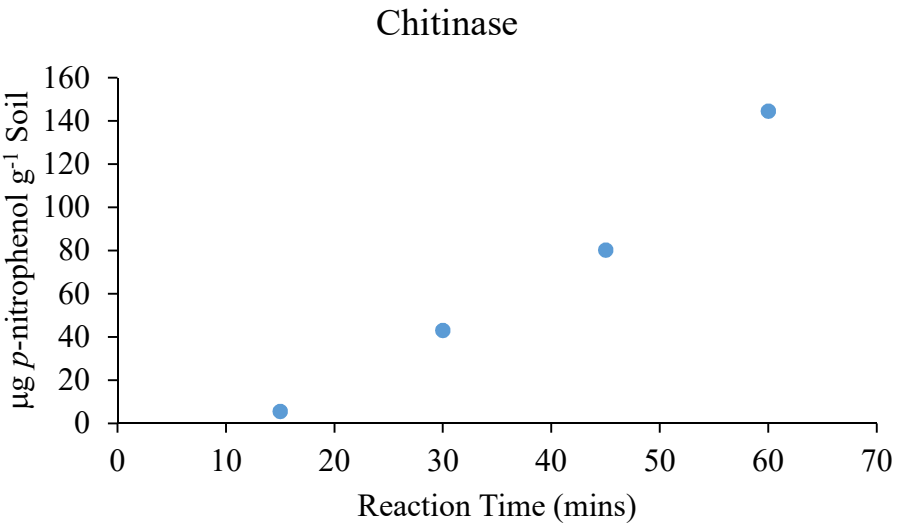

Figure S-2 Chitinase activity measured at four different reaction times

Trial experiment for Glucose induced respiration

The aim of this trial experiment was to test response of soil CO_2 to five different levels of glucose addition. 15 g of moist soil (equivalent to 13.31 g of dry soil) was weighed into 50 ml incubation vials (Centrifuge tube). Glucose solution (2 ml) was added at five concentrations namely; 0, 0.1, 1, 10, 100 mg g^{-1} soil, thus bringing the soil to 58 % of its water holding capacity. Three replicates per glucose level (15 samples in total) were used and the soils were incubated at 26 °C for 1 hour.

Following soil-substrate mixing, the tube was ventilated by blowing in lab air with a 20 ml syringe, ensuring air away from the user was extracted to avoid contamination with human-generated CO_2. The tubes were sealed with septum stoppers and 15 ml of lab air was injected. The headspace was flushed by moving the syringe plunger up and down several times before sampling 15 ml of head space gas and injecting into a 12 ml exetainer vial (T0), creating overpressure, using a tap and needle attached to the syringe. The samples were incubated for one hour at 26°C, at the end of which the process of injecting air, flushing and sampling was repeated (T1). Headspace gas samples were stored at 20 °C prior to analysis by an Agilent 7890B gas chromatograph. The results obtained from the samples were calibrated with CO_2 gas standards (506 ppm, 2542 ppm, 5163 ppm and 19,700 ppm respectively) and the difference in the concentration of CO_2 between T1 and T0 obtained thereafter is presented in Figure S-3, indicative of the actual value for CO_2 flux per hour.

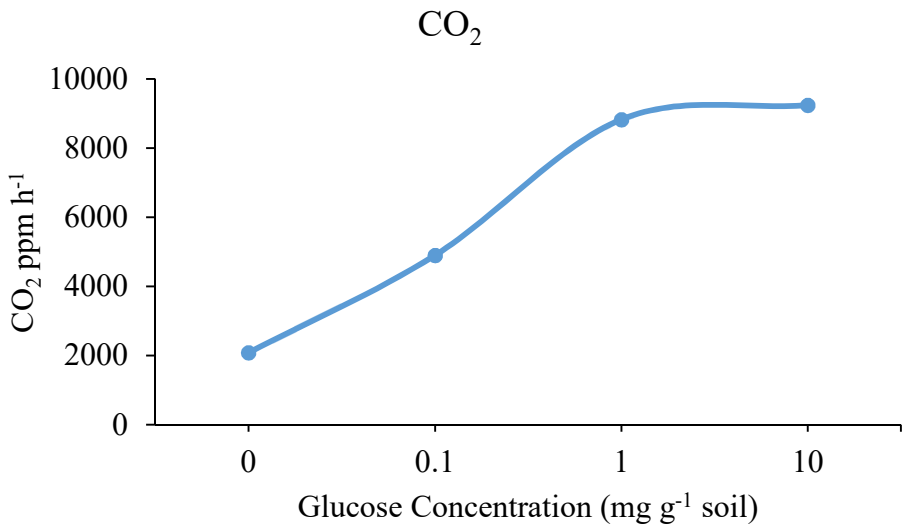

Figure S-3: Soil CO2 measured after incubation with various concentrations of glucose

Appendix 3: Aerial photograph of warming plots during winter 2019/2020 (Chapter 6).

Credit: Professor Kevin White