Temperature responses of nitrogen transformations in grassland soils

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Contents

Acknowledgements

Chapter 1: Introduction

1.1 Overview

The manner in which the soil system functions is a complex web of interactions between changing nutrient balance (stoichiometry), diverse soil microbial, faunal and plant communities, chemically complex soil organic matter, and a complex matrix of spatially and chemically diverse particles and pores. This set of interactions can be pushed and pulled in different directions by many different external factors including abiotic factors such as temperature, precipitation (Koster et al., 2004; Paul, 2007), slope (Lal, 1988; Ribolzi et al., 2011), intrinsic soil properties such as soil texture (Burke et al., 1989; Monreal and Bergstrom, 2000; Silver et al., 2000) as well as biological factors such as invasion by exotic species (Wedin and Tilman, 1990; Hobbie, 1992; Ehrenfeld, 2003; Kourtev et al., 2003; Callaway et al., 2004), or successional community change (Reynolds et al., 2003; Kardol et al., 2006, 2010).

Of growing importance over the past 200 years have been anthropogenic factors such as land use change (Post and Kwon, 2000; Guo and Gifford, 2002), manufacture and use of inorganic Nitrogen (N) fertilizers (Broadbent, 1965; Goulding et al., 1998; Ågren et al., 2001; Galloway et al., 2008; Gruber and Galloway, 2008), use of herbicides and pesticides (Burrows and Edwards, 2002), disruption of soil structure and compaction during tillage (Young and Ritz, 2000; Pagliai et al., 2004; Ussiri and Lal, 2009) and the spread of irrigation for agricultural purpose (Mubarak et al., 2009). These factors often vary concomitantly and, as such, changes in ecosystem functions and services become difficult to attribute to particular drivers, making mitigation strategies difficult to design. Another factor that will have a large bearing on how the soil system

functions is temperature. Already, observations indicate rising global temperatures of both air and soil. Considerable debate remains, however, on how temperature affects soil processes.

Temperature is of intrinsic importance to the rate of chemical reactions for thermodynamic reasons (this will be expanded-upon in section 1.4), temperature is also of great significance in terms of growth and activity of both the microbial and plant populations which drive the cycling of nutrients within the soil system via the inputs of organic matter (OM) and secretion of OM degrading enzymes. The work presented here will consider the effects of changing temperatures on a set of chemical reactions involved in the early stages of the soil N-cycle, focusing particularly on temperatures likely to be experienced in the field currently and under predictions of climate change.

1.2 Global Change

1.2.1 Temperature trends

Since 1750 there have been large increases in global atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) such that these concentrations now considerably exceed pre-industrial levels (IPCC 2007). With increases in concentrations of radiatively forcing gases[1] there have been increases in the total global surface temperature (an average of +0.74 °C over[1] the period 1906 to 2005 (IPCC 2007) due to the efficiency with which these gases trap long-wave terrestrial radiation emitted from the earth's. These temperature changes have been

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[1] radiative forcing factors are a measure of influence on the balance of incoming and outgoing energy in the earth-atmosphere system, these are relative to pre-industrial levels and expressed as Watts m^{-2}.

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more rapid since the 1950's, with warming over the period 1950 to 2005 accounting for more than half of the overall change mentioned above (IPCC 2007). Global changes are not just limited to temperature increase; weather patterns are also changing with an increased likelihood of extreme temperature events (Hansen et al., 2012) as well as changes in rainfall patterns (Plummer et al., 2006; Hayhoe et al., 2007, 2008).

Food and environmental security in relation to climate change could therefore be affected considerably by changes in soil temperature. Despite the significance of this problem, a dearth of information exists in the literature. Currently the literature on terrestrial temperature trends is focused primarily on air and surface temperature measurements, often corresponding to a Stephenson screen 1.25 – 2 m above the ground (World Meteorological Organisation www.wmo.int). A systematic literature search of three commonly used search engines, Web of Science, Google Scholar and CAB Direct, was conducted using the search tems below:

1. "surface temperature trends" NOT models NOT sea

2. "air temperature trends" NOT models NOT sea

3. "soil temperature trends" NOT models

The return from this search reveals a lack of publications relating to observed soil temperature trends (Figure 1.1). This lack of information in the current literature about how soil temperature is changing with time presents challenges for experimental design and accurate parameterization of models, and therefore

compromises the ability of scientists to advise policy makers on how to mitigate potential future problems.
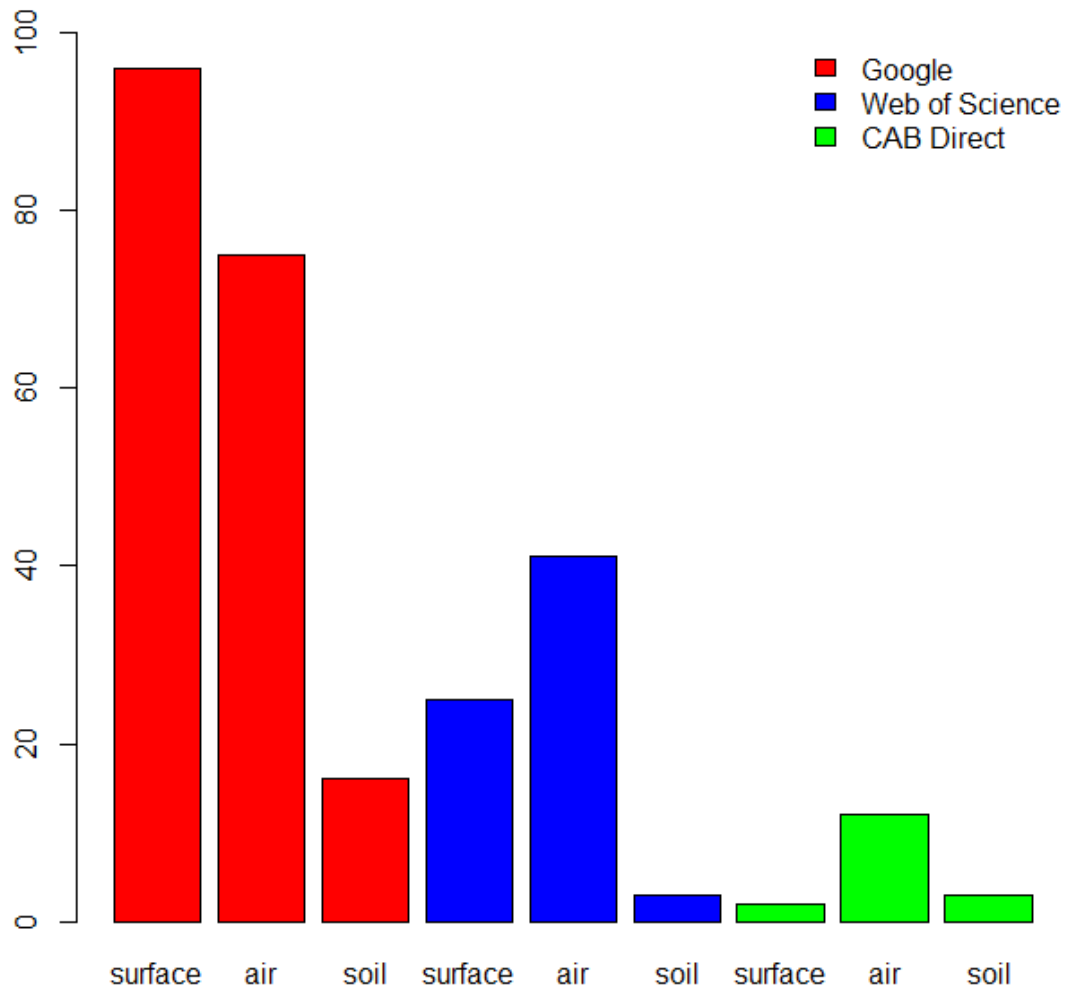
Figure 1.1: Numbers of articles retrieved from 3 commonly used search engines during a systematic literature search using the terms: 1) "surface temperature trends" NOT models NOT sea, 2) "air temperature trends" NOT models NOT sea, and 3) "soil temperature trends" NOT models.

1.2.2 Global variability

Global data sets show that both surface and tropospheric temperatures have consistently increased for the past 30 years (IPCC 2007; Figure 1.2). However, there is evidence to suggest that changes in climate have been and will continue to be more severe at high latitudes than at lower latitudes (Hobbie et al., 2000; McBean et al., 2005 IPCC 2007). Data suggests that over a similar period of time, mean Arctic temperatures have increased up to 1.5 times more than global mean surface temperatures (+0.5 to 2.0 °C increase in the Arctic (McBean et al., 2005) compared with +0.56 to 0.92 °C globally (IPCC 2007) – the so called "Arctic Amplification" (Lesins et al., 2012). This illustrates that the responses to changes in atmospheric chemistry are likely to be non-uniform across the globe. This Arctic Amplification is likely to be the result of a positive feedback loop as a result of more generalized global warming; higher average temperatures result in a greater loss of snow and ice (particularly during the summer) resulting in a decreased albedo effect of the pole and consequently yet higher temperatures (Chapin et al., 2005; McBean et al., 2005). This will potentially have major consequences for future release of CO_2, CH_4 and N_2O to the atmosphere due to the large stocks of C and N contained in Arctic tundra soils (Post et al., 1982; Ping et al., 2008) which in turn may result in even greater warming. The efflux of these gases is potentially increased through a variety of warming-induced changes such as increased microbial activity (Schimel and Clein, 1996; Schimel et al., 2004), melting of permafrost (Schuur et al., 2008, 2009) and priming by shifting the tree line (Field et al., 2007; Liao et al., 2008; Hartley et al., 2012) or invasion of shrubs

in otherwise low growing alpine plant communities (Strum et al., 2005; Tape et al., 2006).
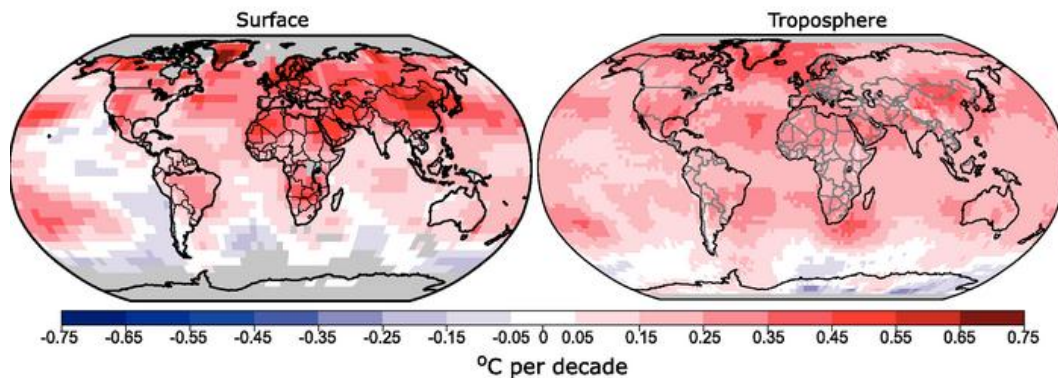
Figure 1.2: Patterns of linear global temperature trends from 1979 to 2005 estimated at the surface (left), and for the troposphere (right) from the surface to about 10 km altitude, from satellite records. Grey areas indicate incomplete data. Note the more spatially uniform warming in the satellite tropospheric record while the surface temperature changes more clearly relate to land and ocean.

Due to the potential consequences listed above, as well as for thermodynamic reasons (section 1.4), much of the literature focuses on the effects of temperature on soil processes at high latitudes. More recently predictions about possible climate change have been made specifically for the UK and temperatures are predicted to increase under all greenhouse gas emissions scenarios (low = SRES[2] B1, medium = SRES A1B and high = SRES A1F1) up to the end of the 2080's. These changes are predicted to vary and to be lower (4.0 (low emissions) to 5.7 °C (high emissions)) during winter compared with summer (5.1 (low emissions) to 8.1 °C (high emissions)) (Murphy et al.,

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[2] SRES = Special Report for Emissions Scenarios by the IPCC the A1 family of emissions scenarios are based on projections of quick uptake of new and efficient technologies, global population reaching 9 billion by 2050 then decreasing and rapid economic growth; A1FI emphasises use of fossil fuels for energy and A1B emphasises a balanced use of all energy sources. B models are similar to A modes but with more focus on the service and information industries for economic growth but the word as a whole is more integrated and environmentally friendly (IPPC 2007).

2009). Similar predictions have been made for the northeast of the United States and Canada (Plummer et al., 2006; Hayhoe et al., 2007, 2008). Warming, and changing precipitation, in these environments is likely to have severe consequences both for C-sequestration in forest (Bonan, 2008; Melillo et al., 2011) or peat-land areas (Belyea and Malmer, 2004), on N_2O emissions from soil due to increased numbers of freeze-thaw cycles (Matzner and Borken, 2008) and on sustainable agriculture and therefore food production (Goulding et al., 1998; Schmidhuber and Tubiello, 2007; Patil et al., 2010).

1.2.3 Anthropogenic effects

Recent evidence has suggested that not only is climate changing but over the past 5 decades this can be convincingly attributed to anthropogenic activities (Thompson et al., 2009). It has also become clear that these changes in climate are having a significant impact on both physical and biological systems globally (Rosenzweig et al., 2008). Recent work has also shown that it is possible to attribute recent temperature extremes directly to climate change (Hansen et al., 2012) although progress has been made towards predicting future weather events (Keenlyside et al., 2008; Wood, 2008)

1.3 Nitrogen Cycling

1.3.1. Overview

The N cycle is a set of redox reactions linking N in different oxidation states (Figure 1.3). These reactions govern the availability of both organic and inorganic forms of nitrogen to soil microbial and plant communities as nutrients flow through ecosystems; this determines the effects, both positive and negative, of different forms of nitrogen on terrestrial and aquatic systems as well as potential effects on atmospheric chemistry.
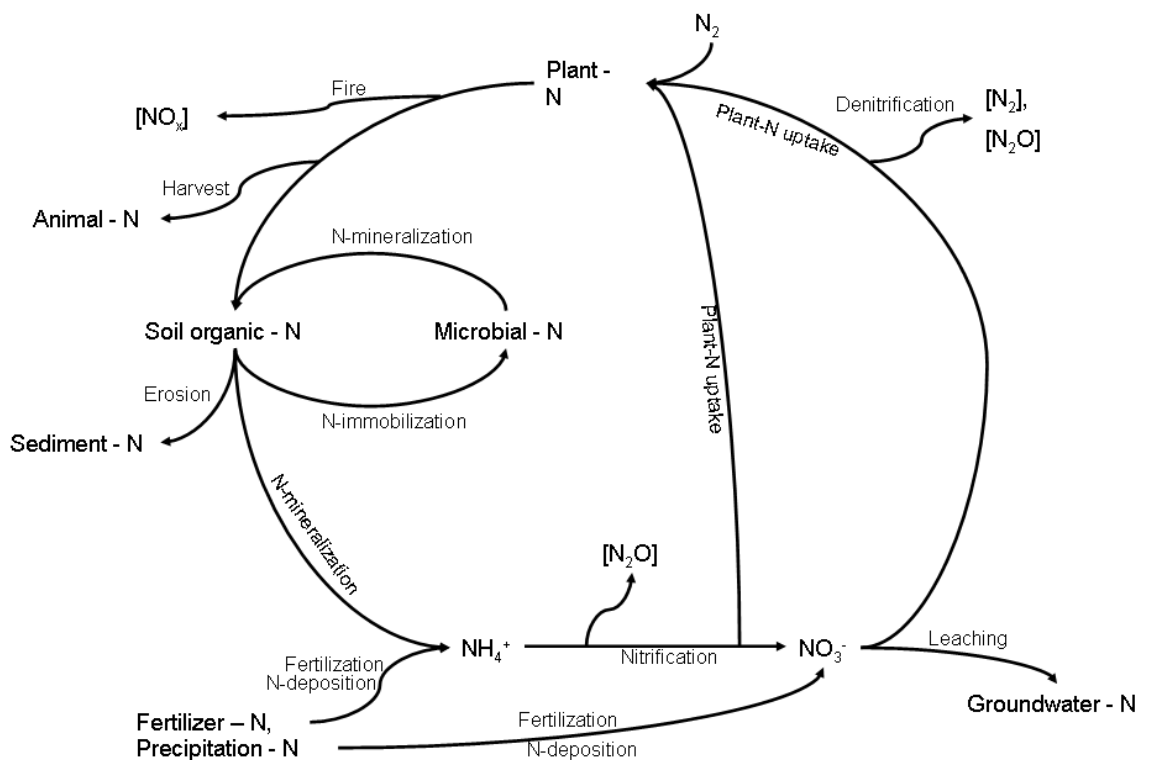
Figure 1.3: The Nitrogen Cycle; showing inputs, outputs and the processes involved in moving nitrogen through the soil, gases in brackets (redrawn from (Paul, 2007) chapter 13 page 343)

Each of the steps involved in the N-cycle are catalysed by enzymes usually produced by the microbial community (Table 1.1). The temperature sensitivities of each of these

sets of reactions have been considered previously although this has often involved using temperature ranges in laboratory incubations that are unlikely to be experienced in the field. These studies have used a variety of methods which usually rely on measuring extractable pools of N containing compounds; usually by extracting inorganic, or sometimes organic, compounds at different points in time to work out rates of turnover but sometimes isotopic pool dilution studies are used. These pools are continually being increased (i.e. as a result of fixation, mineralization and nitrification) and depleted (i.e. as a result of fixation, mineralization and denitrification). N-fixation, nitrification and denitrification reactions are well characterized and highly defined; however, N-mineralization occurs as a result of myriad different reactions catalysed by a wide variety of enzymes. Few studies have examined the effects temperature on soil enzymes and very few of these have examined those enzymes specifically involved in N-mineralization.

Table 1.1: Nitrogen cycle reactions and the enzymes associated with them.

Nitrogen cycle stage	Reaction	Enzymes involved
Nitrogen Fixation	$N_2 \rightarrow NH_3^+$	Nitrogenase
Nitrogen Mineralization	Complex organic mater Long chain polymers Short chain polymers Organic monomers $NH_4^+ \rightarrow NH_3^+$	Proteases Amidases Deaminases
Nitrification Phase 1 = Ammonia oxidation Phase 2 = Nitrite oxidation	$NH_3^+ \rightarrow NO_2^-$ $NO_2^- \rightarrow NO_3^-$	Ammonia monooxygenase Hydroxylamine oxidoreductase Nitrite oxidoreductase
Denitrification	NO_3^- $\searrow$ $\quad NO_2^-$ $\quad \searrow$ $\qquad NO$ $\qquad \searrow$ $\qquad\quad N_2O$ $\qquad\quad \searrow$ $\qquad\qquad N_2$	Nitrate reductase Nitrite reductase Nitric oxide reductase Nitrous oxide reductase

1.3.1. Climate change and the nitrogen cycle

Not only has the global climate changed but global nutrient cycles have also been altered, primarily by human activities (Vitousek et al., 1997; Galloway et al., 2008; Gruber and Galloway, 2008; Erisman et al., 2011), these include a combination of land use changes, increases in the burning of fossil fuels, use of the Haber-Bosh process to create N-fertilizers and their subsequent use. The extra N fixed by Haber-Bosh process and increased burning of fossil fuels has resulted in increased levels of N-deposition across the terrestrial realm (Dise and Wright, 1995; Goulding et al., 1998).   This has resulted in increased rates of acidification (Hogg et al., 1995; Curtis et al., 2005), N-saturation of ecosystems – even those which are not cultivated (MacDonald et al., 2002; Adams, 2003) and increased levels of NO_3^- found in freshwater systems (Simmelsgaard, 1998; Smolders et al., 2010). Use of man-made, inorganic-N fertilizers

can affect the N-cycle through a variety of mechanisms. The addition of vast amounts of NH_4^+ and NO_3^- will obviously change the availability of inorganic N to both plant and microbial communities, which is often reported to have involved a reduction in species richness of both communities (Galloway et al., 2004; Wardle et al., 2004; Bobbink et al., 2010; Parfitt et al., 2010). This can be seen in the effect that N-deposition apparently has on nutrient poor soils such as encouraging colonisation by exotic species to the detriment of native species (Weiss, 1999; Stevens et al., 2004) or imbalance of nutrition in plants already growing on site (Van Dijk and Roelofs, 1988; Power and Collins, 2010; Liu et al., 2011; Fritz et al., 2012).

Land use change can have a variety of effects on the soil system. It has been shown, for example, that soil Carbon (C) stocks change with land use (Guo and Gifford, 2002) which, given the intimately linked nature of biogeochemical nutrient cycles, makes it almost certain that other important soil nutrients will also vary with land use. Much of the work looking at land use change in relation to N-cycling has examined the conversion of forested areas to grassland, resulting in increased accumulations of NH_4^+ (Reiners et al., 1994; Murty et al., 2002; Booth et al., 2005) and often a decrease in the accumulation of NO_3^- (Reiners et al., 1994). It is possible that lower concentrations of NO_3^- are due to lower rates of nitrification as a result of changing microbial community (Singh et al., 2011) but it is probable that there is a higher rate of leaching from grassland systems resulting in greater transport of NO_3^- to the fresh water system (Johnes, 1996; Downing et al., 1999; Harris, 2001; Baron et al., 2012). Even when agricultural fields are abandoned and allowed to revert to their "natural" state, models predict that not only are C-accumulation rates dependent on N-accumulation rates but

that recovery of pre-agricultural levels is likely to take decades or even hundreds of years (Knops and Tilman, 2000). Lengthy recovery of nutrient cycling systems is also seen in forest systems which have experienced disturbance.

1.3.2 Measuring specific reactions

Much of the previous work looking at how changes in environment affect N stocks and transformations has focused on measuring rates of mineralization, nitrification and denitrification via concentrations of NH_4^+ and NO_3^- pools and fluxes of N_2O. These are useful but they miss much of the detail that might be resolved by quantifying changes in individual reactions (Burns et al., 2013). Most reactions in soil are catalysed by enzymes produced by the microbial community (Kandeler et al., 1996), and measurements of how changes in environment affect the rates of enzyme activity can fill gaps needed to parametarize global circulation models.

1.3.2.1 Soil Enzymology

Ultimately the microbial community mediates the majority of reactions taking place in the soil system (Kandeler et al., 1996; Burns et al., 2013), usually through the use of enzymes. Activities of enzymes are controlled by a variety of different factors, both biotic (e.g. rates of synthesis and secretion or rates of denaturation) and abiotic such as temperature (German et al., 2011; Steinweg et al., 2012), pH (Frankenberger Jr and Johanson, 1982; Quiquampoix et al., 1993; Leprince and Quiquampoix, 1996) and moisture availibility (Stark and Firestone, 1995; Sardans and Peñuelas, 2005; Steinweg et al., 2012). As such changes in climate are likely to have an effect on the rates of enzyme activity and therefore on ecosystem function.

Much of the work involving the response of soil enzymes to temperature has been done using laboratory incubations. These often involve incubation of soils over a range of temperatures, many of which are unlikely to be experienced in the field (Ladd and Butler, 1972; Frankenberger and Tabatabai, 1979; Kandeler and Gerber, 1988). Soils are then assayed for rates of activity at one common temperature (often the previously determined optimum (e.g. Ladd and Butler, (1972))) to assertain the potential rate of activity for a soil incubated at a particular temperature. These types of experiments have been used in conjunction with other process measurements such as respiration, gross N-mineralization, nitrification and denitrification as well as measures of microbial community size and composition such as phospholipid fatty acid anaylsis (PLFA), chloroform fumigation-incubation, substrate induced respiration, ATP extraction and genetic methods. These experiments often assess how soil systems will make use of different pools of organic matter (OM) (Moorhead and Sinsabaugh, 2000; Schimel and Weintraub, 2003; Trasar-Cepeda et al., 2007; Sinsabaugh, 2008; Baldrian et al., 2013), respond to changes in moisture levels (Song et al., 2012; Baldrian et al., 2013); land use (Patra et al., 2006; Kaiser et al., 2010; Miralles et al., 2012; Zhao et al., 2012), vegetation type (Kardol et al., 2010; Elgersma et al., 2011; Wu et al., 2012), and N-depostion (Saiya-Cork et al., 2002). Work has also been conducted on the soil systems response to sewage sluge ammendment (Perucci, 1992; Garcia-Gil et al., 2000; Kızılkaya and Bayraklı, 2005), heavy metal contamination (Fließbach et al., 1994; Brookes, 1995) and how microbial community structure and any changes in this might alter the way enzymes in the soil system function (Elgersma et al., 2011; Brockett et al., 2012; Weedon et al., 2012).

1.3.2.2 Enzymes in the field

Unfortunatly, few field studies exist where enzyme activities have been measured in soil from sites where the soil has been subjected to temperature manipulation. Those studies that have used a range of heating methods include plastic tents (Allison and Treseder, 2008), open-top chambers (Weedon et al., 2012), infra-red heaters (McDaniel et al., 2013; Zhou et al., 2013), heating by night-time insulation (Sardans et al., 2008), exploitation of temproral varriation (Bell and Henry, 2011), or a mixture of methods used at different sites (Brzostek et al., 2012). Others have used increased CO_2 concentration to simulate future climates (Chung et al., 2007; Henry, 2007; Jin and Evans, 2007). Those which do look at the direct effects of temperature change have usually done so by using very few sampling events (Burns et al., 2013), often only over one season, and obtained variable results with some studies finding increased rates (Brzostek et al., 2012; Zhou et al., 2013), others decreased rates (Allison and Treseder, 2008; Weedon et al., 2012; McDaniel et al., 2013; Zhou et al., 2013) and some finding no effect of temperature (Bell and Henry, 2011). This unclear relationship between soil system properties and temperature is not restricted to soil enzymology but has been common in studies examining both C and N cycling in soils (Rustad et al., 2001; Kirschbaum, 2006; Conant et al., 2011).

1.4. Temperature Sensitivity

This body of contrasting evidence suggests that the observed temperature sensitivity of soil reactions could be mediated by several other factors. This section will initially consider the chemical basis for increasing reaction rates as a result of increasing

ambient temperature and how this effects enzyme catalysed reactions. It will then move on to consider these thermodynamic principles in conjuction with soil factors including soil organic matter availibility and quality as well as the ability of the microbial community to acclimate to changing temperatures.

1.4.1 Thermodynamics

Reactions proceed at different rates at varying temperatures, even when the concentrations of reactants are held stable. The value of the rate constant of a reaction will therefore be dependent on the temperature at which the reaction takes place; this relationship can be defined using the Arrhenius equation (Equation 1.1),

Eqn 1.1: $$K = Ae^{-E/RT}$$

where k = rate constant, T = temperature in degrees Kelvin (K), R = gas constant, A = the "Arrhenius constant" and E = the activation energy (kJ mol^{-1}).

Studies have shown that A and E are not entirely independent from temperature. As they remain approximately constant over a reasonable range of temperatures, however, they can still be treated as constants (Morris, 1974).

Arrhenius and van't Hoff proposed Collision Theory to explain how the rate constant varies with temperature; this states that the reaction in question will only occur if the molecules of reactants collide with more than a given level of energy when they are in the correct orientation. When these additional considerations are brought into account Equation 1.1 becomes Equation 1.2,

Eqn 1.2: $$K = PZe^{-E/RT}$$

where P = the steric factor – a constant that allows for unsuccessful collisions with the correct energy levels to take place, and Z = the collision frequency, together $P + Z = A$. Although

Collision Theory has aided greatly in the understanding of chemical reactions, it is not entirely satisfactory; firstly using this equation the activation energy is not predictable as P and E cannot be calculated using measurable properties of interacting molecules and, secondly, values of Z calculated using kinetic theory tend not to agree with values of A derived experimentally, particularly for reactions in solution (Morris, 1974).

A more modern interpretation of the manner in which the rate constant varies with temperature is the Transition State Theory, developed by Eyring, (1935) in parallel with Evans and Polanyi, (1935). Transition State theory asserts that the variations in rate constants suggest an initial energy barrier which the reactants must surmount to allow the reaction to take place. When this activation energy is achieved the reactants then form the transition state complexes which can decompose to form the products or to reproduce the reactants. It is proposed that the concentration of the transition complex, and more particularly, the speed of breakdown, determine reaction rate. This means the rate constant is governed by the difference in standard Gibbs free energy (ΔG) between the reactants and the transition complex. This is the free energy of activation therefore changes in free energy are related to changes in enthalpy and entropy as illustrated by Equation 1.3,

Eqn 1.3: $$\Delta G^{\vartheta} = \Delta H^{\vartheta} - T\Delta S^{\vartheta}$$

where ΔG^{ϑ} = change in Gibbs free energy, ΔH^{ϑ} = change in standard enthalpy, ΔS^{ϑ} = change in standard entropy and T = temperature in degrees Kelvin (K). Overall reaction rate is therefore dependent on both standard entropy (ΔS) and standard enthalpy (ΔH). The theory suggests that A involves the entropy of activation and the value of E (experimentally derived) is related to the enthalpy of activation (Equation 1.4),

Eqn 1.4: $$E = \Delta H + RT.$$

1.4.2. Thermodynamics and catalysis

Catalysts accelerate reactions but do not have an effect on the basic thermodynamic properties of the reaction (i.e. ΔG, ΔH and ΔS). The reaction will reach equilibrium more quickly in the presence of the catalyst than without it, but can still take place in the absence of said catalyst. The rate of a catalysed reaction depends heavily on the concentration of the catalyst supplied. In line with the Arrhenius Equation there are two possible explanations for the faster rates of catalysed reactions:

1) a lower activation energy or

2) 'A' has a larger value than in the rate limiting step of the un-catalysed reaction.

Generally the first explanation is correct; catalysed reactions tend to have lower activation energies. The degree to which the activation energy is lowered is determined by the catalyst but is independent of the amount of catalyst available. There are, however, reactions where the catalysed form has the same activation energy as the un-catalysed form but the rate is greater due to a higher value of A (Morris, 1974).

1.4.3 Temperature sensitivity of soil organic mater decomposition

Similar to the situation with soil enzymology described in section 1.3.2.1, studies examining the temperature sensitivity of SOM have variously found that recalcitrant SOM is less temperature sensitive (Liski et al., 1999; Giardina and Ryan, 2000; Fang et al., 2005), equally temperature sensitive (Jones, McConnell, et al., 2005), or more temperature sensitive (Ågren et al., 2001; Knorr et al., 2005; Davidson and Janssens,

2006; Vanhala et al., 2007; Conant, Steinweg, et al., 2008) than SOM in the labile pool. This is likely to be due, at least in part, to some of the potential confounding issues explored bellow.

1.4.3.1 Organic matter quality

The quality of organic matter has been proposed as a potential driver or constraint on the decomposition of residues found in soils (Bosatta and Ågren, 1985; Ågren and Bosatta, 1987; Kirschbaum, 1995, 2006). A theory of continuous quality (*q*-theory) was first developed by Bosatta and Ågren, (1985), who proposed that decomposition could be viewed as a continuum starting with inputs of fresh organic matter (litter) which are then sequentially broken down leaving compounds which become more difficult for microbes to make use of. This would mean organic matter quality could be treated as a single continuous variable, *q*, where the freshest, most easily-decomposable materials would have a *q* of 1, and the oldest, most difficult to use materials a *q* of 0 (Bosatta and Ågren, 1985).

In practice, a method of defining quality of individual substrates was still required; describing OM quality thermodynamically enabled further integration of *q*-theory into experimental work. It has been suggested that quality can be defined as the number of enzymatic steps required to release, as CO_2, one carbon atom from an organic compound (Bosatta and Ågren, 1999). The higher the number of enzymatic steps required, the lower the quality of the material in question; $q = {}^1/_n$ where *n* = number of enzymatic steps (Equation 1.5). Assuming that evolution has selected for efficient enzymes, the free energy profile for the breakdown of organic matter would resemble Figure 1.4. Low activation energies make the formation of activated complexes easy,

and unstable associations within these complexes allow reactions to continue smoothly (Rees and Farrelly, 1990). Given that the peaks and troughs are small and of similar size (Figure 1.4), the Gibbs Free Energy (*ΔG*) of each enzymatic step is similar. This means high energy compounds will require a greater number of steps compared to low energy compounds, provided that *ΔG*'s of different decomposition steps of different compounds are comparable. This also suggests that as quality decreases (increased degree of decomposition or increased degree of physical protection) the substrate should become more energy dense (Bosatta and Ågren, 1999; Lõhmus and Ivask, 1995).
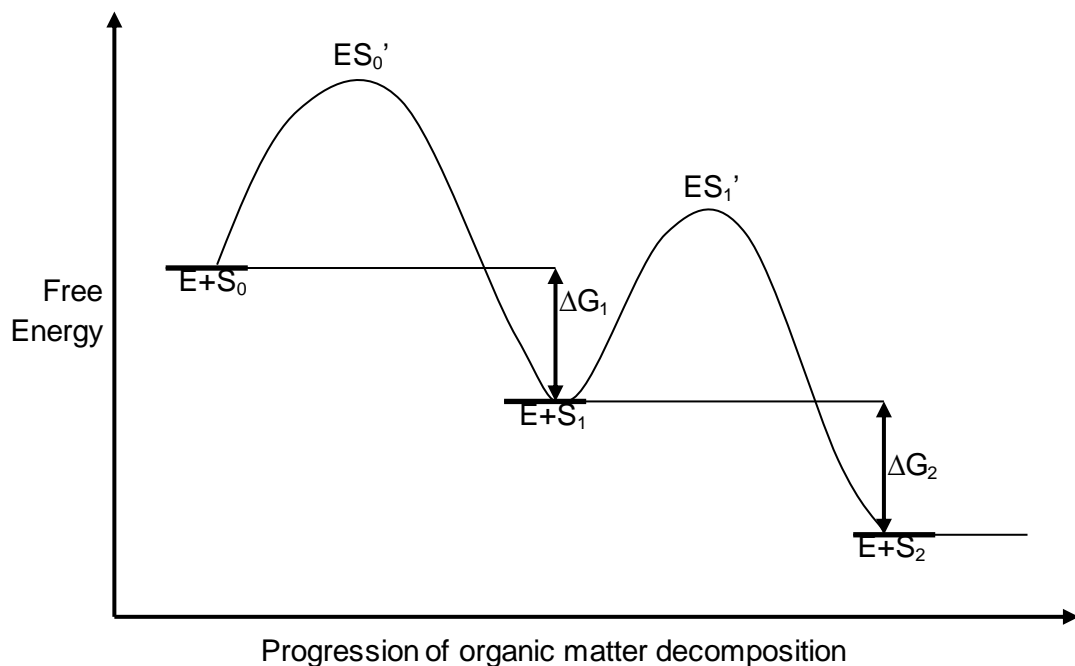
Figure 1.4: Free energy profile for a "model" enzyme catalysed reaction (redrawn from (Bosatta and Ågren, 1999).

Simply put, if these theories are correct, stable organic matter pools with longer turnover times should be more temperature sensitive than less stable, labile pools (Knorr et al., 2005). If this is true there may be severe consequences of increasing

global temperatures, with a potential positive feedback loop where the soil system warms up, releases increased amounts of CO_2, CH_4 and N_2O to the atmosphere and thereby further fuels the greenhouse effect, further warming the global system (Raich and Schlesinger, 1992; Rustad et al., 2000), although it is debatable how strong this feedback is likely to be (Kirschbaum, 2000) and whether this will be attenuated by increases in cycling of other nutrients (Melillo et al., 2011). Understandably, this has received a great deal of research attention and results have been widely published; however, no consensus has yet been reached, arguably, due in part, to the limited number of critical experiments which overcome issues of substrate depletion through time during lab experiments.

1.4.3.2 Substrate availability

Rates of reaction are also limited by the chemical availability of decomposable substrate in the soil matrix, as OM binding or physical occlusion can result in protected and unprotected fractions of otherwise chemically similar material having very different turn-over times (Sørensen, 1972). Binding and unbinding of OM is a chemical process and as such is subject to the same thermodynamic processes as decomposition and therefore will vary with temperature (Conant et al., 2011). In line with Le Chatelier's Principle ("A system in equilibrium reacts to any change in its conditions in a manner that would tend to abolish this change" (Morris, 1974, pp 221) the equilibrium constant (K_{eq}) of an exothermic reaction will decrease (shifting towards the reactants) and therefore promoting desorption of OM from the mineral soil matrix. Increased desorption of organic compounds from minerals with increased temperature has been shown in some experiments (Gianfreda et al., 1995; Kalbitz et

al., 2000; Moore and Dalva, 2000), temperature is also known to increase rates of diffusion and dissolution again making substrate more available to plants and micro-organisms or alternatively more likely to be leached from the system (Xu and Saiers, 2010).

1.4.3.3 Microbial Adaptation

The literature documents many studies where the response of the soil system (often measured as increased respiration rate) to increased soil temperature is short-lived (Peterjohn et al., 1994; Luo et al., 2001; Rustad et al., 2001; Melillo et al., 2002; Eliasson et al., 2005; Bradford, Fierer, et al., 2008). It has been proposed that a possible reason for this is compensatory thermal acclimation of microbial respiration to sustained temperature change (Bradford, Davies, et al., 2008; Bradford, Fierer, et al., 2008; Crowther and Bradford, 2013).

Thermal acclimation of respiration or "the subsequent adjustment in the rate of respiration to compensate for an internal change in temperature" (Atkin and Tjoelker, 2003) has been identified in plants (Atkin and Tjoelker, 2003) and both arbuscular (Heinemeyer et al., 2006) and ectomycorrhizal fungi (Malcolm et al., 2008), as well as in lab grown saprotrophic basidiomycetes (Crowther and Bradford, 2013). In plants, however, the exact response is thought to be variable and is dependent on the species and temperatures being compared (Atkin et al., 2005). Possible reasons for this acclimation to new temperatures include the maintenance of isoenzymes with different temperature optima (Grzymski et al., 2008) or changes in enzyme conformational flexibility (Clarke, 2004; D'Amico et al., 2006). There are also trade-offs in plant evolutionary strategies between potential growth rates, resource

acquisition, reproductive effort and timing and longevity (Grime and Pierce, 2012), which make plant acclimation to rising temperatures a logical adaptive strategy.

However, apparent evidence of compensatory acclimation within microbial communities as a result of increased soil temperatures is a highly debated issue (Hartley et al., 2007, 2008, 2009; Bradford, Davies, et al., 2008; Allison et al., 2010; Bradford et al., 2010; Tucker et al., 2013). Many studies attribute this phenomenon to substrate depletion rather than a change in microbial physiology (Kirschbaum, 2004; Eliasson et al., 2005; Knorr et al., 2005; Hartley et al., 2007, 2008; Rousk et al., 2012). Bárcenas-Moreno et al., (2009) propose three potential mechanisms that might result in the change in community temperature response resulting in the observed decrease in respiration after an extended period of heating: firstly, acclimation as stated above where growth at a given temperature results in a phenotypic advantage with no genotypic change. Secondly, evolution within a species (i.e. genotypic adaptation) and thirdly, species sorting. Species sorting (i.e. selecting for those species that thrive best at a particular temperature over those who work less well) is the most likely cause of the observed changes as even small genotypic changes take several hundred generations to become evident (Bennett et al., 1990).

1.5 Summary and aims

This literature review has identified a lack of information regarding how soil enzymes will respond to climate change, especially those involved in N-mineralization. There is scope for work on how specific reactions involved in N-mineralization respond to temperature both those experienced at present and those predicted to result from climatic change. There is also a big gap in the literature concerning trends in soil

temperature and how these compare with trends in air temperature. There are important implications of both soil temperature and rates of N-mineralization in terms of food security, the ability of the soil system to sequester carbon and for nitrate leaching into surface waters. To try to address some of these highlighted problems, the main aims of this thesis are:

1. To determine long term trends in soil temperatures from a variety of sites and to compare these to trends in air/surface temperatures.

2. To determine the effect of temperatures regularly experienced in the field on the measured enzyme activity of the soil in the lab.

3. To assess the effect of differing organic matter quality on enzyme activity and how this combines with the effects of temperature to influence N-cycling in this soil.

4. To measure the effects of seasonal temperatures and elevated temperatures (as a simulation of climate change) on N-mineralization in the field.

Chapter 2: Trends in long-term soil temperature data from around the world.

2.1. Introduction

Soil warming has been proposed as a driver for the increased release of greenhouse gases (GHGs), such as CO_2 and N_2O, from terrestrial stores (Kirschbaum, 1995; Cox et al., 2000a; Agehara and Warncke, 2005; Knorr et al., 2005; Davidson and Janssens, 2006). To date, debate continues as to whether any effects of increased soil temperatures will be on-going or short-lived (particulary with respect to soil respiration (Liski et al., 1999; Giardina and Ryan, 2000; Bradford, Fierer, et al., 2008; Hartley et al., 2009; Gillabel et al., 2010). Many other soil processes important to ecosystem functioning are also likely to be affected by changes in soil temperature. For example, soil temperature is of high importance to organic matter decomposition (Kirschbaum, 1995; Bosatta and Ågren, 1999; Cox et al., 2000a; Kirschbaum, 2006; Bradford, Fierer, et al., 2008; Hartley et al., 2008), see section 1.4 for further detail), nutrient cycling (Chapin et al., 1979; Schmidt et al., 1999 Cookson et al., 2002; Deslippe et al., 2005), and primary production (Weih and Karlsson, 2001; Mellander et al., 2004; Euskirchen et al., 2006; Dawes et al., 2011).

Although many laboratory experiments have been carried out to assess the effects of temperature on soil processes it is often unclear how laboratory (Bradford, Fierer, et al., 2008) incubation temperatures are chosen (Andrews et al., 2000; Barrett and Burke, 2000; Steinweg et al., 2008). Some studies state use of mean annual temperature (e.g. Bradford, Fierer, et al., 2008), but it is sometimes unclear if this is

derived from air or soil temperature (e.g. Barrett and Burke, 2000). Other studies deploy a wide range of temperatures but they miss the cold extremes and greatly exceed the hot extremes that could be expected in soils. Rarely have such studies extended as low as 0 °C but and often they include high maximum values that are unlikely to be experienced beyond the upper few centimetres of the soil profile in temperate regions (Horton et al., 1996).

The poor selection of sensible soil temperatures in the literature may be due to a lack of good quality, readily accessible, long-term soil temperature data sets. In comparison with air/surface temperatures, there are not only few soil temperature data sets but those that are available are often shorter and more prone to missing data than their air/surface counterparts. Collecting soil temperature data is more difficult than collecting air temperature data for a variety of reasons, for instance, availability of an appropriate site to be left unused by more traditionally productive purposes, the expense of monitoring temperature at various soil depths, and even taking decisions regarding which depths to record temperature.

The soil temperature data sets that do exist often contain a systematic shift. For example, if records are old enough (prior to 1961) in the UK, common recording depths were measured in inches and were changed with the conversion to metric from 4, 8, 12, 24 and 48 inches (10.2, 20.3, 30.5, 61 and 121.9 cm respectively), typical prior to 1961, to 5, 10, 20, 30, and 100 cm in common use today (BADC 2011 www.badc.nerc.ac.uk).

Completeness of soil temperature data sets is also more difficult to achieve than for air temperature; for example, recording soil temperatures year round can be difficult

as suspended thermometers may become frozen into the ground or covered with snow in winter. The daily removal of these thermometers from the ground increases the likelihood of breakages; this could result in extended periods of missing data due to delays in the replacement of equipment (for example, the paper records of the Mylnefield Meteorological Station show delays prior to restarting data collection after thermometers are broken (personal observation)).

Soil temperature data collected from networks within FLUXNET (http://fluxnet.ornl.gov/) have been used to look at the effects of soil temperature on when in the year forests become net carbon sinks (Baldocchi et al., 2005), rates of evapotranspiration (Fisher et al., 2008) and C-flux partitioning within ecosystems (Misson et al., 2007). Data from this network have also been used to assess changes in soil moisture dynamics over extended periods of time (years to decades) (Miller et al., 2007; Zhang et al., 2009) but there has been no analysis of soil temperature trends through time despite temperatures being recorded (at 2, 5, 8, and 10 cm depths) at many sites (Baldocchi et al., 2005).

Those studies of soil temperatures which do exist often focus on soil temperature in relation to changing distribution of permafrost (Walegur and Nelson, 2003; Chudinova et al., 2006), look at soil temperature trends in one particular geographic location ((He and Zhang, 2013; Maich et al., 2011) or consider only average data rather than trends in daily observations (Gilichinsky et al., 1998). None of the published work identified during the literature search discussed in section 1.2.1 examines daily soil temperature trends over an extended period of time using high quality data sets collected at a variety of sites under different climatic regimes. This chapter will focus on using 5

such data sets to determine both annual and seasonal soil and air temperatures over several decades. This will provide information about how soil temperatures have changed at mid-latitude sites over time and then discuss how this might affect important ecosystem services such as food security in the future.

2.2. Hypotheses

1. In line with recorded changes in air temperature, soil temperatures have increased over time.

2. Air temperatures and soil temperatures recorded at the same site will not, however, change at the same rate.

3. Changes in soil temperature will differ between depths, reflecting contrasts in soil thermal conductivity and heat capacity.

4. Changes in soil temperature will differ between sites.

5. There will be seasonal differences in the rates of change of temperatures.

2.3. Materials and Methods

2.3.1. Data sets

Data were obtained from five mid-latitude sites, three in Canada and two in the UK. The distribution of these sites represents a variety of different climatic conditions including maritime, continental and high altitude (Figure 2.1). These sites were specifically chosen not only for the representation of different climatic regimes but also because they each had long-term records of both soil and air temperatures.
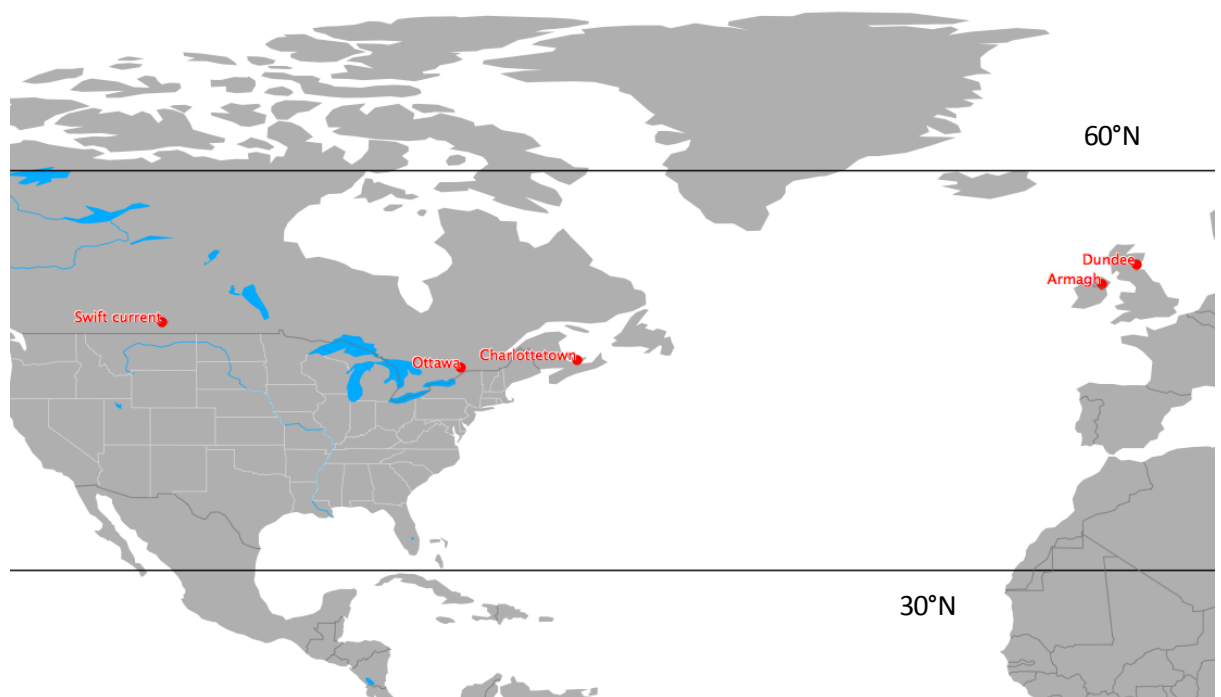
Figure 2.1: Map showing distribution of sites from which meteorological data were obtained.

Dundee

Data from the Mylnefield Meteorological Station (situated at the James Hutton Institute's Dundee site (56.45°N, 3.07°W, altitude 32 m above sea level (a.s.l)) was obtained from the British Atmospheric Data Centre (BADC; badc.nerc.ac.uk). Data is

recorded daily at 9 am (GMT) at this station for a range of climate variables including air temperatures (maximum and minimum), soil temperatures (at 10, 20, 30, 50 and 100 cm depths), grass temperatures, wind speed and direction, solar radiation and precipitation. Data are available for all parameters from the 1st of January 1959 to the 31st of December 2012.

Armagh

The meteorological station at the Armagh Observatory (54.21°N, 6.39°W, altitude 60 m a.s.l) has been collecting weather data since 1795 (Butler et al., 2005; García-Suárez and Butler, 2006). Currently this site is home to an automatic weather station but also continues to take daily observations manually. Measurements made include: air temperature (maximum and minimum); humidity; solar radiation; wind speed and direction; air pressure; precipitation; ground and soil temperatures (at 30 and 100 cm). All observations are made daily at 9 am (GMT). Data were obtained from the Armagh Observatory Meteorology Data Bank website (climate.arm.ac.uk) where data for soil temperature are available from 21st of April 1904 to 31st December 2003 and air temperature data is available from the 1st of January 1844 to the 31st of December 2004.

Ottawa

The meteorological station at the central experimental farm in Ottawa (45.23°N, 75.43°W, altitude 79 m a.s.l) reports daily measurements (8 am EST) of air temperature (maximum and minimum), soil temperature (at 5, 10, 20, 50 and 100 cm depths) as well as precipitation and snow depths. These measurements have been

recorded and reported since the 1st of January 1958 to the 31st of December 2003; data were provided by Environment Canada.

Charlottetown

The meteorological station in Charlottetown, Prince Edward Island (46.25°N, 63.13°W, 23 m a.s.l.) reported daily measurements (8 am, Atlantic Time) of maximum and minimum air temperature, precipitation, snow depth and soil temperatures (at 5, 10, 20, 50, 100 and 150 cm depths) from the 1st of January 1961. Reporting of soil temperature ceased in May 1998 but was also fragmented in the 2 – 3 years prior to this; the air temperature data used here also ends on the 31st of May 1992. All data were provided by Environment Canada.

Swift Current

The meteorological station at Swift Current, Saskatchewan (50.17°N, 107.41°W, altitude 825 m a.s.l.) has been reporting daily measurements (8 am, Central Time) of air temperature (maximum and minimum), total precipitation, snow depth, and soil temperatures (at 5, 10, 20, 50 and 150 cm depths) since the 1st June 1962 up to the 31st of December 2003. Maximum and minimum air temperature data were available from the 1st January 1959 until the 31st December 2006; all data were provided by Environment Canada.

For a summary of sample site information see table 2.1.

Table 2.1: Summary table containing information about data collection sites

Site location	Site code	Latitude	Longitude	Altitude (m.a.s.l)	Start year	End year	Measurement depths (cm)	Winter snow cover?
Armagh, UK	AR	54.21°N	6.39°W	60	1904	2003	30, 100	No
Dundee, UK	DD	56.45°N	3.07°W	32	1959	2012	10, 20, 30, 50, 100	Rarely
Charlotte-town, Canada	CT	46.25°N	63.13°W	23	1961	1992	5, 10, 20, 50, 100, 150	Yes
Ottawa, Canada	OTW	45.23°N	75.43°W	79	1958	2003	5, 10, 20, 50, 100	Yes
Swift Current, Canada	SC	50.17°N	107.41°W	825	1962	2003	5, 10, 20, 50, 100, 150	Yes

2.3.2. Data Set Preparation

Data from each site were visually inspected to look for systematic errors and large periods of missing data. Soil temperature data from Dundee was found to have a large number of duplicate or triplicate entries particularly towards the latter stages of the series. These were individually inspected and if all entries were identical the first was retained and subsequent entries deleted. If entries were not identical, those with improbable values (e.g. 40.0 °C compared to the more sensible value of 4.0 °C) were removed. Data from Armagh had previously had -888 entered for days where data was unavailable and -999 for data that did not exist (e.g. days that did 'not exist', such as the 29th of February in non-leap years). Data from Ottawa had -999 for data that were unavailable so these were replaced with NA.

Air temperature data from each site were collated into one spread sheet and a loop constructed in R (R Development Core Team, 2010) to compare the date of each observation with a standardised list of dates. In this way each entry for each site was checked for missing-ness or multiple entries. In all data sets, for missing values an "NA" was inserted and the first of any multiple entries was retained. Again, any entries equal to -999 or -888 were replaced with NA.

2.3.3. Time series length

The length of available data differed for each site; data had initially been obtained for Armagh, Dundee and Ottawa. Based on these sites it was decided to analyse data from the 1st of January 1959 to the 31st of December 2003 as this was the longest period common to all three datasets. Data obtained for Charlottetown and Swift Current had been recorded over a shorter time period; it was decided to maintain the length of the original three data sets so as to lose as little information as possible.

2.3.4. Data Analysis

Soil and air temperature data – Periodic Regression

Each set of soil or air temperature data were analysed using periodic regression analysis (Bliss, 1958; Pelat et al., 2007). Periodic regression incorporates a partial Fourier series into a linear regression where the relationship between the dependent and the independent variables is known to be periodic (e.g. the regular, cyclical pattern of temperatures per year). Trigonometric functions of the independent variable are used instead of fitting a polynomial (Equation 2.1) (Bliss, 1958). This breaks the periodic component of the data down into simple trigonometric functions

that can be summarized using a smaller number of parameters; the harmonics are also orthogonal and as such have no issues of auto-correlation (Bliss, 1958). Using this type of analysis allows the specification of any number of harmonics per cycle (here assumed to be 365 days) which means that much of the variation in the data can be captured in the model. There is only one oscillation in temperature annually but adding a biannual harmonic takes into account the asymmetry of the annual peak and further harmonics of shorter time periods will help account for any remaining variation. It is possible to add many of these harmonics to the model – even though they are likely to become less meaningful – without affecting the results of important harmonics because each is completely independent of all others. Use of periodic regression is superior to linear regression for analysing long term temperature data sets due to the incorporation of a sine function which helps to take into account the issue of temporal autocorrelation.

Selecting harmonics of known frequency (here 12, 6 and 3 months) allows examination of the amplitude (Equation 2.2) and phase (Equation 2.3) of each harmonic for each time period (shown in Figure 2.2). Assigning a periodic series to these few, biologically meaningful, parameters has been practically used to examine the seasonality of biological patterns (Bliss, 1958; Olsson and Eklundh, 1994; Rogers et al., 1996). However, a single model of temperature for any given depth and site showed changes in the amplitude and phase over the full 45 year time range. This temporal non-stationarity of the mean can confound the estimates of the errors so must be controlled. By examining each year separately this was eliminated, but also allowed linear models to be constructed for the phase (timing of the peak of temperature

which tells us about the timing of seasons) and the amplitude (the height of the peak which tells us about seasonal temperature variability) for each site and depth (this can be seen in Figure 2.2). To illustrate this further, if a harmonic is found to have a larger amplitude than the same harmonic for the previous year this indicates an increase in the peak temperature. If a harmonic is found to have a larger phase than the same one the year before this means that the rate of temperature change has slowed (during one year).

The fitted values from the periodic regression were subsequently used to calculate the mean, minimum, maximum and proportional range ((maximum–minimum) / mean). The proportional range was used to examine changes to the temperature range relative to the annual mean. In communities experiencing a narrow range of temperatures, the same absolute change might be more biologically (or chemically) significant than the same absolute change for communities experiencing a wider range of temperatures. This is because those communities already subject to large fluctuations in temperature throughout the year may be less susceptible to changes in annual temperature range than those under temperature regimes where the fluctuation is smaller. Because the proportional mean is skewed by the mean (i.e. the same absolute range is proportionally larger as the annual mean approaches 0 °C given that the Celsius scale was used) it describes a curve that emphasizes temperature changes in the range when the mean annual temperature is lower. The bias at 0 °C is potentially convenient, though an arbitrary point, as this corresponds to the physical changes in water availability, however, given this bias the statistical significance of these results must be interpreted cautiously. A linear regression was then fitted

through each of these variables and this was used to calculate the annual trend in temperature and the absolute change in °C over the entire time period.

Equation 2.1:

$$Y = m + \cos(1 * w * t) + \sin(1 * w * t) + \cos(2 * w * t) + \sin(2 * w * t) +$$
$$\cdots \cos(n * w * t) + \sin(n * w * t)$$

Where m = mean, w = 2*π/k (where w =frequency and k = length of one cycle (e.g. 365 days)), t = time, and 1 and 2 are the number of harmonics per cycle.

Equation 2.2:

$$amplitude = \sqrt{(\sin^2 + cos^2)}$$

Equation 2.3:

$$phase = \tan^{-1}(\sin - \cos)$$

2.3.5. Seasonal trends

In order to analyse trends in seasonal temperatures, each year was broken down into four parts consisting of 3 months each: Spring = March, April and May; Summer = June, July and August; Autumn = September, October and November; and Winter = December, January and February. Because the definition of winter means that this season straddles two years, data from December was attributed to the following year, for example; December 1999 contributed to the winter of 2000.

The mean temperature of each 3 month period was calculated and a linear regression fitted to each seasonal data set. This was then used to calculate the changes in seasonal air and soil temperature over the entire time series.
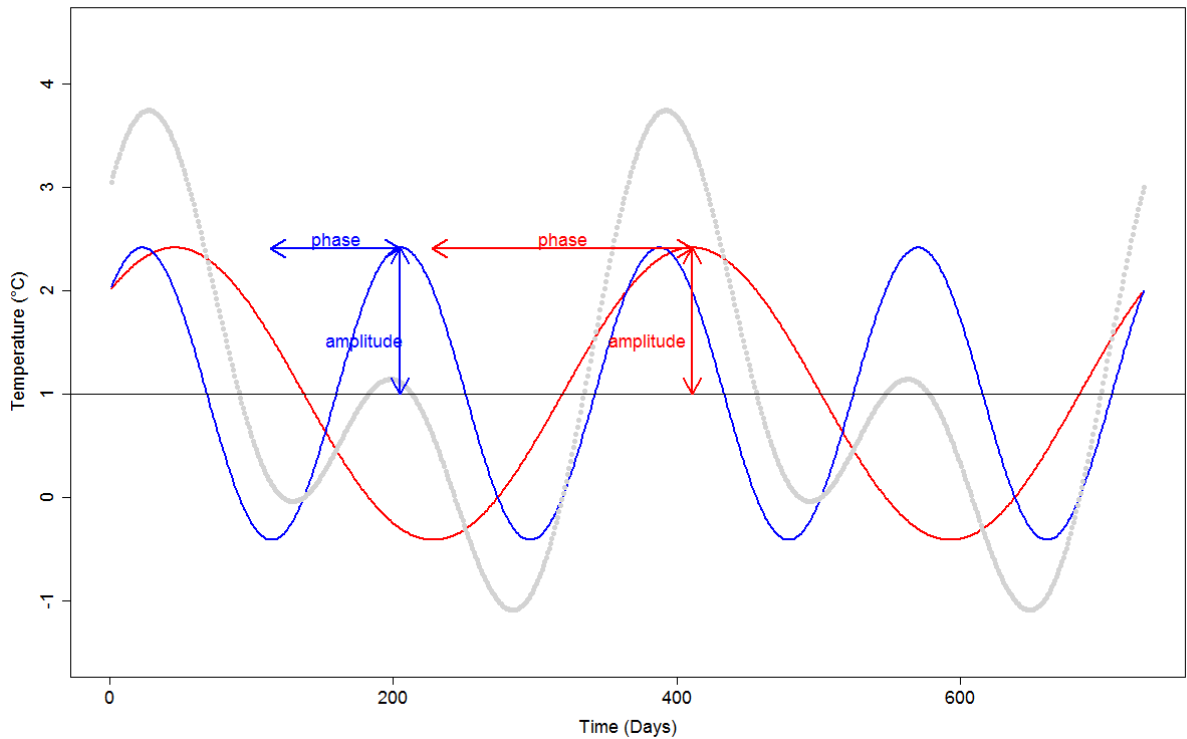
Figure 2.2: illustration of periodic regression built using 2 harmonics of known frequency and a mean of 1. Red line shows an harmonic with one oscillation per cycle (annual in this case), blue line shows an harmonic with 2 oscillations per cycle (biannual in this case) and grey points show how these are combined to fit a periodic regression line fitted to the observations.

2.3. Results

2.4.1. Periodic regression

2.4.1.1. Soil temperatures

Table 1 shows the R^2 values for the periodic regressions fitted to temperatures collected from each depth at each. The regression fits closely, with a minimum R^2 of 0.7834 at 100 cm in Armagh and a maximum R^2 of 0.9987 at 150 cm in Swift Current. The model fitted here had 3 harmonics – an annual, a biannual and a quarterly – the phases and amplitudes for each of which were determined using the periodic regression and then used in a linear regression to check for changes over the time series to assess temporal non-stationarity in the annual variation (amplitude) and seasonal timing (phase). The quarterly harmonic showed no consistent change in either phase or amplitude over the length of the time series, while the biannual harmonic generally showed no differences over time with two exceptions; the phase at 100 cm in Dundee showed a significantly negative trend (p = 0.0237), i.e. the peak became earlier over time. The amplitude at 150 cm in Swift Current also showed a significantly negative trend (p = 0.0251) suggesting the temperature range narrowed over time. Changes in the annual harmonic over time are more substantial and widespread; the amplitudes changed significantly in the deeper layers (30 – 100cm) in Dundee and Armagh as well as the shallower and mid-layers at Swift Current (5 – 50 cm) (Figure 2.3). Phases of the annual harmonic also changed significantly over time in a few places; the shallower layers at Dundee (10 and 20 cm) and the deeper layers at Swift Current (100 and 150 cm), as well as two depths at Charlottetown (20 and 100 cm), were changed over time (Figure 2.4).

2.4.1.2. Air temperatures

The same analysis was conducted using mean air temperatures from each of the five sites; Table 2.2 shows the fit of the models to the data. Although poorer than for soil temperature, the regression has a good fit to the air temperature data with a minimum R^2 of 0.6833 at Armagh and a maximum R^2 of 0.9020 at Swift Current. When examining the amplitude and phase values for air temperatures, few systematic trends were found. For the amplitude the only statistically significant result was a negative trend in the biannual harmonic for Ottawa (p = 0.042), for the annual harmonic there were negative trends at Ottawa and Swift Current; these were not quite statistically significant at the 95% level but may still be biologically relevant (p = 0.0618 and 0.0561 respectively) (Figure 2.5). The phase for air temperature data was similar with no statistically significant results at the 95% level although for the biannual harmonic Armagh shows a negative trend (p = 0.0640) and for the quarterly harmonic Charlottetown also shows a negative trend (p = 0.0795) (Figure 2.6).

Table 2.2: The R^2-values relating to the year where the periodic regression has the best fit (maximum R^2), the poorest fit (minimum R^2) and showing the average fit overall years (mean R^2) for soil temperatures at each depth at each site.

Site	Depth (cm)	Minimum R^2	Mean R^2	Maximum R^2
Armagh	30	0.9440	0.9620	0.9751
	100	0.7834	0.9817	0.9944
Dundee	10	0.8410	0.9000	0.9317
	20	0.8681	0.9217	0.9521
	30	0.9295	0.9586	0.9748
	50	0.9535	0.9738	0.9868
	100	0.9827	0.9901	0.9963
Charlottetown	5	0.9279	0.9621	0.9794
	10	0.9520	0.9710	0.9847
	20	0.9620	0.9768	0.9886
	50	0.9694	0.9862	0.9941
	100	0.9817	0.9910	0.9972
	150	0.9848	0.9933	0.9974
Ottawa	5	0.9342	0.9538	0.9724
	10	0.9458	0.9594	0.9749
	20	0.9546	0.9676	0.9823
	50	0.9717	0.9811	0.9911
	100	0.9826	0.9893	0.9943
Swift Current	5	0.8848	0.9230	0.9587
	10	0.8976	0.9362	0.9665
	20	0.9189	0.9525	0.9762
	50	0.9651	0.9793	0.9892
	100	0.9871	0.9926	0.9977
	150	0.9916	0.9954	0.9987

Table 2.3: the R^2 values relating to the year where the periodic regression has the best fit (maximum R^2), the poorest fit (minimum R^2) and showing the average fit over all years (mean R^2) for air temperatures at each site.

Site	Minimum R^2	Mean R^2	Maximum R^2
Armagh	0.6834	0.7625	0.8181
Dundee	0.6853	0.7775	0.8424
Charlottetown	0.7957	0.8571	0.8994
Swift Current	0.8121	0.7931	0.9020
Ottawa	0.6960	0.8626	0.8580
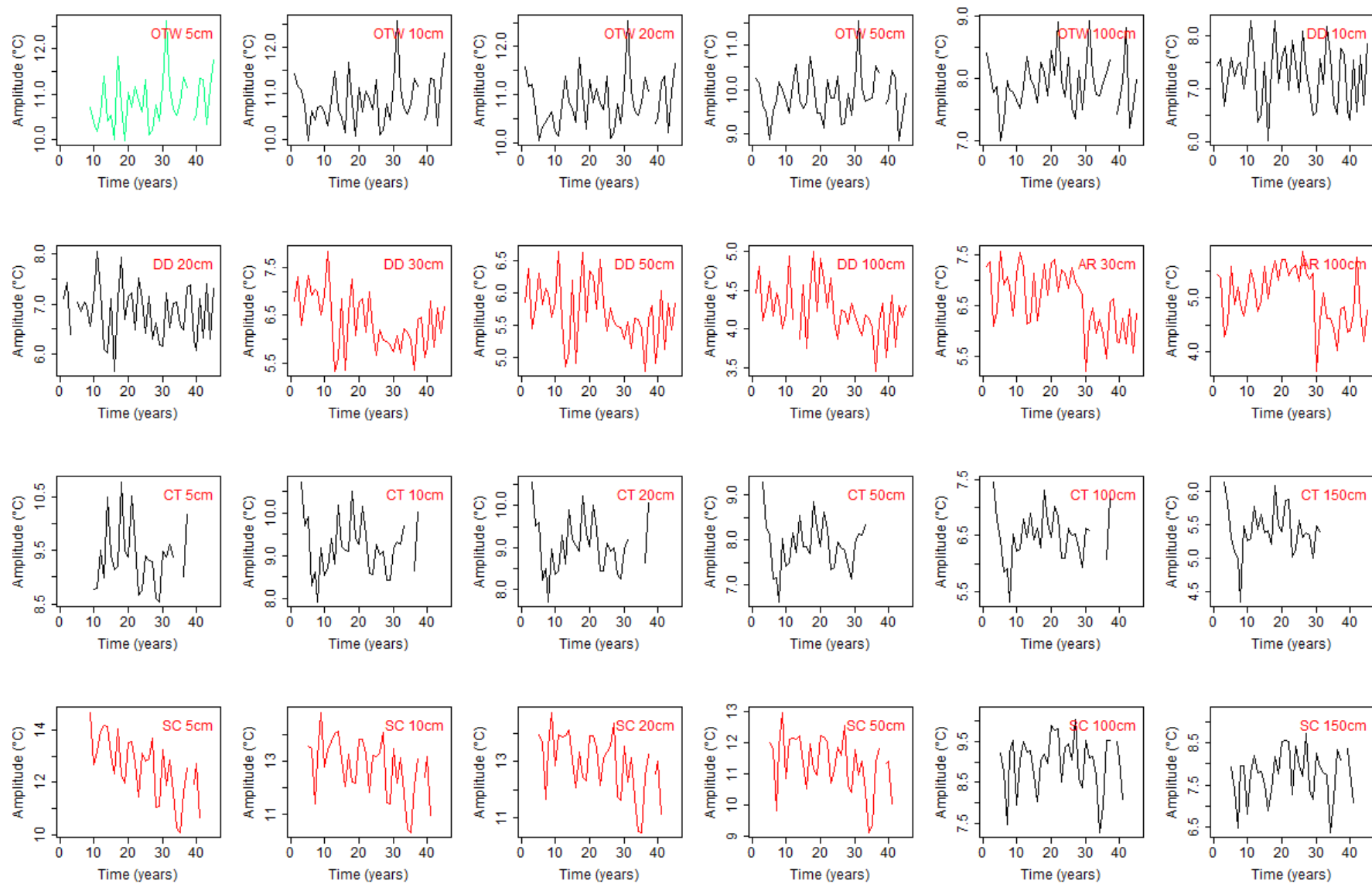
Figure 2.3: amplitudes for the annual harmonic in the periodic regression for each depth at each site, red lines are those with statistically significant trends over time (p<0.05, green lines are those which are close to being statistically significant over time (p < 0.10)). OTW = Ottawa, DD = Dundee, AR = Armagh, CT = Charlottetown and SC = Swift Current.
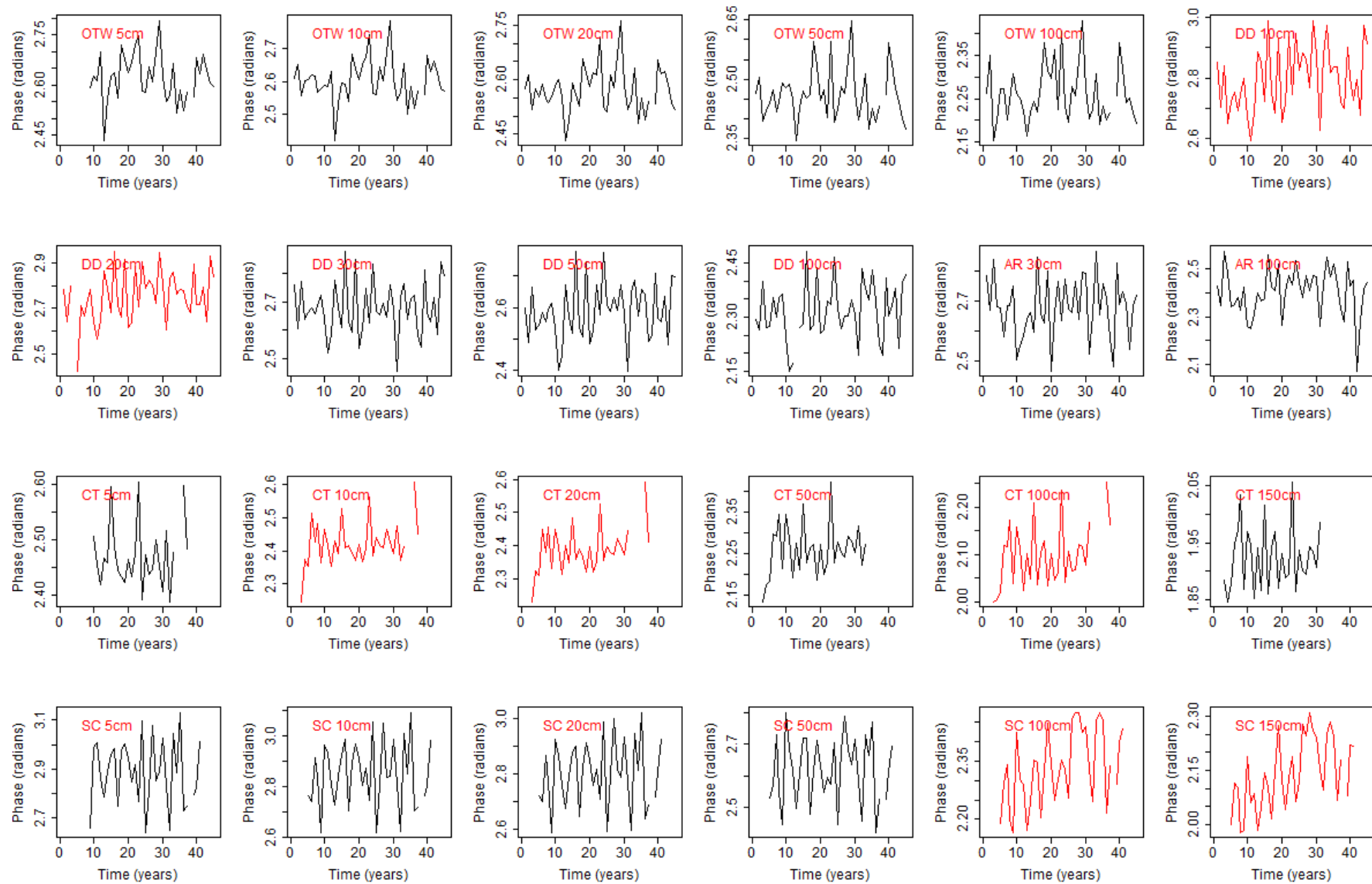
Figure 2.4: phases for the annual harmonic in the periodic regression for each depth at each site, red lines are those with statistically significant trends over time (p<0.05). OTW = Ottawa, DD = Dundee, AR = Armagh, CT = Charlottetown and SC = Swift Current.
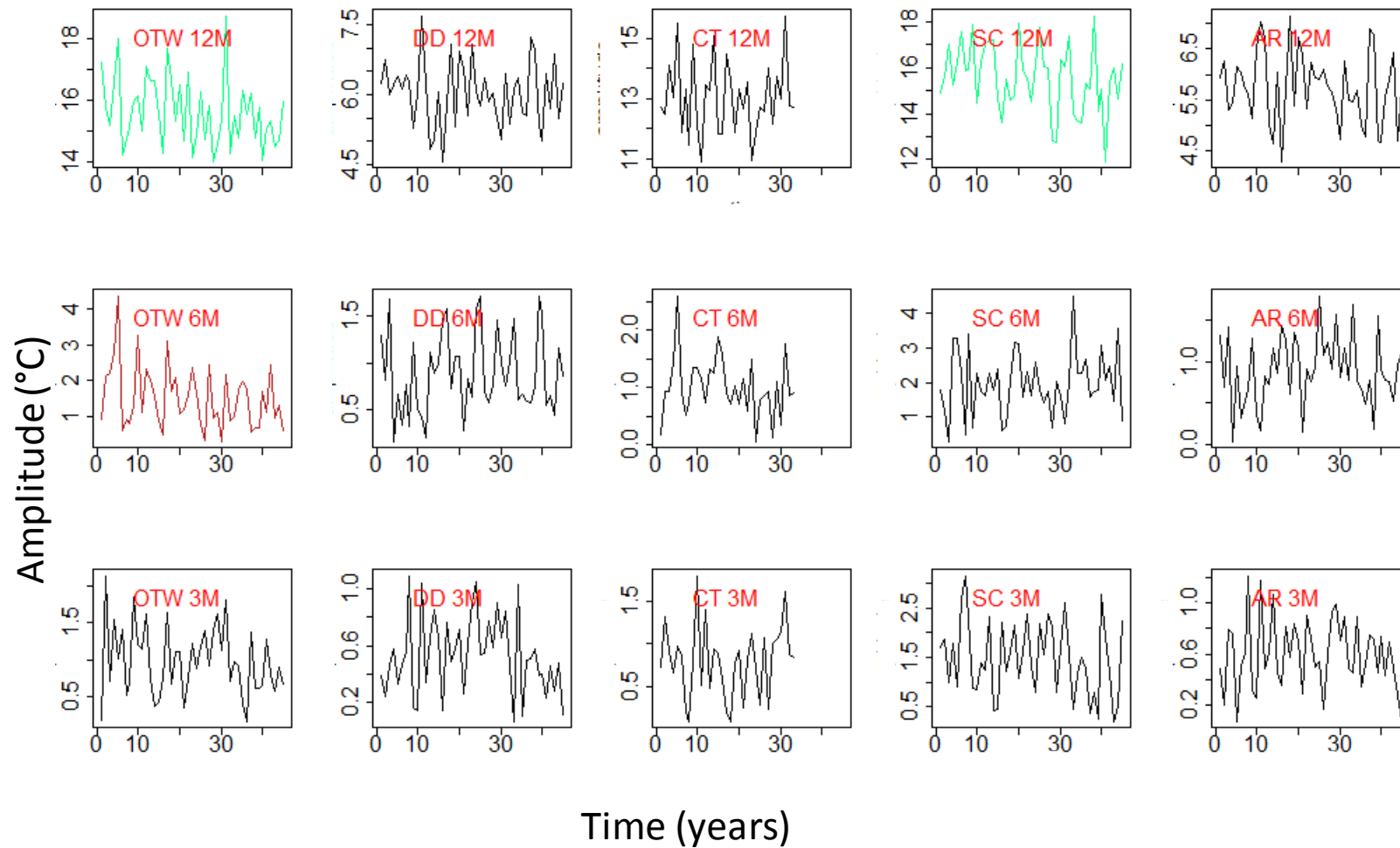
Figure 2.5: amplitudes in the periodic regression for air temperatures at each site, red lines are those with statistically significant trends over time (p<0.05) green lines are those which are close to being statistically significant over time (p < 0.10). Results are included for each harmonic, annual = 12M, biannual = 6M and quarterly = 3M. OTW = Ottawa, DD = Dundee, AR = Armagh, CT = Charlottetown and SC = Swift Current.
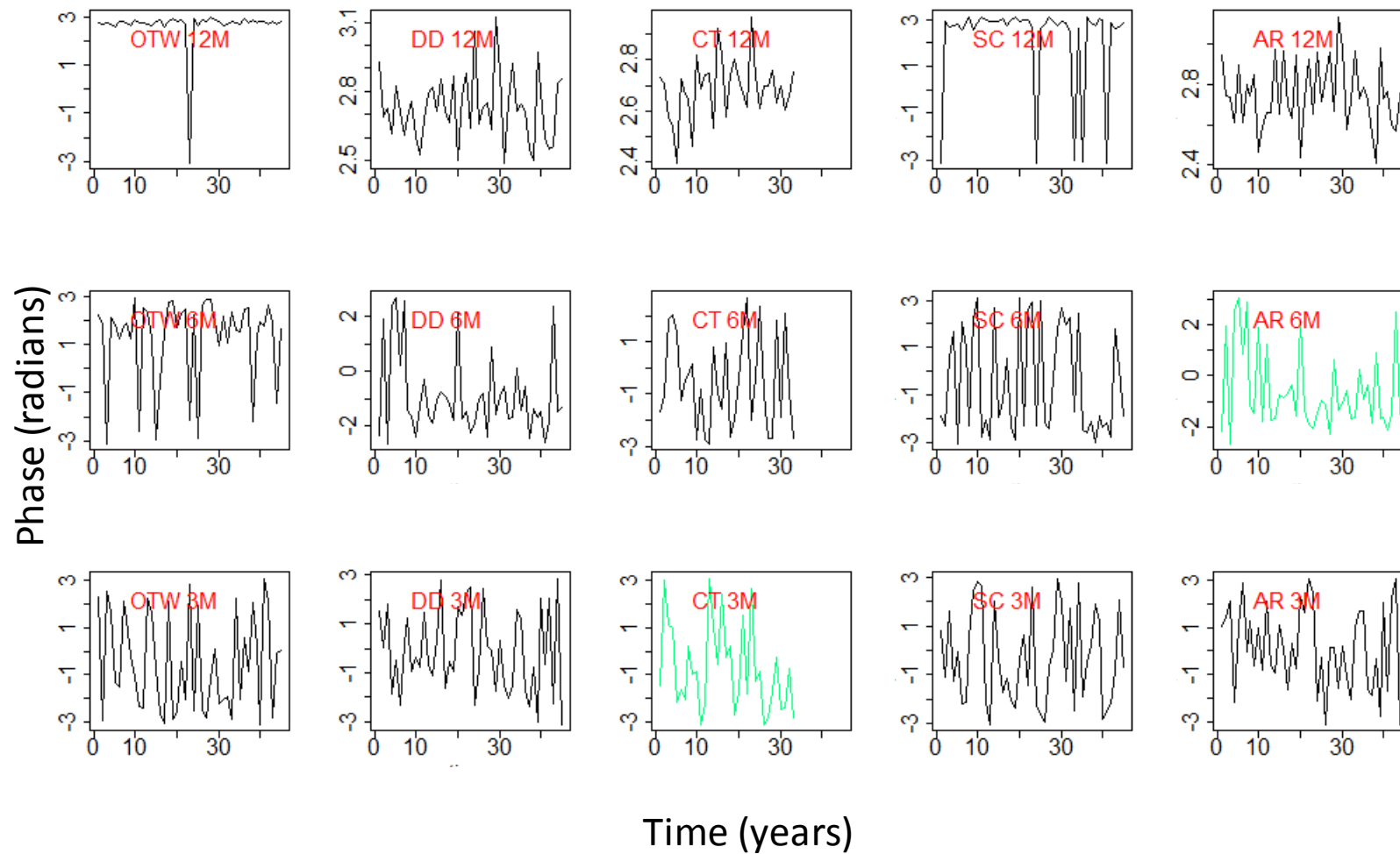
Figure 2.6: phases for the annual harmonic in the periodic regression for air temperatures at each site. Red lines are those with statistically significant trends over time (p<0.05), green lines are those which are close to being statistically significant over time (p < 0.10). Results are included for each harmonic; annual = 12M, biannual = 6M and quarterly = 3M. OTW = Ottawa, DD = Dundee, AR = Armagh, CT = Charlottetown and SC = Swift Current.

2.4.2. Linear Regression of fitted values

2.4.2.1. Soil temperatures

Figures 2.7 – 2.11 show the fitted annual values for mean, maximum and minimum temperatures, and the proportional annual range. A linear regression was then fitted to each of the sets of fitted data. Generally, the mean, minimum and maximum annual temperatures were found to be increasing across all soil depths and all sites. The annual proportional range showed less consistency between depths and sites; some ranges decreased over time (e.g. Swift Current; 5, 10 and 20 cm), some increased (e.g. Ottawa; 5 and 50 cm) but many were stationary (e.g. all depths at Dundee). Table 2.4 gives the annualised gradient (the slope of the regression line) at each depth at each site and the absolute change in °C for each over the length of each data set. The majority of the gradients for mean, minimum and maximum annual temperatures were positive (Figures 2.7 – 2.11). Most of the trends in the mean temperatures were statistically significant; this was also true for minimum, and, to a lesser extent, the maximum temperatures. The proportional ranges showed mainly negative trends; many of these were not statistically significant (hence they appear stable in Figures 2.7 – 2.11), those that were statistically significant tended to be from maritime climates (i.e. Dundee, Armagh and Charlottetown).

2.4.2.2. Air temperatures

Linear trends were calculated for the fitted values from the periodic regression. As with soil temperatures, the annual mean, maximum and minimum air temperatures consistently increased across all sites, but again the response of the proportional range over time was variable between sites (Figure 2.12). Table 2.5 gives the

annualized gradients (the slopes of the regression lines) and absolute changes in °C for mean air temperature at each site. All sites showed increasing mean annual temperatures, and these were all significant with the exception of Charlottetown (which has a shorter time series); this was the same for minimum temperatures although this was only significant at Armagh. The picture was similar for maximum air temperatures, although the absolute changes were smaller and these trends were statistically significant for both Armagh and Dundee. The proportional ranges all had negative trends (statistically significant for Ottawa) but the range of absolute change was wide; from -0.12 °C in Armagh to -3.37 °C in Swift Current.

Figure 2.7: Annualised mean (green), maximum (red), minimum (blue) and proportional range (brown) values for all depths at Armagh. Lines of matching colour are the linear regression lines used to determine the rate of change of each variable.

Figure 2.8: Annualised mean (green), maximum (red), minimum (blue) and proportional range (brown) values for all depths at Dundee. Lines of matching colour are the linear regression lines used to determine the rate of change of each variable. Breaks in data indicate years dropped from the analysis due to missing data.
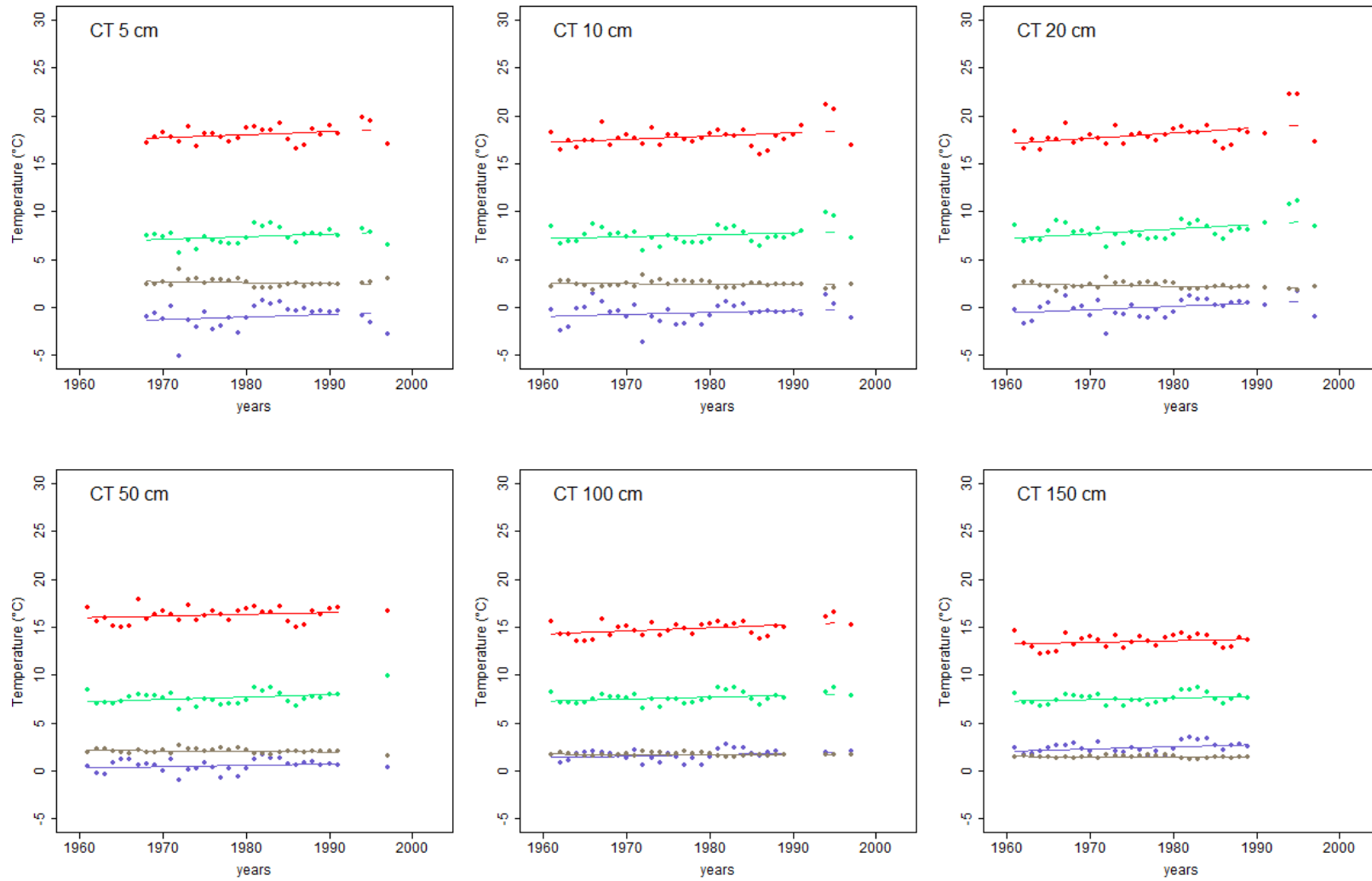
Figure 2.9: Annualised mean (green), maximum (red), minimum (blue) and proportional range (brown) values for all depths at Charlottetown. Lines of matching colour are the linear regression lines used to determine the rate of change of each variable. Breaks in data indicate years dropped from the analysis due to missing data.
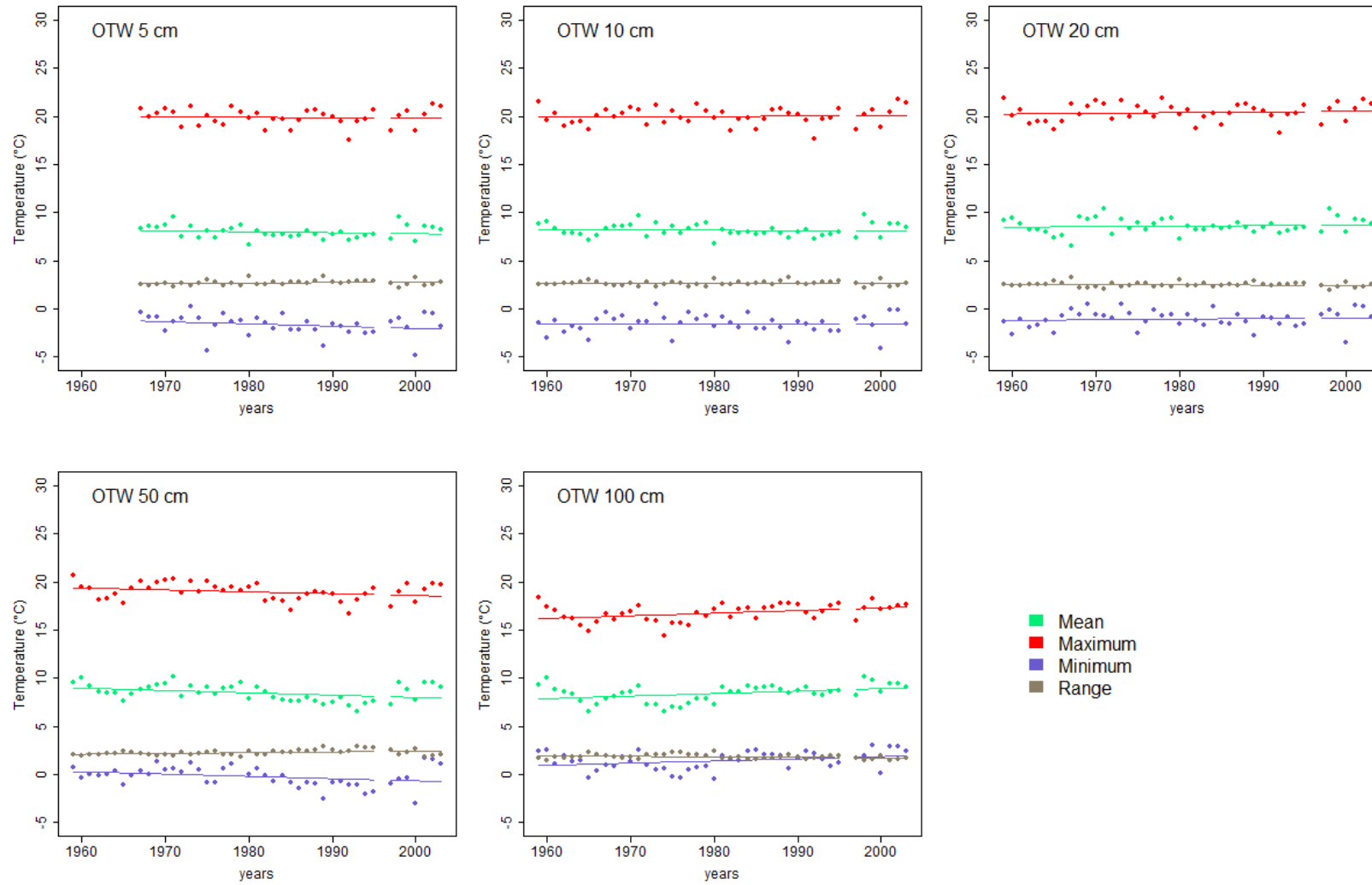
Figure 2.10: Annualised mean (green), maximum (red), minimum (blue) and proportional range (brown) values for all depths at Ottawa. Lines of matching colour are the linear regression lines used to determine the rate of change of each variable. Breaks in data indicate years dropped from the analysis due to missing data.
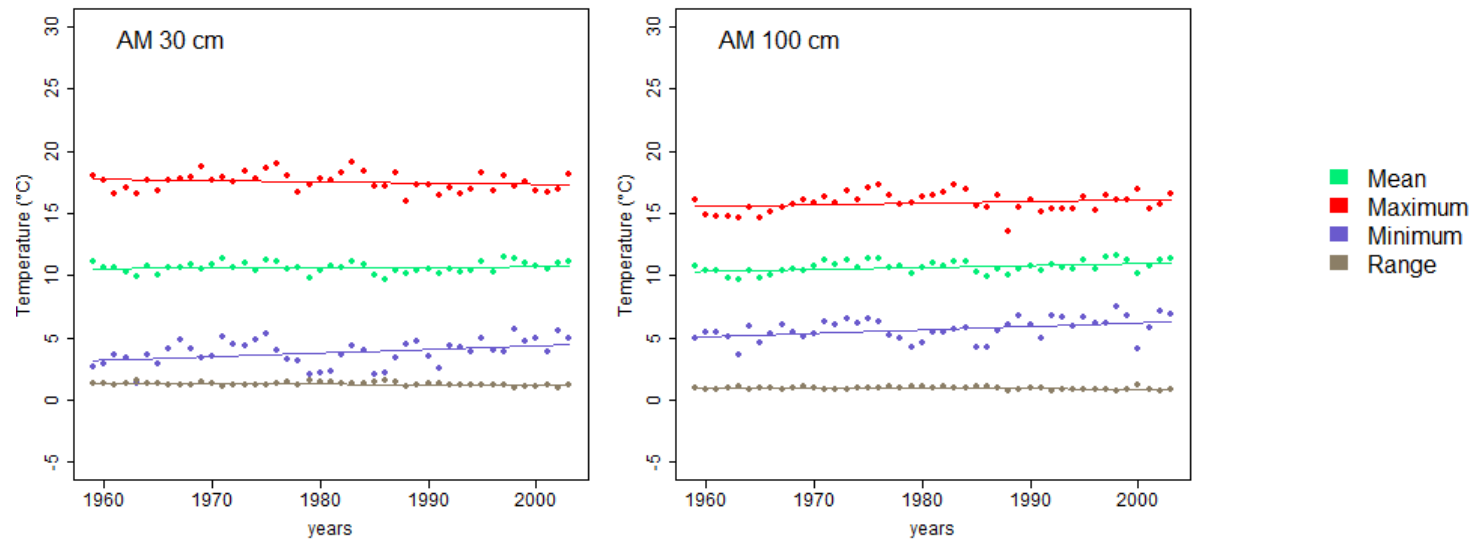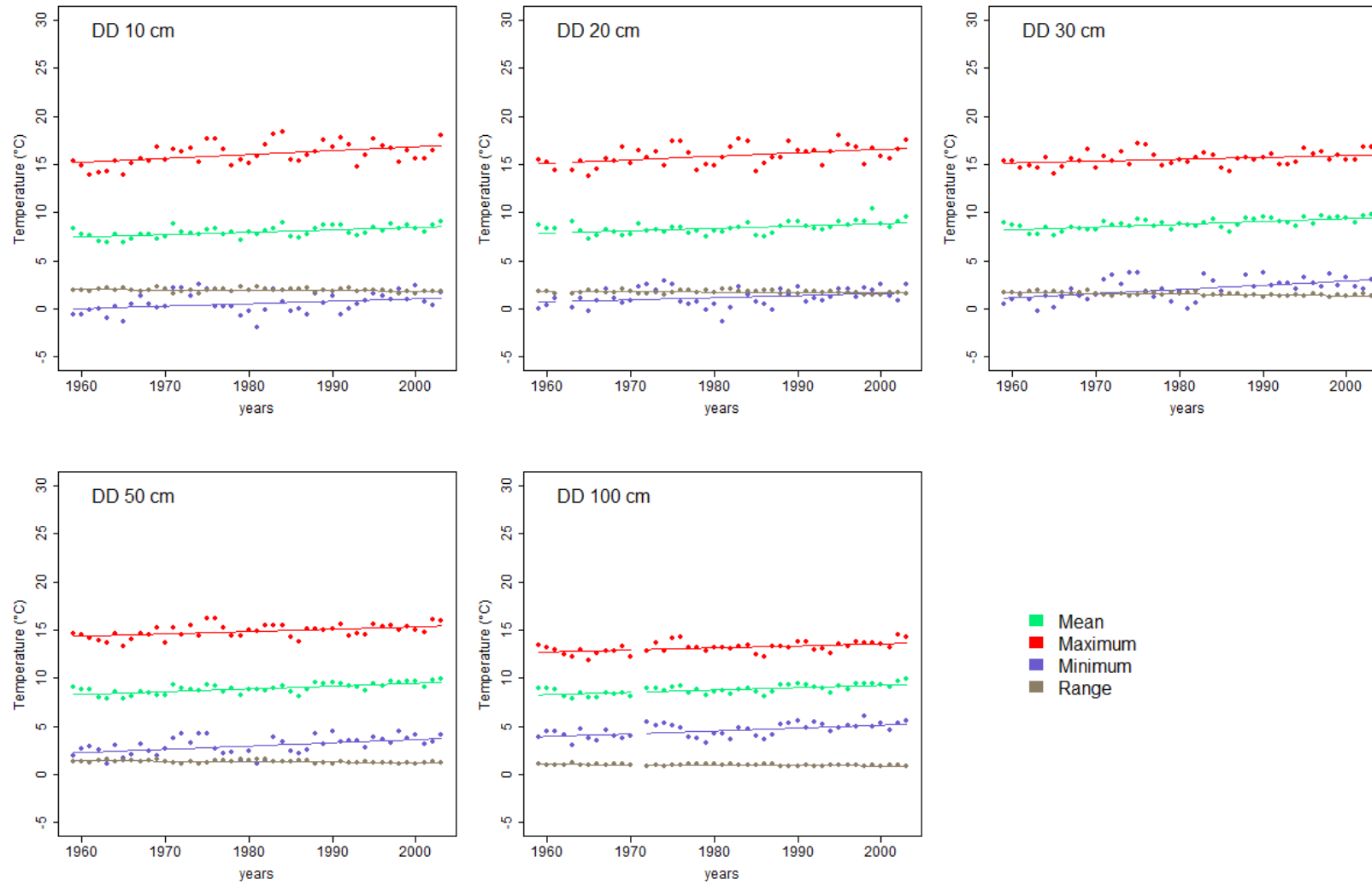
Figure 2.11: Annualised mean (green), maximum (red), minimum (blue) and proportional range (brown) values for all depths at Swift Current. Lines of matching colour are the linear regression lines used to determine the rate of change of each variable. Breaks in data indicate years dropped from the analysis due to missing data.

Table 2.4: Annual trends and total change in temperature over the length of the time series for mean, minimum and maximum temperatures and annual proportional temperature range for each site and each depth. Red cells denote significant positive gradients, blue cells significant negative gradients.

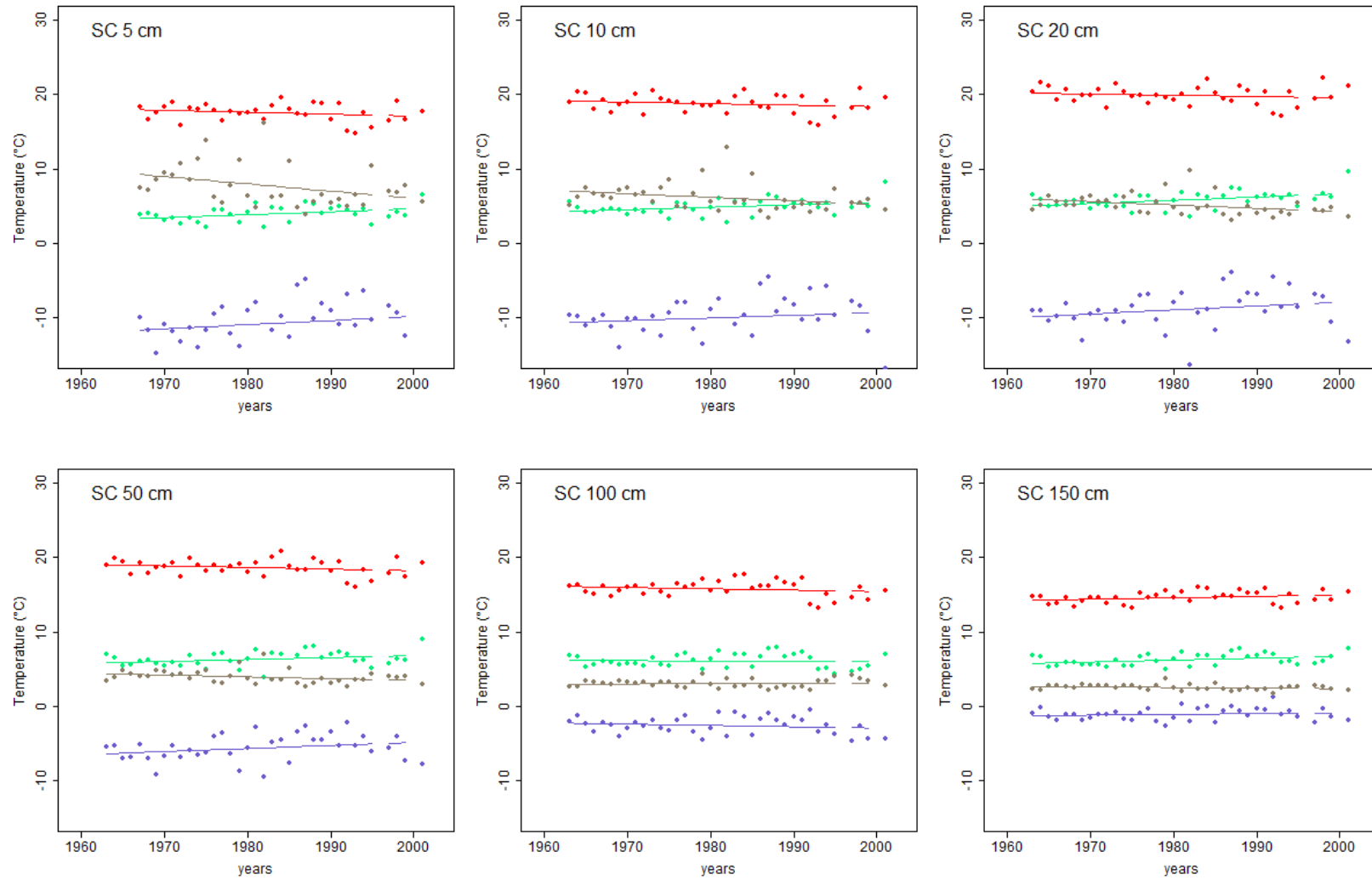| Site	Depth (cm)	N	Mean annual temperatures		Minimum annual temperatures		Maximum annual temperatures		Annual proportional temperature range	
			Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)
Armagh	30	45	0.0034	0.1543	0.0288	1.2938	-0.0098	-0.4406	-0.0040	-0.1788
	100	45	0.0153	0.6905	0.0280	1.2605	0.0132	0.5944	-0.0027	-0.1196
Dundee	10	45	0.0244	1.0979	0.0254	1.1420	0.0409	1.8413	-0.0040	-0.1804
	20	45	0.0245	1.1024	0.0219	0.9876	0.0367	1.6497	-0.0031	-0.1399
	30	45	0.0292	1.3137	0.0431	1.9406	0.0192	0.8662	-0.0079	-0.3548
	50	45	0.0290	1.3028	0.0337	1.5152	0.0247	1.1122	-0.0053	-0.2380
	100	45	0.0246	1.1063	0.0299	1.3464	0.0209	0.9408	-0.0037	-0.1671
Charlottetown	5	29	0.0275	0.7975	0.0268	0.7771	0.0311	0.9016	-0.0106	-0.3072
	10	35	0.0205	0.7184	0.0205	0.7192	0.0341	1.1925	-0.0049	-0.1719
	20	35	0.0487	1.7044	0.0351	1.2294	0.0552	1.9314	-0.0105	-0.3687
	50	35	0.0258	0.9047	0.0140	0.4916	0.0179	0.6261	-0.0057	-0.1987
	100	35	0.0204	0.7144	0.0161	0.5635	0.0327	1.1446	-0.0024	-0.0837
	150	35	0.0168	0.5865	0.0235	0.8230	0.0170	0.5937	-0.0039	-0.1360
Ottawa	5	36	-0.0101	-0.3645	-0.0239	-0.8606	-0.0057	-0.2038	0.0060	0.2146
	10	45	-0.0029	-0.1299	0.0016	0.0713	0.0032	0.1435	0.0013	0.0602
	20	45	0.0053	0.2365	0.0064	0.2883	0.0068	0.3081	-0.0017	-0.0784
	50	45	-0.0245	-1.1019	-0.0235	-1.0572	-0.0199	-0.8939	0.0078	0.3511
	100	45	0.0259	1.1670	0.0225	1.0129	0.0275	1.2375	-0.0053	-0.2405
Swift Current	5	36	0.0396	1.4261	0.0557	2.0043	-0.0261	-0.9389	-0.0983	-3.5379
	10	39	0.0337	1.3137	0.0360	1.4049	-0.0204	-0.7956	-0.0475	-1.8524
	20	39	0.0434	1.6941	0.0534	2.0840	-0.0166	-0.6482	-0.0453	-1.7686
	50	39	0.0257	1.0031	0.0409	1.5963	-0.0215	-0.8396	-0.0241	-0.9411
	100	39	-0.0041	-0.1599	-0.0177	-0.6919	-0.0179	-0.6988	0.0053	0.2085
	150	39	0.0254	0.9902	0.0100	0.3899	0.0194	0.7579	-0.0085	-0.3316
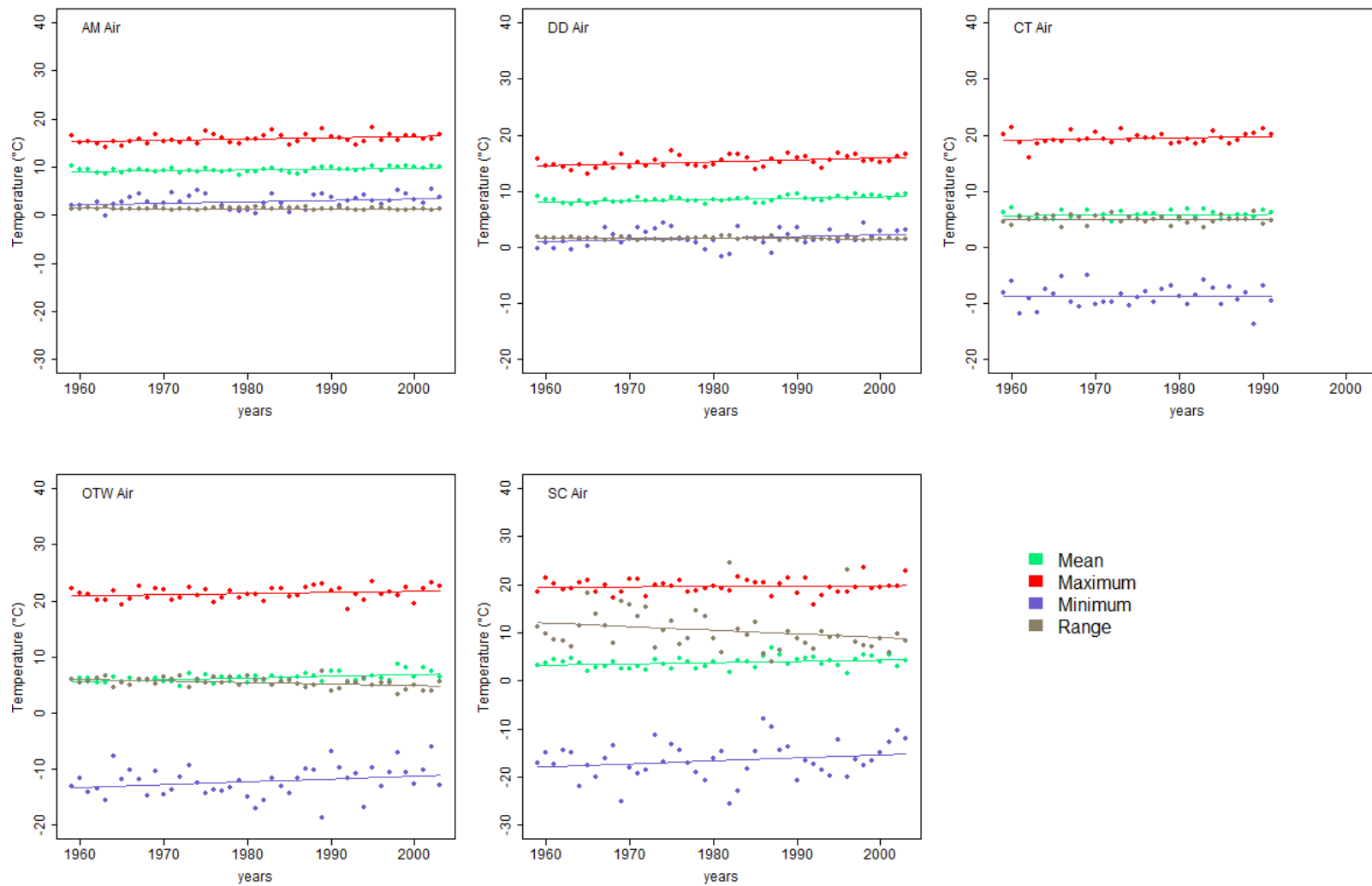
Figure 2.12: Annualised mean (green), maximum (red), minimum (blue) and proportional range (brown) values for mean air temperatures at all sites., Lines of matching colour are the linear regression lines used to determine the rate of change of each variable.

Table 2.5: annual trends in mean air temperatures and total change in temperature over the length of the time series for mean, minimum and maximum temperatures and annual proportional temperature range for each site. Red cells denote significant positive gradients, blue cells significant negative gradients.

Site	N	Annual mean temperatures		Annual minimum temperatures		Annual maximum temperatures		Annual proportional temperature range	
		Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)
Armagh	45	0.0171	0.7688	0.0310	1.3936	0.0272	1.2226	-0.0027	-0.1235
Dundee	45	0.0235	1.0587	0.0295	1.3279	0.0349	1.5707	-0.0036	-0.1633
Charlottetown	32.4	0.0074	0.2385	-0.0045	-0.1464	0.0206	0.668	-0.0027	-0.0874
Ottawa	45	0.0310	1.3945	0.0510	2.2932	0.0202	0.9103	-0.0277	-1.2479
Swift Current	45	0.0264	1.1887	0.0629	2.8316	0.0078	0.3498	-0.0749	-3.3722

2.4.3. Seasonal temperature trends

2.4.3.1. Soil temperatures

Figures 2.13 – 2.18 show the calculated trends in seasonal temperature for each site. The data for Armagh and Dundee (Figures 2.14 and 2.15 respectively) suggested increases in temperature for each depth at each site (with the exception of 30 cm depth at Armagh, which appeared stable or even to have decreased slightly). Generally for these two sites winter and spring temperatures increased more rapidly than summer and autumn temperatures; this was supported by the absolute changes in temperature shown in Table 2.6, where winter changes were almost always greater than those in other seasons but were mostly closely matched by changes in spring temperatures. Charlottetown (Figure 2.16) showed increases for each depth but with a greater emphasis on spring temperature changes (Table 2.6). The data for Ottawa (Figure 2.17 and Table 2.6) showed no trends except at the 50 and 100 cm depths; at 50 cm temperatures in all seasons decreased whereas the 100 cm temperatures in all seasons appeared to increase. Summer temperatures in the upper 50 cm at Swift Current decreased before stabilizing at 100 cm and beginning to increase again at 150 cm (Figure 2.18). The winter temperatures in the upper 50 cm increased but seemed to be decreasing at both 100 and 150 cm. Spring temperatures at Swift Current generally appeared to be increasing whilst autumn temperatures remained largely stable (Figure 2.18 and Table 2.6).

2.4.3.2. Air temperatures

Air temperature rates of change in Armagh and Dundee were roughly equivalent in different seasons and all increased slightly; for Ottawa and Swift Current, however,

winter temperatures seemed to increase more rapidly than those in spring, summer and autumn (Figure 2.19). Charlottetown was different, with both winter and autumn temperatures showing negative trends. Table 6 shows that both Dundee and Ottawa had statistically significant positive trends in summer, autumn and winter temperatures, though the absolute change was greatest in winter temperatures. Armagh and Swift Current also showed statistically significant positive trends in winter temperatures   (table   2.7).

Figure 2.14: Mean seasonal soil temperatures; spring = green, summer = red, autumn = brown, winter = blue for each depth at Armagh. Lines of corresponding colour are the linear regression line used to calculate temperature change.
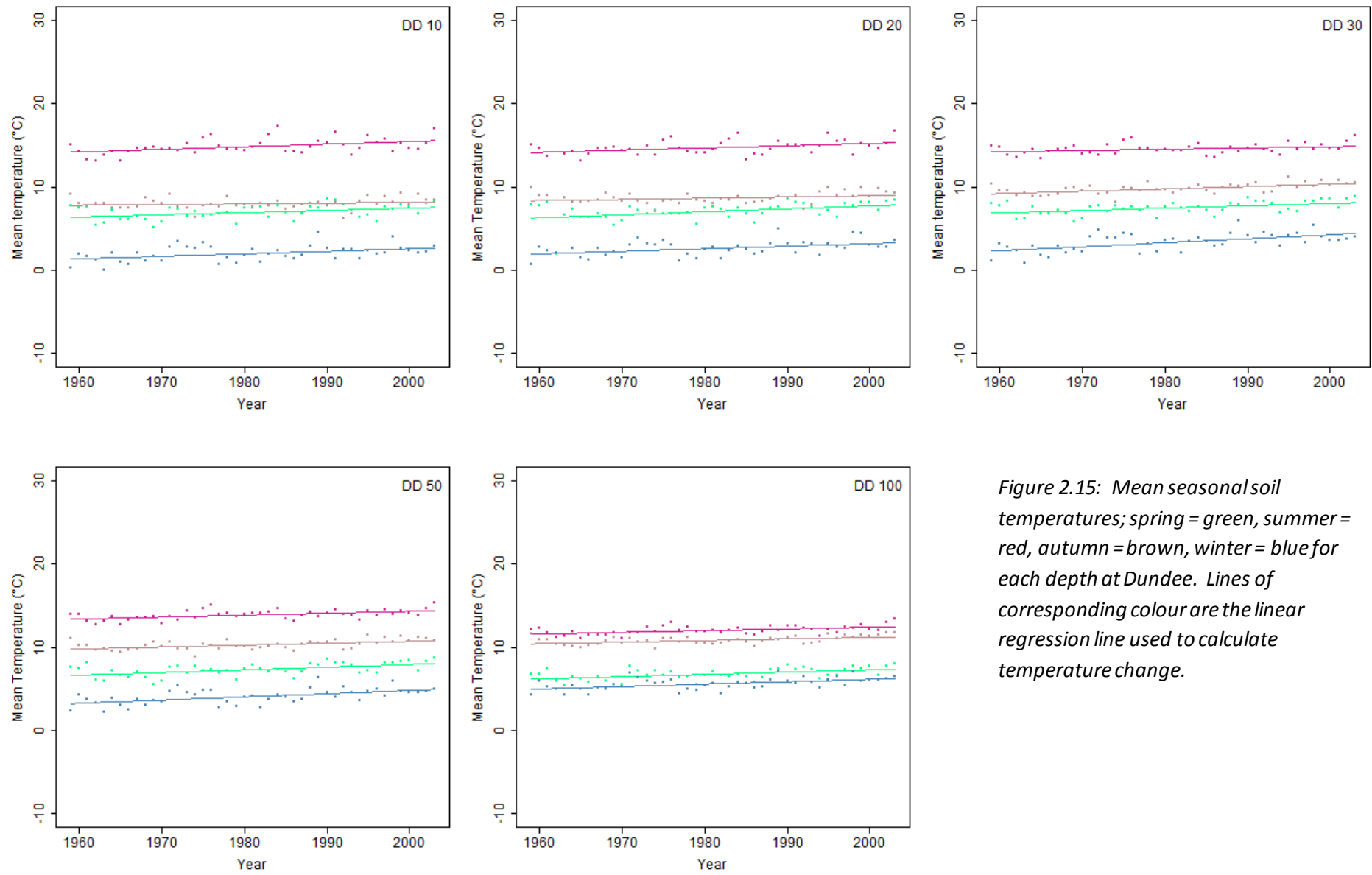
Figure 2.15: Mean seasonal soil temperatures; spring = green, summer = red, autumn = brown, winter = blue for each depth at Dundee. Lines of corresponding colour are the linear regression line used to calculate temperature change.
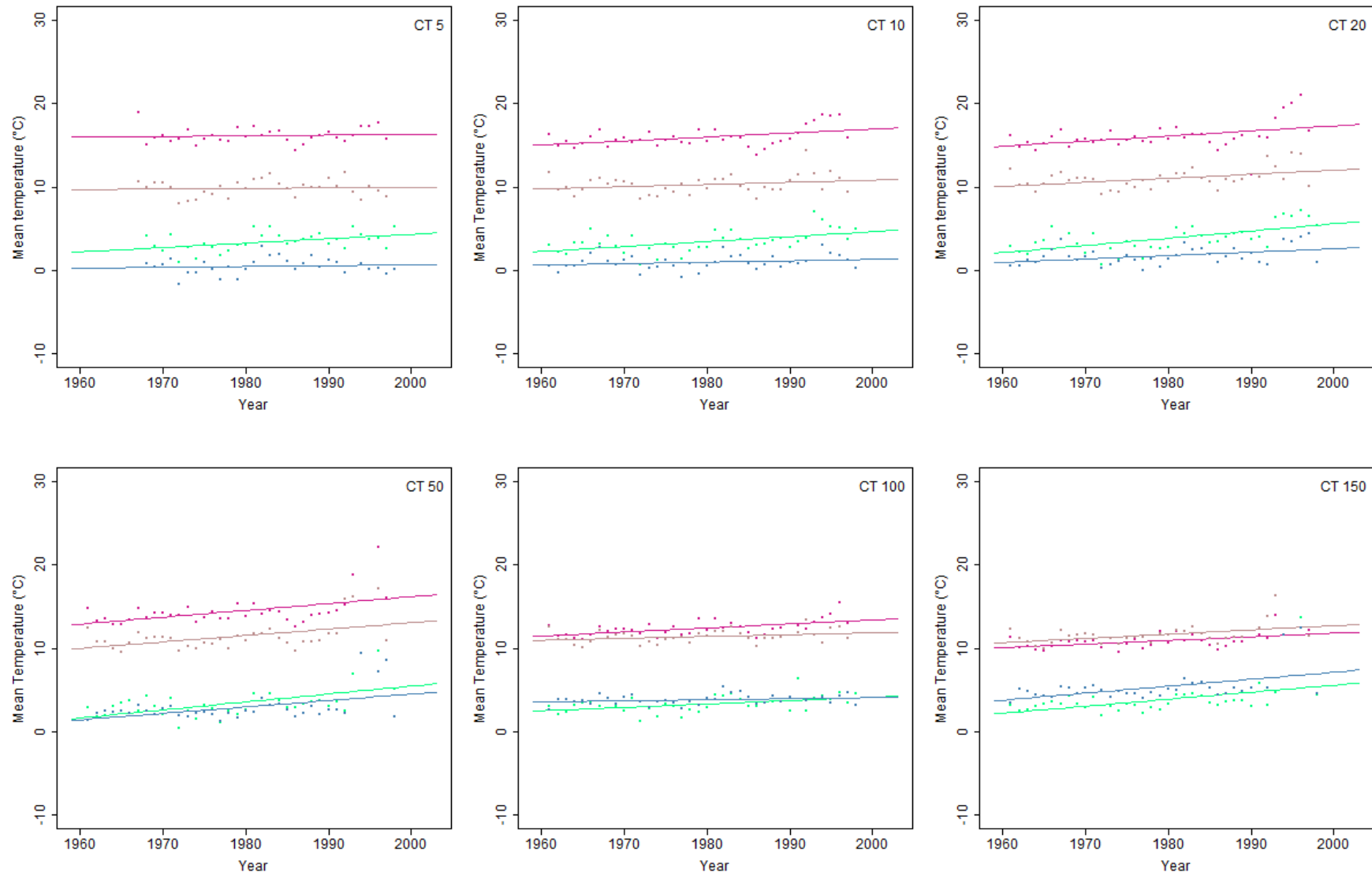
Figure 2.16: Mean seasonal soil temperatures; spring = green, summer = red, autumn = brown, winter = blue for each depth at Charlottetown. Lines of corresponding colour are the linear regression line used to calculate temperature change.

Figure 2.17: Mean seasonal soil temperatures; spring = green, summer = red, autumn = brown, winter = blue for each depth at Ottawa. Lines of corresponding colour are the linear regression line used to calculate temperature change.

Figure 2.18: Mean seasonal soil temperatures; spring = green, summer = red, autumn = brown, winter = blue for each depth at Swift Current. Lines of corresponding colour are the linear regression line used to calculate temperature change.

Table 2.6: Mean seasonal soil temperature trends and the absolute change in °C; red cells show positive gradients, blue cells show negative gradients and bold type shows statistically significant results.

Site	Depth (cm)	N	Spring		Summer		Autumn		Winter	
			Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)
Armagh	30	45	0.0044	0.1959	-0.0162	-0.7274	-0.0003	-0.0113	0.0272	1.2253
	100	45	0.0185	0.8306	0.0072	0.3239	0.0091	0.4090	0.0283	1.2718
Dundee	10	45	0.0265	1.1914	0.0314	1.4132	0.0083	0.3757	0.0302	1.3591
	20	45	0.0355	1.5994	0.0266	1.1982	0.0141	0.6334	0.0310	1.3942
	30	45	0.0286	1.2856	0.0153	0.6879	0.0279	1.2553	0.0479	2.1551
	50	45	0.0322	1.4489	0.0225	1.0142	0.0227	1.0203	0.0384	1.7294
	100	45	0.0269	1.2116	0.0205	0.9229	0.0190	0.8546	0.0290	1.3062
Charlottetown	5	29	0.0530	2.3838	0.0086	0.3869	0.0068	0.3067	0.0100	0.4510
	10	35	0.0595	2.6763	0.0467	2.1029	0.0260	1.1698	0.0168	0.7555
	20	35	0.0861	3.8726	0.0607	2.7315	0.0484	2.1797	0.0413	1.8578
	50	35	0.0957	4.3056	0.0823	3.7039	0.0767	3.4512	0.0774	3.4813
	100	35	0.0410	1.8432	0.0486	2.1859	0.0221	0.9933	0.0123	0.5555
	150	35	0.0839	3.7775	0.0435	1.9588	0.0513	2.3071	0.0836	3.7624
Ottawa	5	36	-0.0191	-0.8598	-0.0029	-0.1302	0.0076	0.3398	-0.0269	-1.2119
	10	45	-0.0053	-0.2365	0.0047	0.2101	-0.0059	-0.2665	-0.0094	-0.4228
	20	45	0.0060	0.2679	0.0087	0.3898	0.0009	0.0427	-0.0023	-0.1021
	50	45	-0.0315	-1.4160	-0.0198	-0.8931	-0.0243	-1.0931	-0.0261	-1.1731
	100	45	0.0203	0.9156	0.0306	1.3753	0.0247	1.1113	0.0273	1.2297
Swift Current	5	36	0.0202	0.9104	-0.0298	-1.3428	0.0165	0.7442	0.0758	3.4097
	10	39	0.0348	1.5669	-0.0089	-0.3985	0.0088	0.3951	0.0509	2.2896
	20	39	0.0490	2.2039	-0.0050	-0.2263	0.0150	0.6757	0.0647	2.9129
	50	39	0.0264	1.1876	-0.012	-0.5353	0.0072	0.3243	0.0335	1.5064
	100	39	0.0088	0.3964	-0.0032	-0.1444	-0.0334	-1.5044	-0.0368	-1.6568
	150	39	0.0365	1.6422	0.0376	1.6931	0.0074	0.3315	-0.0124	-0.5558
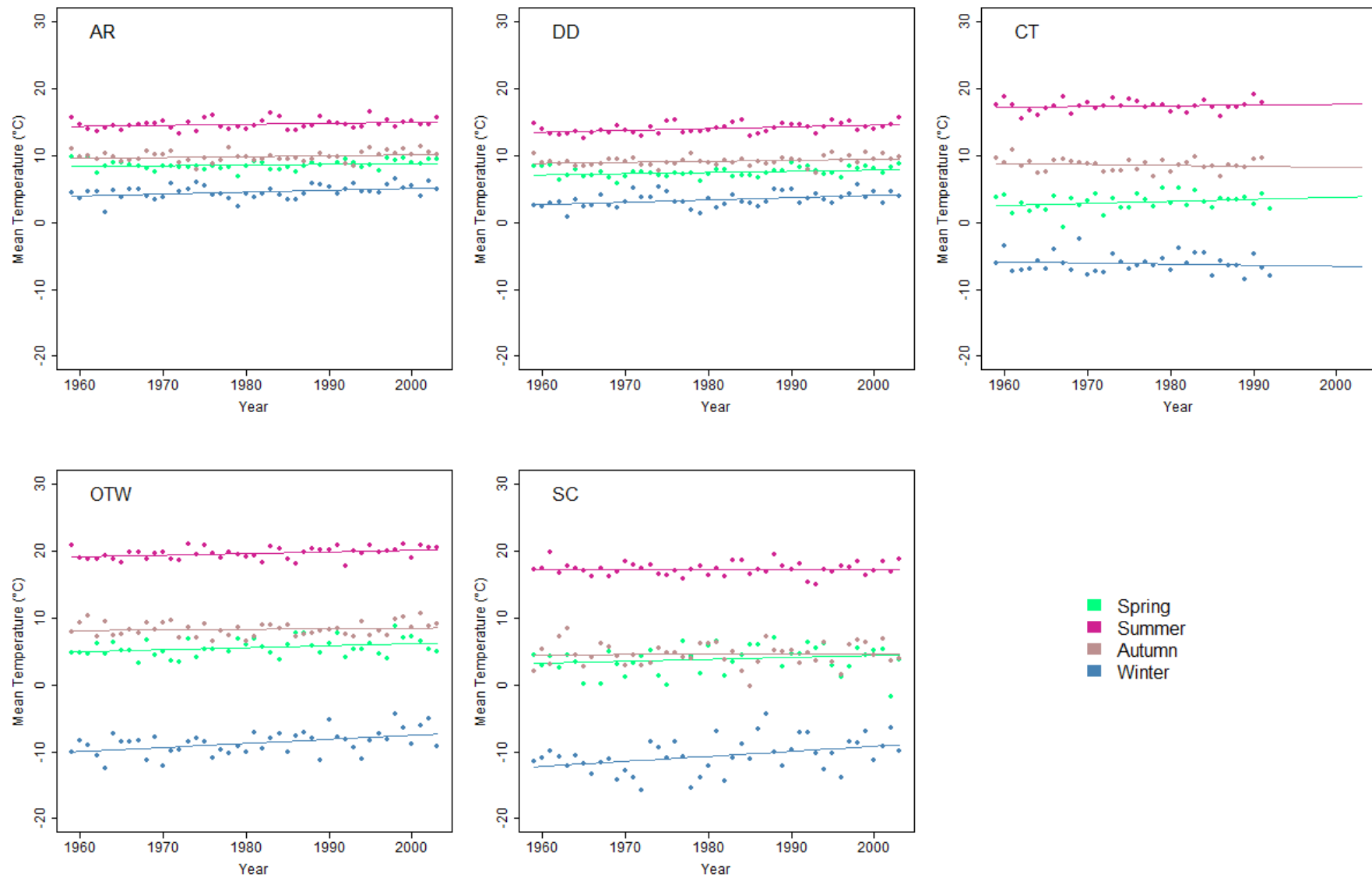
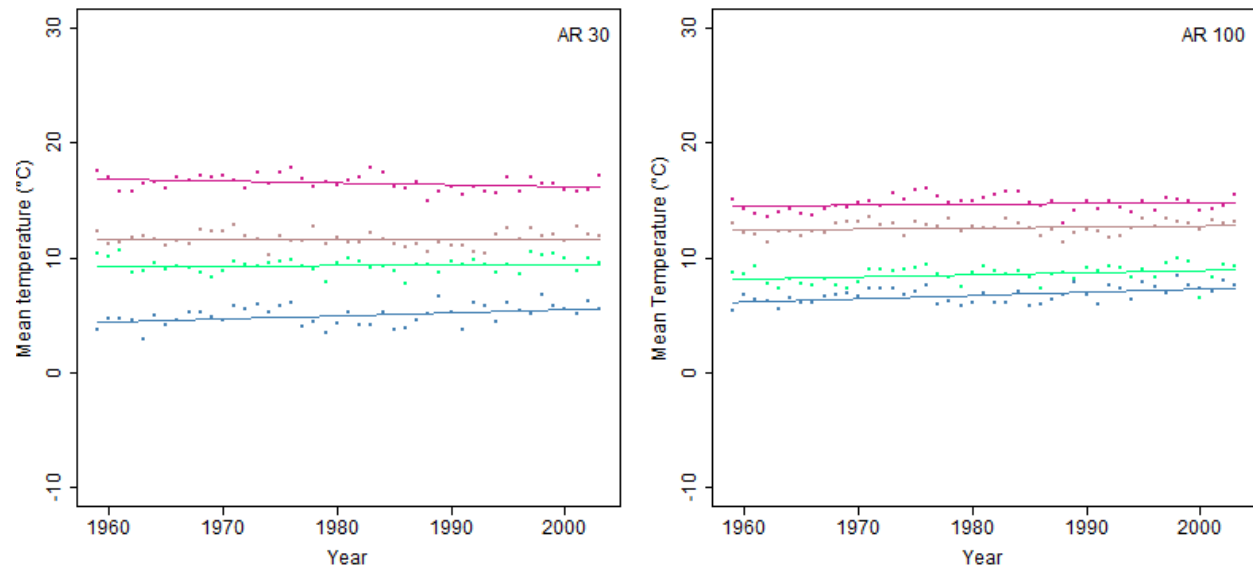*Figure 2.19: Mean seasonal air temperatures; spring = green, summer = red, autumn = brown, winter = blue for each site. Lines of corresponding colour are the linear regression line used to calculate temperature change.*

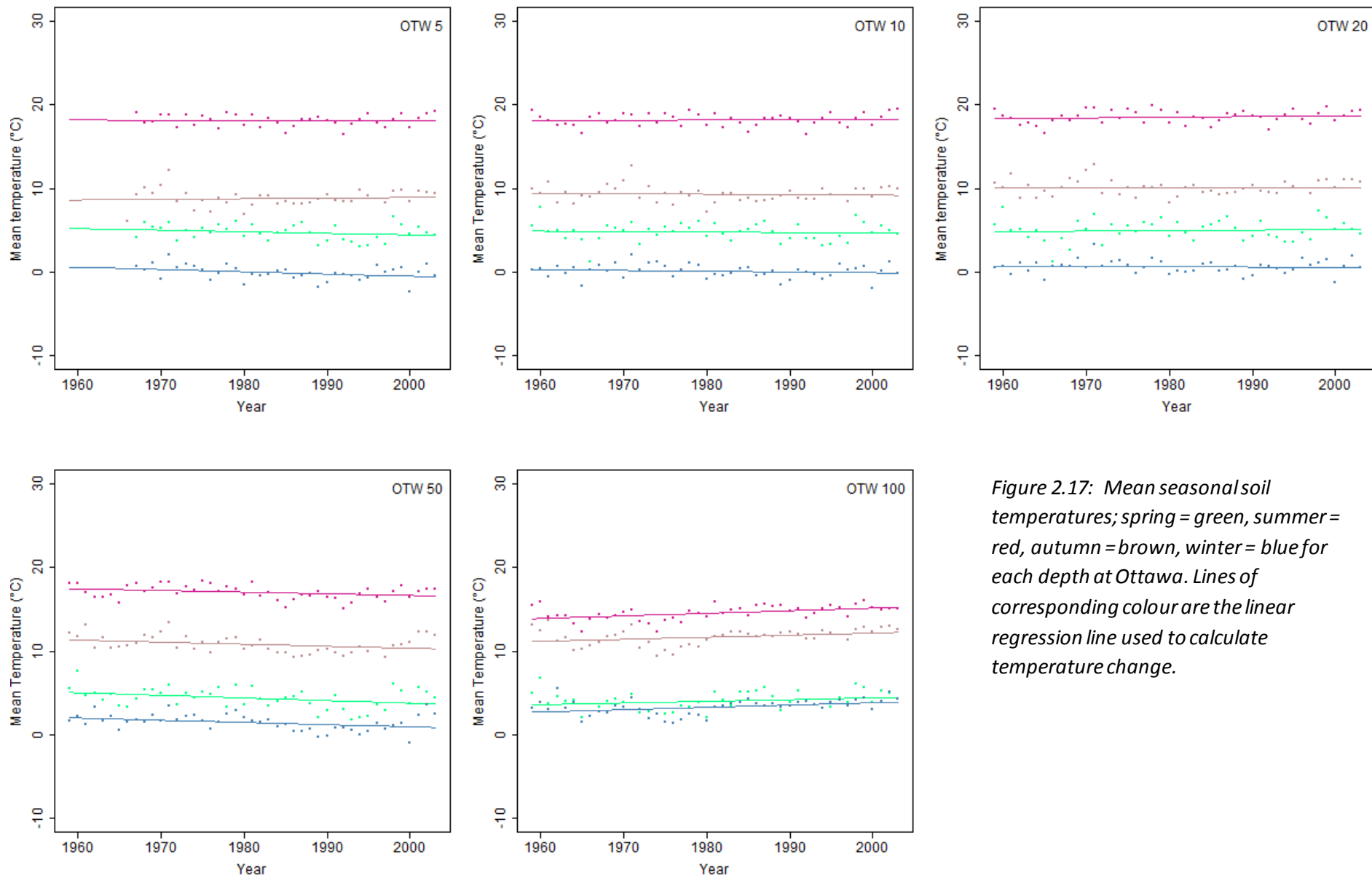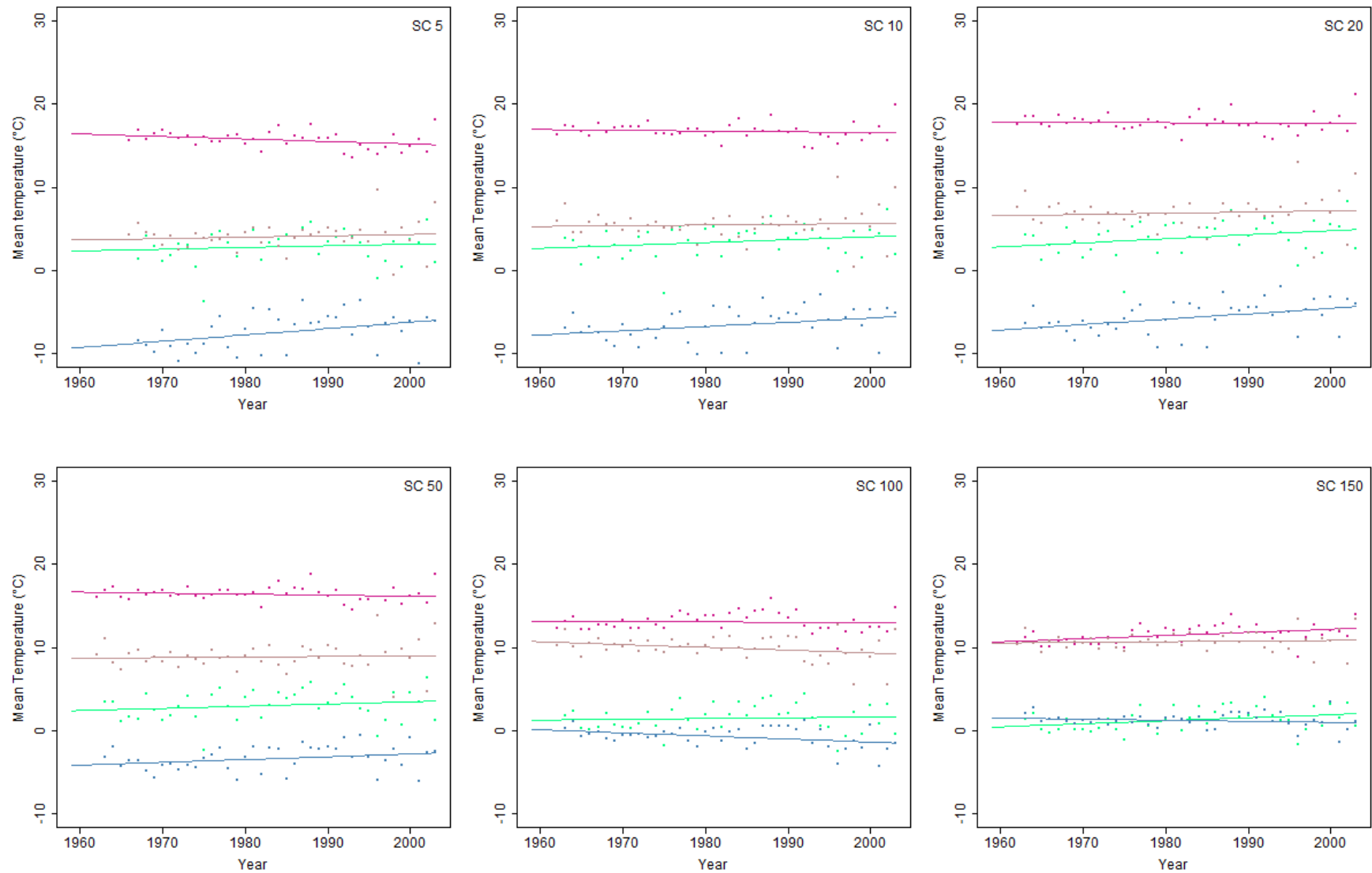*Table 2.7: seasonal air temperature trends and the absolute change in °C, red cells show significant positive gradients, blue cells show significant negative gradients.*

Site	N	Spring		Summer		Autumn		Winter	
		Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)	Gradient	Change (°C)
Armagh	45	0.0134	0.6044	0.0110	0.4954	0.0154	0.6917	0.0282	1.2700
Dundee	45	0.0151	0.6816	0.0180	0.8121	0.0268	1.2071	0.0343	1.5433
Charlottetown	32.4	-0.0145	-0.653	0.0305	1.3745	0.0108	0.4874	-0.0161	-0.7266
Ottawa	45	0.00889	0.4001	0.0300	1.3478	0.0242	1.0901	0.0610	2.7458
Swift Current	45	0.00286	0.1287	0.0289	1.3000	0.0001	0.0056	0.0745	3.3520

2.5. Discussion

The results presented here show that soil and air temperatures increased at all five of these sites over the period covered by the data. These results are not, in themselves, unexpected (because of the correlation between air and soil temperatures) but few published studies have reported trends in soil warming previously (Figure 1.1).

2.5.1. Amplitudes and Phases

The periodic regression fitted well to the data for both air and soil temperatures; it also facilitated the examination of amplitude and phase for each harmonic over time. For soil temperatures the biannual and quarterly harmonic showed little or no change in either the phase or the amplitude; the annual harmonic, however, showed decreasing amplitudes in the mid- and deeper layers at Dundee and Armagh and a similar change in the shallow and mid-layers at Swift Current. It is possible that Charlottetown may have also experienced this reduction in amplitude over time – each depth does show a decrease similar to that seen in Dundee and Armagh during the early 1980s - however, because the Charlottetown data does not extend beyond the early 1990s this trend is not significant in the linear regression. The change in amplitude suggests a change in the range of temperatures at these depths and sites. A similar pattern is seen where the annual phase is increasing in the shallow layers at Dundee and the deeper layers at Swift Current. Charlottetown also shows evidence of increasing phase, but, again, because the data ends early, it is unclear whether these may have occurred in all layers or rather a particular subset. The change in phase is most clearly expressed in the annual harmonic but also to a slightly lesser extent in the

biannual harmonic. When analysing data from temperate climates for seasonality, the role of the biannual harmonic is normally to regulate the width and symmetry of the peak of the annual harmonic, which carries the over-riding signal about temperature patterns in these areas. These results indicate that the annual and biannual harmonics are both changing (though this is more pronounced in the annual) and as such add up to give an altered over-all fit. This means that the timing of annual temperature events (e.g. *maxima* and *minima*) is changing but also that rates of warming and cooling pre- and post- summer may be changing too. When the fitted annual values from the periodic regression were analysed using linear regression all sites show oscilations of these values over time, these cannot be an artefact of fitting the periodic regression because each year was fitted independently and as such there can be no temporal autocorrelation.

The results indicate that the deeper layers of maritime soils, such as those in Dundee and Armagh, are behaving in a similar way to mid- and shallow layers of continental soils, such as those at Swift Current; their amplitudes are decreasing and their phases are increasing so the ranges of temperatures they are experiencing are reducing and the timing of the harmonic peak is getting later in the year. When air temperatures were considered few patterns were found with no statistically significant systematic changes in phase or amplitude. This difference in the patterns found by the periodic regressions for air and soil temperatures suggests that, by using air or surface temperatures rather than soil temperatures to parametarise global feedback or cycling models, such as Century or RothC, important patterns may be missed.

Ottawa does not conform to the pattern of continental shallow and mid-soils behaving similarly to maritime deeper soils; it is likely that this is due to changing levels of precipitation; indeed there have been reports of decreasing snow depths across much of the south of Canada during the measurement period reported here (Qian et al., 2011; Trenberth, 2011). As Ottawa is the most southerly site here it is possible that its snow cover is most affected; a reduction of snow cover in winter would likely lead to a reduction in soil temperatures during that season, because the insulation from lying snow is diminished, and therefore an increase in the range of temperatures is experienced.

2.5.2. Seasonality

Trends in the proportional range over time were overwhelmingly negative, meaning that the range was narrowing, further investigation of this shows that minimum temperatures tend to be increasing more rapidly than maximum temperatures. The same is true of mean air temperatures, although only one of these sites was statistically significant (Ottawa). The results for soil temperature proportional ranges also suggest that the small changes in maritime climates are more significant than larger changes occurring at continental sites.

For further clarity, data sets were broken down to consider seasonal changes. Winter temperatures were found to have shifted most over the total time period, both for soil and air. Spring, summer and autumn temperatures show considerable change over all sites for soil, but air temperatures show no statistically significant changes anywhere during spring and only at Dundee and Ottawa for summer or autumn. For Dundee and Armagh the rates of soil warming during spring are very similar to those during winter;

this is in stark contrast to air, where winter warming is approximately twice as fast as spring warming. Winter warming is likely to have substantial consequences for a wide variety of soil and plant processes. Soil may be less likely to freeze, and, as such, processes like nutrient cycling will continue throughout this period when, traditionally, it is thought that little decomposition or cycling of nutrients occurs (Breland, 1994; Schöll et al., 1997; Andersen and Jensen, 2001). This, coupled with the likely increase in soil moisture in mid- to high latitudes (Rosenzweig et al., 2008; Trenberth, 2011) and the expectation that an increased proportion of winter precipitation will fall as rain rather than snow in alpine and tundra environments (Solomon et al., 2007; Wipf et al., 2009) increases the likelihood that nutrients liberated from soil organic matter over this period will be leached into ground/surface waters rather than being used for biomass production (Andersen and Jensen, 2001).

2.5.3. Greenhouse gases

Warming during parts of the year, when the plant and microbial community is less active (late autumn, through the winter to early spring), has the potential to change the frequency of soil freeze–thaw cycles (Henry, 2008). This, in turn, may lead to changes in the structure of the microbial community (Schimel and Clein, 1996; Sharma et al., 2006) and the stability of organic matter or soil aggregates (Herrmann and Witter, 2002; Oztas and Fayetorbay, 2003). Lysing of cells and break up of soil aggregates by freezing releases organic molecules, and when soil subsequently thaws this has been reported to increase the fluxes of GHGs (particularly N_2O) (Neilsen et al., 2001; Dörsch et al., 2004; Grogan et al., 2004). There is confusion, however, as to whether this will be borne out in practice; many of the results showing increased

fluxes from studies where snow was removed in order to increase the frequency of freeze-thaw cycles over winter; these do not take into account the concomitant changes in air temperature that might result in fewer days where air temperatures are below zero and therefore possibly fewer sub-zero soil days (Henry, 2008). It is likely that the timing of snowfall is more important than just the presence or absence of snow. If snow falls prior to soil freezing the ground below the snow pack may remain un-frozen (Campbell et al., 2005; Burn and Kokelj, 2012) and potentially more active (Brookes, 1995; Schimel et al., 2004; Kuhnert et al., 2012) than if soil freezes prior to snowfall when the snow cover may then prevent early thawing thereby potentially reducing activity in soil (Schimel et al., 2004; Borner et al., 2008). Freeze-thaw cycles in soil are also potentially very important when considering the release of N_2O from soil; Molecule-for-molecule N_2O has 297 times more climate forcing potenial than CO_2 and freeze-thawing of soil is thought to account for between 50 and 66% of N_2O emissions from agricultural soils (Duxbury et al., 1982; Röver et al., 1998). It is possible that these emissions could be diminished by growing catch crops over winter which would reduce the pool of nitrate in soil which would then be unavailible for denitrification (Teepe et al., 2000, 2001; Dietzel et al., 2011). It is important to note that forest soils and some more northerly arable soils do not show the same responses to freeze–thaw events as stated above. This suggests that loss of snow cover and increased numbers of freeze–thaw cycles at higher latitudes might not result in increased emissions of N_2O and CO_2 (Grogan et al., 2004; Koponen et al., 2006; Matzner and Borken, 2008).

2.5.4. Soil-borne pathogens

Increased winter temperatures are also likely to have implications for soil-borne pathogens/ pests with increased numbers of individuals surviving year round or increased numbers of generations per year. In maritime climates this may result in increased problems for winter crops which have previously been relatively protected from pests. In cooler, continental climates, however, this increase in winter temperature may have effects that could be viewed as benificial by increasing the length of growing season or the potential to over-winter crops resulting in an increased potential for food security.

Soil provides a conduit for many plant pathogens important in agriculture; two examples are *Phytophthora infestans* (potato blight) and wheat soil-borne mosaic virus (WSBMV). Both pathogens are known to be temperature sensitive with current forecasting algorithms for *P. infestans* that are used in temperate regions predicting no pathogen development below 7, 8 or 10°C (Grünwald and Flier, 2005). Similarly WSBMV is found to infect crops mainly during the autumn or when soil temperatures are between 10 and 15 °C. Increasing winter/spring soil temperatures are likely to mean that these lower limits will be exceeded for longer during (future) growing seasons resulting in a greater window of infection for these pathogens. Pathogen problems are unlikely to stop at farm boundaries; *Armillaria* species ('honey' fungi) cause root rot in many economically important tree species. The growth of rhizomorphs, through which the fungus infects new trees/root systems, is thought to be slowed by low soil temperatures (e.g. below 10°C) (Rishbeth, 1978) but with increasing soil temperatures over winter and spring these constraints may be partially removed presenting problems for the forestry/timber industry.

2.6. Conclusion

The work presented here represents a first step towards quantifying long-term trends in soil temperatures which can then be used to feed into models of how this change will affect different ecosystem functions and services. This work shows that changes in soil temperature are often more significant than those in air temperature, and that they occur at different times of year. The changes in temperature are likely to have substantial consequences for a whole range of ecosystem services, including food production, organic matter decomposition and production/storage of greenhouse gases. These results also show that further investigation of the effects of soil temperature on soil processes is warranted, explicitly considering season and soil depth. In line with this, the following chapters will focus on assessing the impact of changing soil temperatures on soil nitrogen transformations.

The changes seen in mean annual soil temperature have likely been driven by increases in lower soil temperatures rather than increases in higher temperatures. This was reflected in the significant narrowing of the annual temperature range at various soil depths at most sites (table 2). Seasonal soil temperature change appears to have been driven by increases in winter air temperatures which results not only in increased winter soil temperatures but also in increased spring soil temperatures (tables 5 and 6).

Chapter 3: The effects of temperature on enzyme activity during short term laboratory incubations

The work presented in this Chapter forms the basis of a paper entitled "How do enzymes catalysing soil nitrogen transformations respond to temperature change?" This was published in Soil Biology and Biochemistry in 2012, a copy of this paper can be found in Appendix 1.

3.1. Introduction

N-transformations in soil - including mineralization - are well studied; however, in comparison with the many studies on the temperature sensitivity of soil respiration (see for example Hartley et al., 2007, 2008, 2009; Lützow and Kögel-Knabner, 2009a) the temperature sensitivity of N-mineralization has received comparatively little attention. N-mineralization has been recognised for many years to be a particularly important step in the nitrogen cycle; initially this was because it was widely accepted that plants could only make use of inorganic forms of N (Kielland, 1994). This is now known not to be the case (Näsholm et al., 1998) and it is understood that plants in many ecosystems can compete effectively with the microbial community for both organic and small inorganic molecules (Schimel and Chapin, 1996; Jones and Kielland, 2002; Schimel and Bennett, 2004). Previous work on N-mineralization rates in soil in relation to temperature has tended to focus on measuring changes in the rate at which NH_4^+ accumulates (Cookson et al., 2002; Agehara and Warncke, 2005; Dijkstra et al., 2010; Turner and Henry, 2010). The pool of NH_4^+ will accumulate as the product of a myriad of different reactions carried out by a host of organisms in many parts of the

soil ecosystem. These organisms are unlikely to have a uniform response to temperature changes and as such measuring gross processes such as NH_4^+ accumulation makes it difficult to interpret what changes in pool size actually mean (Schimel and Bennett, 2004; Näsholm et al., 2009). Given predictions of increasing global temperatures as a result of anthropogenic climate change, it is important to acknowledge that specific reactions within nutrient cycles may respond individualistically, significantly altering the availability of nutrients to plant and microbial communities (Schimel et al., 2004; Borner et al., 2008). By measuring the pool of NH_4^+ and using the resulting figure as a measure of mineral-N availability may be misleading because this pool as the starting point for many reactions involved in nitrification can be easily depleted. Measuring N-availability in this way can overlook the fact that N becomes available to both microbes and plants at earlier stages in the decomposition processes (i.e. as organic molecules) (Kielland, 1994; Näsholm et al., 1998, 2009; Jones and Kielland, 2002). For this reason it is likely to be an advantage to measure mineralization as a string of processes rather than one pool of potential substrate.

It is possible to isolate the effects of temperature change on individual soil reactions by studying the effect of incubation temperature on soil enzymes. By estimating the kinetics of soil enzymes it is possible to gain a direct understanding of the consequences of temperature change for individual reactions and an indirect understanding of the consequences of temperature change on the soil population responsible for enzyme production. Many studies have considered kinetic properties of soil enzymes over temperature ranges that rarely extend below 5 °C but sometimes

exceed 50 °C (e.g. Gould et al., 1973; Moyo et al., 1989; Lai and Tabatabai, 1992; Trasar-Cepeda et al., 2007). Such hot temperatures substantially exceed the maxima for many soils under field conditions (Qian et al., 2011), even though some of the enzymes apparently have temperature optima as high as 50 °C (Ladd, 1972; Ladd and Butler, 1972; Bremner and Mulvaney, 1978). This limits the value of data from such studies, except possibly for surface soils which may reach 50–60 °C as a result of direct radiative warming on recently tilled and sparsely vegetated soils (Horton et al., 1996).

The work presented here will examine the rates of activity of 3 enzymes involved in the mineralization processes feeding into the soil NH_4^+ pool. Protease activity catalyses the initial depolymerisation of proteins to polypeptides, oligopeptides and amino acids (Ladd and Butler, 1972), amidase catalyses the hydrolysis of amides to NH_4^+ and the corresponding carboxylic acid (Frankenberger and Tabatabai, 1979) and urease catalyses the hydrolysis of urea to NH_4^+. These three enzymes form links in the chain of reactions which feed into the NH_4^+ which as stated above is commonly measured to define N-mineralization (Figure 3.1).
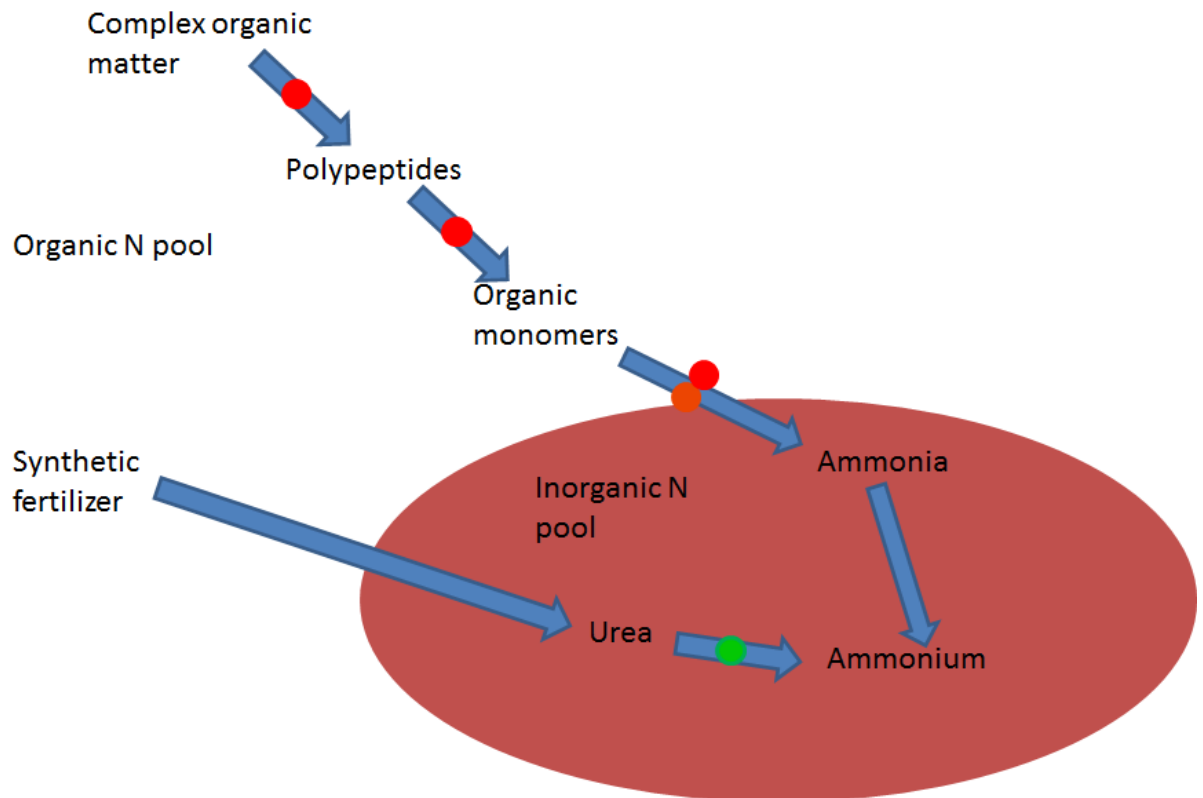
Figure 3.1: Simplified chain of enzymes involved in N-mineralization, red spot = protease, orange spot = amidase and green spot = urease.

The aim of the experiment described in this chapter was to determine the effect of incubation of soils over a realistic temperature range such as might be experienced at the sample site. To this end, samples were collected from an area of undisturbed grassland adjacent to the Mylnefield meteorological station at the James Hutton Institute, Dundee. Daily soil temperature data collected at the met station over the preceding 50 years was used to determine the incubation range for the soil samples. Samples were then incubated under highly-controlled conditions and potential enzyme activities determined at the incubation temperatures.

3.2. Hypotheses

1. Enzyme activity will increase with increasing incubation temperature.

2. The response of enzyme activity to incubation temperature will remain the same throughout the entire incubation period.

3.3. Materials and Methods

3.3.1 Soil sampling

Soil was collected from an area of undisturbed grassland adjacent to the Mylnefield met station. Using a trowel the grass and moss was removed and the soil collected from to 0 to 10 cm layer. The soil had a sandy loam texture, a pH (in water) of 5.7 and C and N contents (gravimetric) of 3.5 and 0.25% respectively; it is classified as a dystric cambisol (FAO classification). The soil was taken to the laboratory and immediately sieved to pass a 2 mm mesh. During sieving all remaining plant and animal biomass (both above ground and roots along with any invertebrates) as well as stones were removed. Samples were weighed into glass tubes ready for incubation.

3.3.2 Soil incubation

Incubation was carried out in a temperature-gradient block, as described by Isaksen et al., (1994) (Figure 3.2). Samples were incubated for either 7 or 14 days at temperatures ranging from -2 to 21 °C; temperatures were checked daily using alcohol thermometers installed in the block (Figure 3.2) and stabilised to +/- 0.2 °C. The incubation range was chosen based on analysis of soil temperature data collected daily at the Mylnefield met station and accounted for 99.7% of the temperature range at the site between January 1959 and December 2009 (Figure 3.3). The design of the incubation block makes it impossible to replicate samples at all temperatures, it does, however, allow for incubation over a wide temperature range meaning that this experiment can effectively describe the shape of the temperature responses of these reactions.
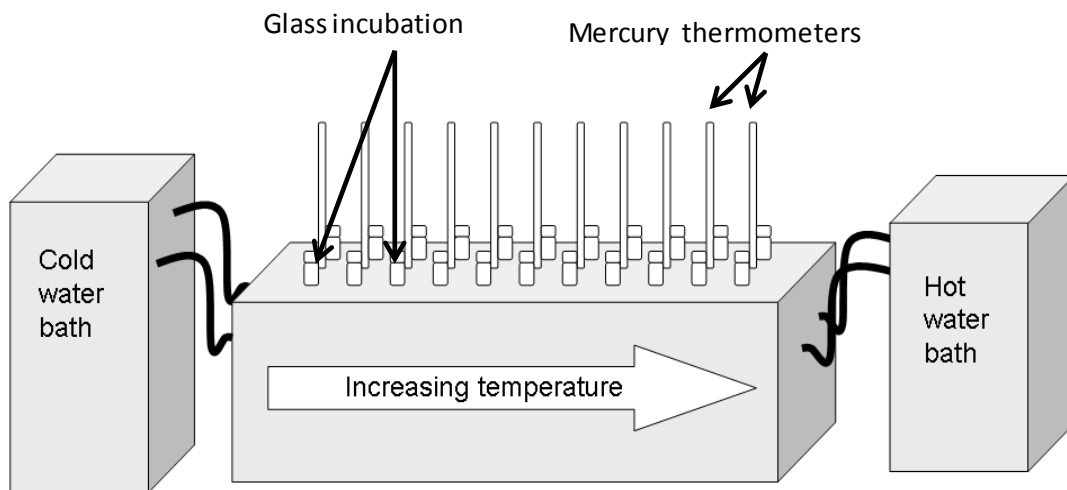
Figure 3.2: Temperature gradient block used for the incubation of field moist soil samples; aluminium block attached to a refrigerated water bath and a heated water bath to create a temperature gradient within the metal. Glass tubes of soil were inserted in the block for incubation and temperatures monitored using the alcohol thermometers also inserted in the block. The entire aluminium block was wrapped in 2 cm thick polystyrene sheets to increase the efficiency of the temperature control.
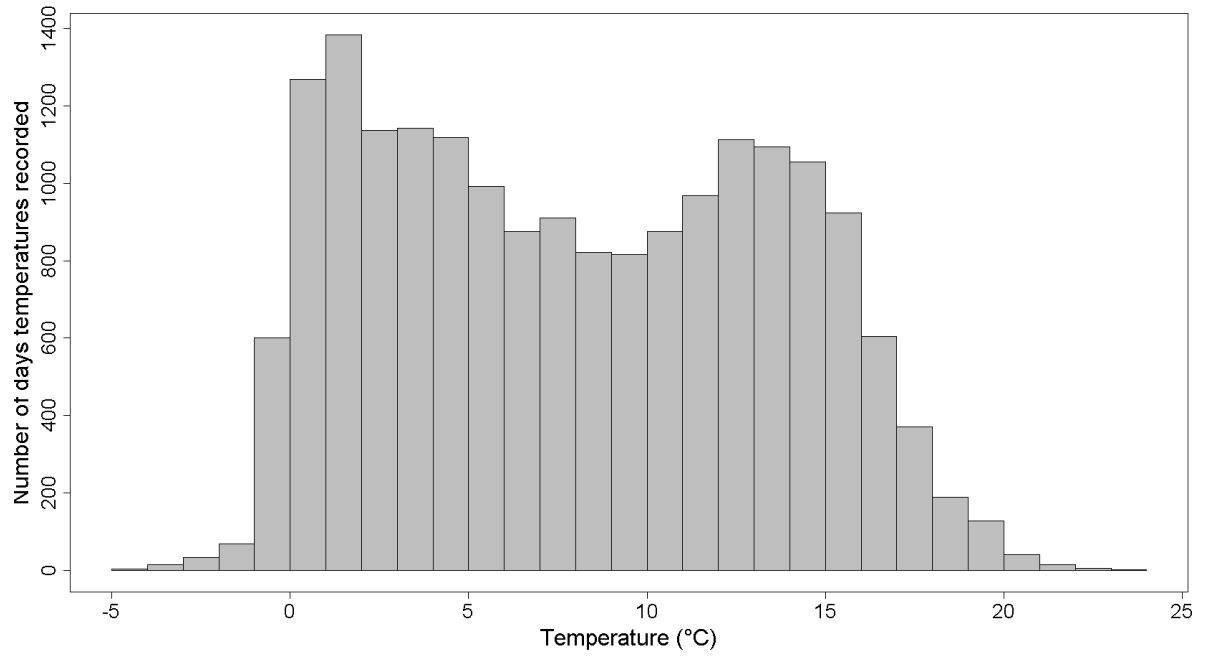
Figure 3.3: Histogram showing the distribution of temperatures recorded at 10 cm depth in the soil between 1959 and 2009.

3.3.3. Enzyme assays

All enzyme assays were carried out at the same temperature that the sample had been incubated at also using the temperature gradient block.

3.3.3.1. Protease activity

Samples were assayed for protease activity using the method described by Ladd and Butler, (1972). One gram samples of soil were weighed into labelled tubes; 5 ml of Tris(hydroxymethyl amino)methane buffer (Tris) adjusted to pH 8.5 using 1M Hydrochloric acid (HCl) and 5 ml of 2% sodium caseinate solution were added to each sample. Tubes were capped, shaken and then incubated at the appropriate temperatures (details of incubation temperatures will be provided for each experiment). Samples were incubated for 4 hours prior to the addition of 5ml of 15% Trichloroacetic acid (TCA) to each tube stopping the reaction. Samples were then centrifuged at 10,000 rpm (12074g) for 5 minutes following which 5 ml of the supernatant was pipetted into a clean tube. Controls were treated identically to samples except that the TCA was added immediately after the sodium caseinate solution. 7.5 ml of an alkaline reagent (50 g sodium carbonate dissolved in 60 ml 1 M NaOH, 940 ml distilled water, 20 ml of 0.02 M copper sulphate and 20 ml 0.03 M potassium sodium tartrate) were added to each supernatant sample and left to stand for 15 minutes before 5 ml of 33% Folin–Ciocalteu's Phenol Reagent was added to each tube, tubes were swirled and left to stand for 1 hour for full colour development. Absorbances were then measured using a WPA Spectawave S1000 spectrophotometer at 700 nm. Standard curves were created by measuring the absorbance of standards

made up using 500 µg ml^{-1} tyrosine solution diluted to 0, 50, 100, 150, 200 and 250 µg ml^{-1} tyrosine concentrations and treated in the same manner as samples and blanks. Absorbance values were then converted into rates of activity using equation 3.1:

Equation 3.1:

$$Activity\ (\mu g\ tyrosine\ equvalent) = \frac{(measured\ concentration * final\ volume)}{dry\ weight\ of\ 1g}$$

3.3.3.2. Urease activity

Samples were assayed for urease activity using the method described by Kandeler and Gerber, (1988). 2.5 g samples of soil were weighed into labelled tubes; 1.25 ml of 0.08M urea (CH$_4$N$_2$O) solution was then added to each sample; the tubes were swirled, capped and incubated at the appropriate temperatures for 2 hours. After incubation, 25 ml of 1M acidified potassium chloride (990 ml - 1M KCl plus 10 ml 1M HCl) solution were added and samples shaken for 30 minutes prior to centrifuging at 10,000 rpm (12074g) for 5 minutes. Controls were treated in the same way as samples except that the acidified KCl was added immediately after the urea and shaken to extract the ammonium (NH$_4^+$) straight away. After centrifuging, 1 ml of supernatant was taken and pipetted into a clean tube, to this was added 4.5 ml of distilled water, 2.5 ml of sodium salicylate solution (1:1:1 ratio mixture of 0.11 M sodium salicylate solution (plus 12 mg sodium nitroprusside), 0.3M sodium hydroxide (NaOH), distilled water) and 1 ml of 0.1% sodium dichloroisocyanurate solution. All tubes were swirled then left to stand for 30 minutes prior to absorbance being measured at 690 nm in the spectrophotometer. A standard curve was created using 1000 µg NH$_4^+$ ml^{-1} ammonium chloride solution (NH$_4$Cl) diluted to 0, 10, 15, 20 and 25 µg NH$_4^+$ ml^{-1} using

acidified KCl solution. Absorbance were then converted into urease activity rates using equation 3.2.

Equation 3.2:

$$Activity = \frac{(measured\ \mu g\ NH_4^+ - N\ ml^{-1} * total\ volume * 10)}{dry\ weight\ of\ 1\ g * 2.5}$$

10 = dilution factor

2.5 = weight of soil used in assay.

3.3.3.3 Amidase activity

Soils were assayed for amidase activity using the method described by Frankenberger and Tabatabai, (1979). Five gram samples of soil were weighed into labelled tubes; 9 ml of Tris buffer adjusted to pH 8.1 using 1M sulphuric acid (H_2SO_4), 0.2 ml toluene and 1 ml of 0.5M propionamide were added to samples, tubes were capped, shaken and incubated at appropriate temperatures for 24 hours. After incubation 39.8 ml of KCl (2.5M) − Uranyl acetate (0.005M) ($UO_2(CH_3COO)_2$) solution was added to each sample to stop the reaction, samples were then filtered through Whatman No. 1 filter papers. All controls were treated in the same way as samples except that the KCl − uranyl acetate solution was added along with the other reagents prior to filtering. Subsequently 0.1 ml of each filtrate was pipetted into a clean tube and 5 ml of solution N1 (34 g sodium salicylate ($C_7H_5NaO_3$), 25 g sodium citrate ($Na_3C_6H_4O_6$), 25 g sodium tartrate ($Na_2C_4H_4O_6$) and 0.12g sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot2H_2O$) dissolved in 1000ml of water) were added, each tube was vortex mixed and left to stand for 15

minutes; 5 ml of solution N2 (30 g sodium hydroxide (NaOH) and 10 ml sodium hypochlorite (NaOCl) dissolved in 990ml of water) were then added, tubes were vortex mixed for a second time and left to stand for 1 hour before absorbances were measured at 655 nm. A standard curve was created using 100 µg ml^{-1} NH$_4^+$ − N ammonium sulphate ((NH$_4$)$_2$SO$_4$) solution diluted to 0, 5, 10, 15, 20, 25, 30, 40 and 50 µg NH$_4^+$ - N. Absorbances were then converted into amidase activities using equation 3.3:

Equation 3.3:

$$Nitrogen\ (\%) = \left(\frac{Measured\ concentration * total\ volume}{dry\ weight\ of\ sample}\right) * 0.0001$$

Where 0.0001 = dilution factor

Using this protocol amidase activity was un-measurable in this soil. Frankenberger and Tabatabai, (1979) also stated that two other amide substrates could be used in the assay; acetamide and propionamide. Assays were carried out using both of these to test for substrate specificity. Amidase assays were also carried out on samples where glucose was added to the soil prior to incubation so as to provide 3.2 µg of C per gram of soil but using formamide as the substrate, these samples were incubated for either 24 or 72 hours so as to measure activity before the glucose−C was depleted.

3.3.4. Kinetic calculations

The rate constants (k) for each enzyme activity were calculated at each temperature assuming zero-order kinetics. From an Arrhenius plot, k was the slope of the straight line between the concentrations (unit of product g^{-1} dry wt soil h^{-1}) recorded at

common temperatures on days 7 and 14. The rate constants were used to calculate the activation energy (E_a) of the reaction; all k values were transformed to natural logarithms and plotted against the reciprocal of the incubation temperatures in Kelvin (1/T). The slope was estimated by linear regression and used to calculate the activation energy (E_a) (equation 3.4):

Equation 3.4:

E_a = - *slope x lnR,* where R is the universal gas constant.   (Morris, 1974)

Q_{10} values were calculated for different parts of the temperature range (4–13, 11–21 and 4 –21 °C for protease, and -2 – 9, 11 –21 and -2 – 21 °C for urease) using equation 3.5:

Equation 3.5:

$$Q_{10} = (k2/ k1)^{10/(T2-T1)}$$

3.3.5. Data analysis

Paired t-tests were used to compare rates of enzyme activity observed on day 7 with those observed on day 14 and exponential regression models were fitted to each day's data. The effects of glucose addition were also investigated using paired t-tests; all statistical tests were carried out using the R statistical package version 2.11.1 (R Development Core Team, 2010).

3.4. Results

3.4.1. Protease and urease assays

3.4.1.1. Effects of temperature and time

Protease and urease activity were detected at all incubation temperatures (Figure 3.4).

Protease activity was found to be significantly greater after 14 compared to 7 days of

incubation (p=0.0227); no significant difference in urease activity was found between

days 7 and 14 (p = 0.1561).
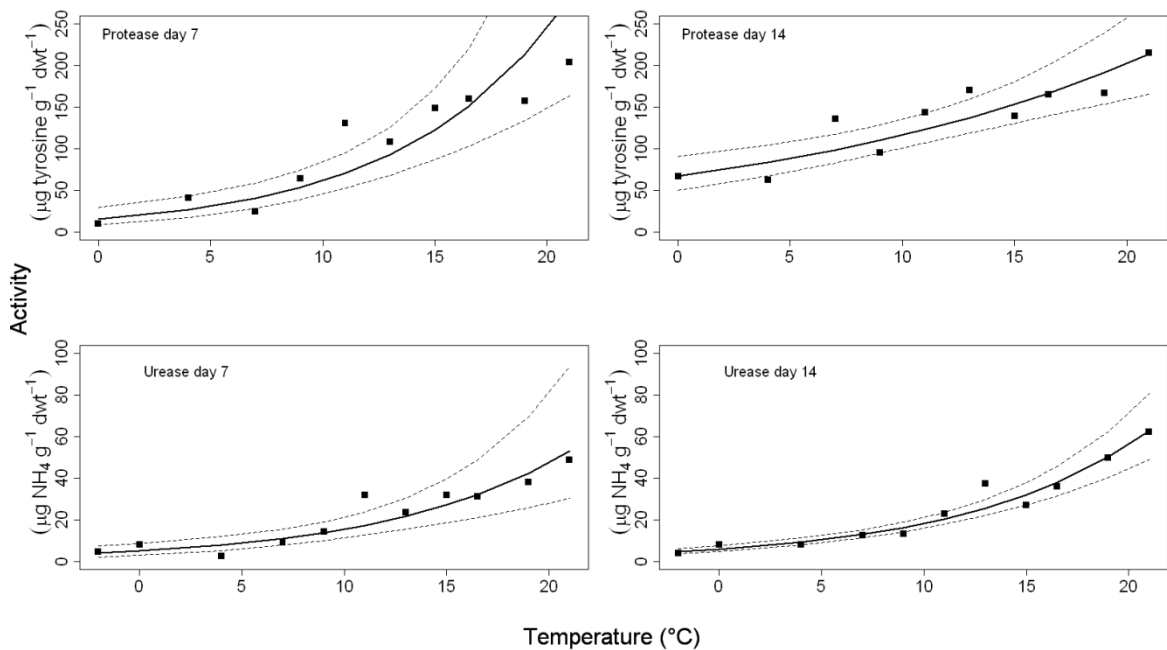
Figure 3.4: Protease and urease activities after 7 and 14 days of incubation; solid lines show the fitted exponential regressions and the dashed lines show the 95% confidence limits of the regressions.

Table 3.1 Exponential regressions fitted to protease and urease activities.

Enzyme	Days of incubation	Adjusted R^2	p - value
Protease	7	0.83	0.00014
	14	0.77	0.00046
Urease	7	0.76	0.00026
	14	0.94	<0.0001

When exponential regression models were fitted to the day 7 and day 14 data for protease and urease (Figure 3.4) between 76 and 94% of the variation was accounted for by incubation temperature (results in table 3.1).

3.4.1.2. Kinetic calculations

Protease and urease activities were then used to calculate the rate constants (k) (table 3.2) and activation energies (E_a) of the reactions (Figure 3.5). Analysis of the Arrhenius plots resulted in E_a = 49.7 kJ mol^{-1} for protease and E_a = 73.4kJ mol^{-1} for urease.

Table 3.2: Rate constants (k) for protease and urease used to build the corresponding Arrhenius plots in Figure 3.5.

Enzyme	Temperature (°C)	k (μg product g^{-1} h^{-1})
Protease	0	0.000932
	3	0.000873
	5	0.001836
	8	0.001308
	10.5	0.001944
	12	0.0023
	14.5	0.001884
	16	0.002231
	19	0.002253
	21	0.002253
Urease	-2	0.000019
	0	0.000035
	4	0.000023
	7	0.000046
	9	0.000059
	11	0.000119
	13	0.000132
	15	0.000128
	16.5	0.000147
	19	0.000191
	21	0.000242
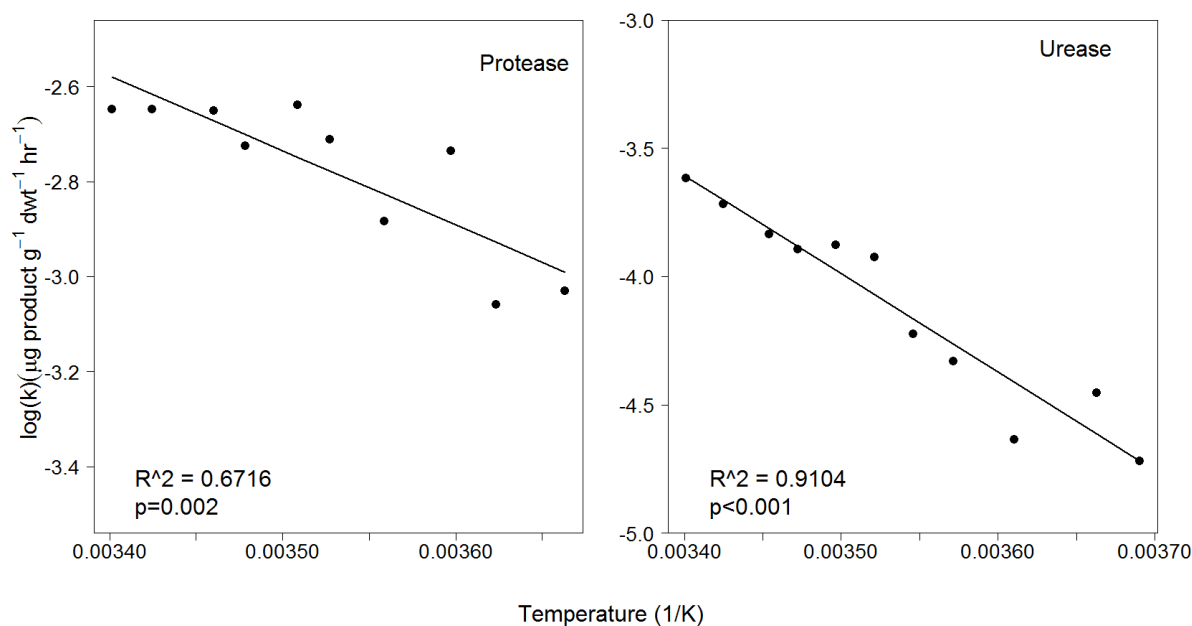
Figure 3.5: Arrhenius plots used to calculate activation energies for protease and urease activities.

The Q_{10} values for protease and urease varied with temperature range (Table 3.3). An increase of approximately 10 °C at the low end of the temperature range used (-2 to 13 °C) resulted in Q_{10} values that were considerably higher (approximately 3.0 for both protease and urease activity) than an increase of 10 °C at the upper end of the temperature scale used, between 11 and 21 °C, (1.6 and 1.5 for protease and urease activities respectively).

Table 3.3: Q_{10} values calculated for different parts of the temperature range.

Enzyme activity	Temperature range (°C)	Q_{10} value
Protease activity	3 to 12	2.969
	12 to 21	1.56
	3 to 21	2.558
Urease activity	-2 to 9	2.765
	11 to 21	1.541
	-2 to 21	2.779

3.4.2. Amidase activity

3.4.2.1. Amide substrates

No significant amidase activity was detected in soil at any temperature when the assay was carried out using formamide as the amide substrate (Figure 3.7.a); this was true even when the assays were carried out at 37°C as per the original protocol (Frankenberger and Tabatabai, 1979). To test whether this lack of measurable activity was due to substrate specificity three different amide substrates were tested; formamide, acetamide and propionamide. Assays were run in triplicate at the mean annual temperature (MAT) at 10 cm depth in soil (8.1°C); acetamide gave 1 positive and 2 negative results, formamide had 2 positive and 1 negative results and propionamide yielded 3 positive results (Figure 3.6). Using a one-way ANOVA there were found to be no statistically significant difference between the amidase activities yielded using the three different substrates ($p = 0.437$).
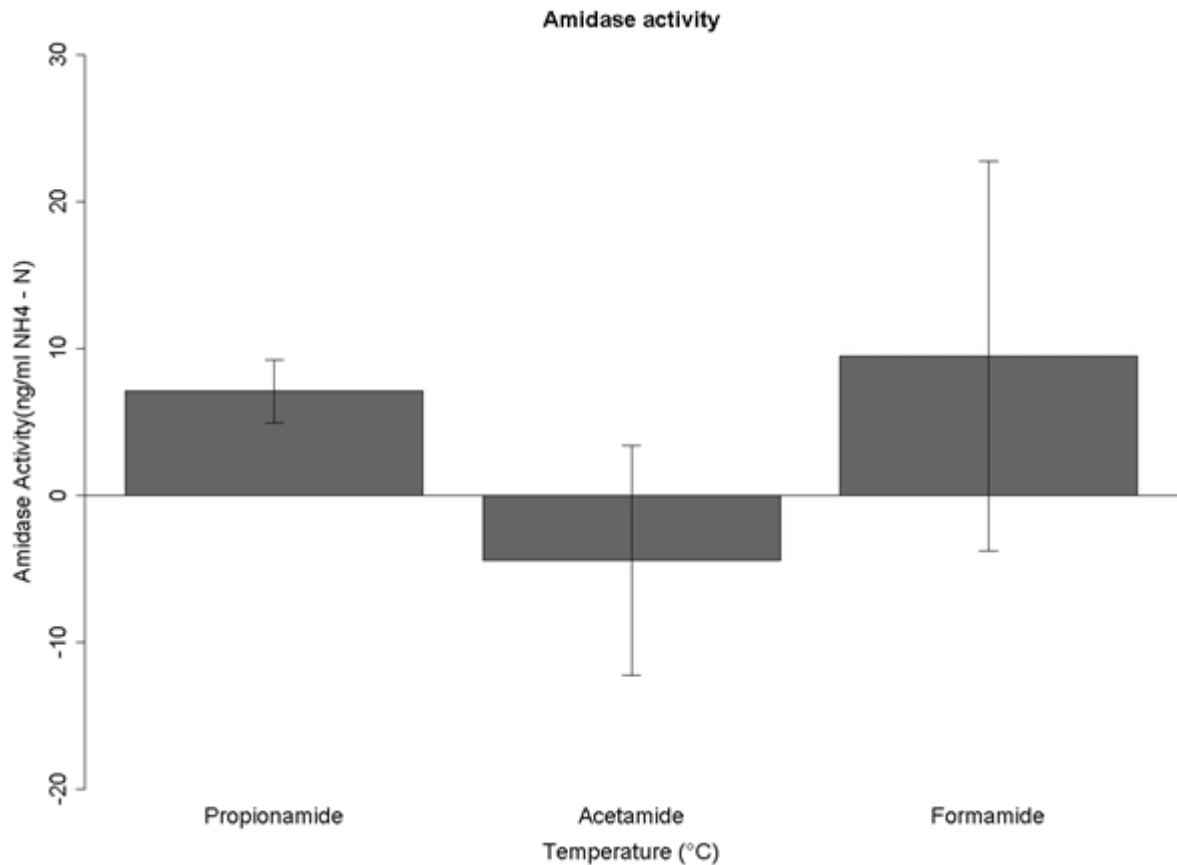
Figure 3.6: Amidase activity detected at 8.1°C (MAT 10 cm soil depth) using 3 different amide substrates.

3.4.2.2. The effect of additional carbon on amidase activity

Amidase assays were re-run in the incubation block but prior to incubation glucose was added to each sample so as to provide 3.2 mg of extra carbon per gram of soil. Assays were carried out at the range of incubation temperatures using formamide as the assay substrate and results were on the whole positive (Figure 3.7.b). Data obtained from soil with and without glucose present were compared using a t-test and those tested without glucose were found to show significantly lower rates of amidase activity (p =0.00124, t = -3.9538). However, even after the addition of glucose to the

samples no relationship was found between the rates of amidase activity and soil

incubation temperature.
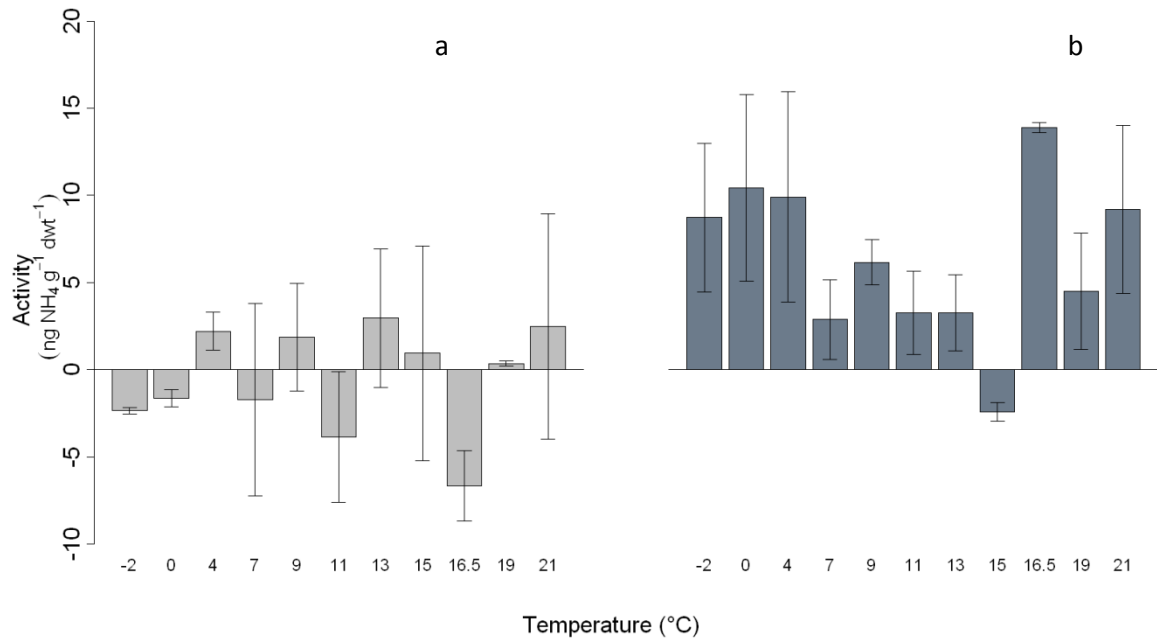
Figure 3.7: Amidase activity measured over a typical temperature range for this soil; graph a shows results for soil incubated without any additions, graph b shows results for soil incubated with glucose added. Error bars are ± 1 standard error.

3.5. Discussion

3.5.1. Temperature range

Assays were carried out according to the protocols detailed previously following generally accepted methods except that all assays were carried out at the incubation temperatures the samples were kept at for either 7 or 14 days. The range of incubation temperatures was based on analysis of long term soil temperature data (as described in section 3.3.2.) and as such is known to reflect every-day field conditions for this soil. The results presented here are actual activities that would be likely to be expressed by this soil, albeit under idealised pH conditions and without inputs from plants, under realistic field temperature conditions. It was decided that to carry out assays in this way would give a more reliable picture of how enzymatic reactions were governed by temperature. The studied temperature range accounts for 99.7% of that experienced at the site over 50 years (Figure 3.2) and is therefore relevant to the debate on climate change, especially as recorded warming of recent decades, as well as predicted changes for the future, indicate that winter warming may be of similar, or greater, magnitude than that in summer (Murphy et al., 2009; Manabe et al., 2011). The 50-year mean temperature at 10 cm in this soil is 8.1 °C, so even at this depth a 3 to 5°C temperature increase (towards the higher end of the predictions in the UK for eastern Scotland by 2080 Murphy et al., (2009) falls well within the range examined.

3.5.2. Protease and urease activities

3.5.2.1. Effects of incubation temperature

Protease activity was less sensitive to temperature increase than urease activity, however, protease activity was affected by length of incubation time whereas urease was not. This may reflect a growth response from the microbes synthesising protease. Urease often acts as an extracellular enzyme (Saiya-Cork et al., 2002) and as such any temperature response does not need to be preceded by a growth or physiological response by soil micro-organisms. The difference between day 7 protease activity and day 14 protease activity is mostly expressed at the lower end of the temperature range (between 0 and 10 °C); this suggests a possible up-regulation of protease activity at lower temperature.

3.5.2.2. Kinetic calculations

It is likely that properties such as E_a and Q_{10}, despite being broadly similar, will differ between soils. Trasar-Cepeda et al., (2007) reported that the E_a values for protease activity in three grassland soils ranged between 33.9 and 44.8 kJ mol^{-1}, which is within 10% of the estimate from the current work (49.7 kJ mol^{-1}). Estimates for urease activation energy ranged from 27.69 to 95.1 kJ mol^{-1}, with a median of 39.1 kJ mol^{-1} (Table 2), which spans the estimates from this experiment. The differences in E_a between different soils could be caused by both biotic and abiotic factors, including: (a) the differing temperature ranges used to incubate soils, (b) the slight modification of carrying out assays at their individual incubation temperatures, (c) rhizosphere effects (i.e. how extensive the root systems are in different soils will affect both

concentrations of enzymes and other root exudates) (Bandick and Dick, 1999), (d) differing microbial (Burns, 1982) or plant communities (Bandick and Dick, 1999), (e) contrasting levels of enzyme adsorption due to soil clay content(Ladd and Butler, 1972; Burns, 1982), (f) pH (Leprince and Quiquampoix, 1996), (g) land management practices(Bandick and Dick, 1999; Garcia-Gil et al., 2000), and (h) soil moisture content (Stark and Firestone, 1995). Whatever the reason, the fact that only one other study could be found for comparison suggests that substantial further work is required to develop a more robust understanding of how soil protease activities are regulated by temperature in terms of E_a, especially in view of their significance for N cycling in a warming world.

The Q_{10} values for protease and urease activity varied with temperature range (Table 3.3). This suggests that, by using a selection of realistically low incubation temperatures, this study gains a considerable amount of information on the temperature responses of soil enzymes that may have been missed in other studies only using higher temperatures. As Q_{10} values are frequently used when building models simulating the effects of climate change on biogeochemical cycles (Cox et al., 2000b; Ryan and Law, 2005; Reichstein and Beer, 2008), it is important that these give a full and accurate picture of possible temperature effects.

3.5.3. Amidase activity

3.5.3.1. Kinetics

There were no consistent trends in amidase activity with temperature; the reasons for this are unclear. There are apparently no reports of E_a or Q_{10} values for amidase activity in soils.

3.5.3.2. Amide substrates and labile carbon addition

The use of three amide substrates in the amidase assay revealed different responses from the microbial community. These were not found to be statistically significant (Table 3.4) but do seem to be biologically relevant as only propionamide gave consistently positive results. It is likely that the differences in carbon content (Figure 3.8) of these substrates is the reason for the observed differences; this was tested by adding glucose to samples at the beginning of incubation and using formamide as the amide substrate during the assay. The lack of response of the soil to the addition of formamide was probably because there is no demand by the soil microorganisms for N so expression of amidase was not energetically expedient in this soil. This presumably occurred because the supply of N amino acids, amino sugars, NH_4^+ and NO_3^- to the soil microorganisms was already adequate without the need to hydrolyse large peptides. However, when excess C is added to the soil this balance is shifted so that more N needs to be made available to satisfy requirements for microbial growth (Hopkins and Dungait, 2010). This is supported by observations that substrate addition can lead to both N and P limitation (Stotzky and Norman, 1961; Allison and Vitousek, 2005; Hopkins et al., 2006).
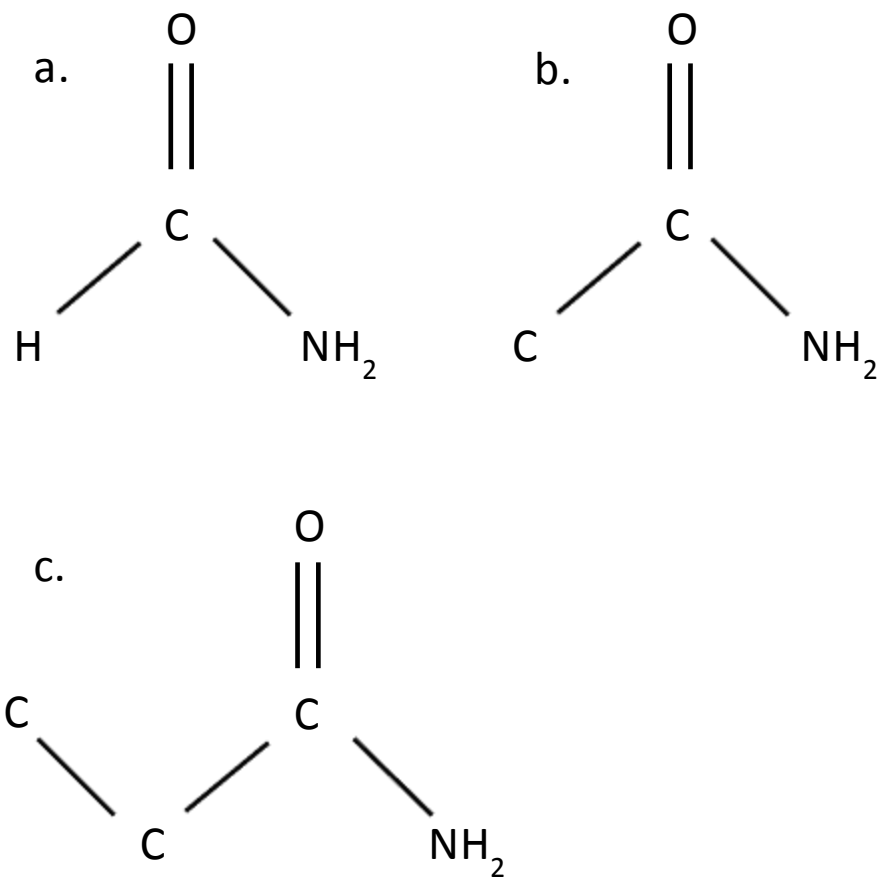
Figure 3.8: Structural molecular formulae of amide substrates showing differences in C-content of a) formamide, b) acetamide and c) propionamide.

3.6. Conclusions

The results presented in this chapter show that the activity of both protease and urease are highly temperature sensitive during tightly controlled, short-term laboratory incubations. Amidase activity is only measurable in this soil when labile C is added in order to drive the need for extra N. This raises questions about how soil enzymes respond to inputs of different types of SOM both in the short and longer term and whether these responses will be conserved in the field rather than under idealised lab conditions.

Chapter 4: The effects of soil temperature and organic matter quality on enzyme activity during laboratory incubations

4.1. Introduction

The previous two chapters have shown that soil temperature is changing (Chapter 2) and changes in soil temperature affect the rates at which important N-transformation-reactions take place (Chapter 3). The availability of particular substrates in the soil can have significant effects on whether reactions take place or not (Chapter 3 – Amidase activity is not measureable in this soil unless labile C is added). In this chapter, the experimental work examines whether organic matter quality can affect the rate of important reactions in soil and whether this, in turn, can be modified by soil temperature.

As described in the introductory chapter (section 1.3), previous studies have set out to elucidate the relationship between temperature and the rates of mineralization reactions.   Briefly, the results of these experiments have frequently proven contradictory (Rustad et al., 2001; Kirschbaum, 2006; Conant, Steinweg, et al., 2008; Conant et al., 2011). Possible reasons for this lack of consensus may be as simple as the variable use of terms such as temperature sensitivity, leading researchers to discuss subtly different issues (Kirschbaum, 2006; Conant et al., 2011). There are also practical considerations when it comes to experimental design, potential confounding factors have already been discussed in detail in section 1.4 but may include which SOM pool decomposition is being measured for (Bradford, Fierer, et al., 2008; Briones

et al., 2010; Heinemeyer et al., 2012), substrate depletion (Bradford, Davies, et al., 2008; Hartley et al., 2008), microbial adaptation (Peterjohn et al., 1994; Bradford, Davies, et al., 2008; Bárcenas-Moreno et al., 2009) or length of experimental period (Conant, Steinweg, et al., 2008; Turner and Henry, 2010; Butler et al., 2012). The differences between the temperature sensitivity of bulk SOM and that of different SOM fractions are, if not taken into account, likely to have major consequences, not only for the interpretation of results (Piñeiro et al., 2006), but also for the integration of these data into models describing or predicting SOM turnover (Knorr et al., 2005; Davidson and Janssens, 2006; Piñeiro et al., 2006; Lützow and Kögel-Knabner, 2009b). Organic matter (OM) quality, though a somewhat abstract concept, can be defined as the accessibility of organic matter to the decomposer/user community in soil (Ågren and Bosatta, 1998). OM tends to enter the soil system as fresh, easily decomposable material which is then sequentially degraded to become more recalcitrant and difficult to use, therefore suffering a decrease in quality (Bosatta and Ågren, 1985). OM quality has been measured in several different ways, including C:N ratios, lignin concentrations (Guggenberger et al., 1994; Rahn et al., 1999), cellulose content (Rahn et al., 1999), carbohydrate concentrations (Gregorich et al., 1994; Guggenberger et al., 1994), molecular weight (Gregorich et al., 1994), degree of physical protection (Gillabel et al., 2010) and association with particular soil fraction sizes (Gregorich et al., 1994; Accoe et al., 2004), as well as other chemical characteristics.

There has been a move towards defining OM quality using thermodynamic principles. It has been suggested that quality can be quantified using the number of enzymatic steps required to release, as CO_2, one carbon atom from an organic compound

(Bosatta and Ågren, 1999). The higher the number of enzymatic steps required, the lower the quality of the material in question. As introduced in Chapter 1, this can be described using the equation q = 1/n where q = quality and n = the number of enzymatic steps required to mineralize one C atom (Equation 1.5 (section 1.4.3.1)). This leads to the conclusion that decomposition rates of litter of poorer quality will have a stronger temperature dependency than that of higher quality materials (for more details see section 1.4).

This question of OM quality is an important one; soil contains the largest store of organic C in the terrestrial biome (Guo and Gifford, 2002) and 133 – 140 Pg of N are stored in the upper 100 cm of the soil alone (Batjes, 1996). According to the thermodynamic principles discussed in section 1.4, recalcitrant, adsorbed or complexed SOM have slow rates of decomposition with high activation energies (E_a) and therefore are highly temperature sensitive (Davidson and Janssens, 2006; Lützow and Kögel-Knabner, 2009b). This means that these slowly decomposing SOM pools ought to be proportionately more affected by temperature change than faster decomposing more labile pools. If these basic chemical principles hold true under the highly complex environmental conditions in the field, this would mean that increased soil temperature could lead to increased decomposition of recalcitrant SOM and therefore increased loss of soil sequestered C and N (Freppaz et al., 2007; Conant, Drijber, et al., 2008; Conant, Steinweg, et al., 2008). Given the potential consequences of release of greenhouse gases (GHGs) such as CO_2, N_2O and CH_4, it is important to understand how OM of different qualities is likely to respond to realistic changes in soil temperature.

To address this question, the work presented here used a long-term grassland soil amended with OM on a continuum of qualities. Samples were incubated over a range of temperatures typically experienced during autumn at the sampling site according to analysis of met office records from the site between January 1959 and December 2009. The temperatures decreased with time, so as to avoid potential problems of substrate depletion likely to be experienced if temperatures were increased over time. To try to separate out the effects of substrate depletion and changing temperature, a second set of samples was incubated at a stable temperature for the entire incubation period, any changes in the enzyme activity of these samples could then be attributed solely to substrate depletion.

The experiment was designed to bridge the gap between laboratory and field incubations, key aspects of this were to use temperatures and time scales in the laboratory that could plausibly be experienced in the field. In order to do this and attempt to avoid substrate depletion over the course of incubation which are likely to occur if incubation follows an increasing temperature pattern it was decided to use temperature commonly recorded at this site during autumn. In order to separate the effects of temperature from an residual effect of substrate depletion, a second set of samples was incubated at a stable temperature for the entire incubation period, this meant that any change in enzyme activity during the incubation could then be attributed solely to substrate depletion. Microbes mediated the mineralization of soil organic matter through the production of enzymes (intra and extracellular), these communities have been shown to be altered by temperature changes (Zogg et al., 1997; Davidson and Janssens, 2005). It is possible that the temperature treatments

imposed during these experiments could affect microbial community size or structure which could in turn cause changes in mineralization rates; as such the microbial community will be monitored along with rates of enzyme activity.

4.2. Hypotheses

1) Enzyme activity rates will change over the incubation period as a result of changing substrate availability.

2) Enzyme activity rates will be proportionally more affected in samples under the changing temperature regime than those under the stable temperature regime due to the combined effects of substrate depletion and temperature change.

3) Rates of enzyme activity will be significantly affected by the quality of organic matter added to samples; specifically enzyme activity will be higher in samples amended with lower quality organic matter (i.e. more complex substrates will provoke a higher rate of enzyme activity than simple substrates).

4) Rates of enzyme activity in samples where more complex organic matter is added will be proportionally more affected by changes in temperature than those in samples amended with higher quality organic matter.

4.3. Materials and Methods

4.3.1. Temperature range and the definition of autumn

Daily soil temperature data from the Mylnefield met station were used to characterise autumn temperatures for the site. The 25th and 75th quantiles of temperatures recorded at 10 cm depth in the soil were established, with any temperatures above the 75th quartile defined as summer temperatures and any below the 25th percentile as winter temperatures. The remaining temperatures were defined as being from spring and autumn (shown in red in Figure 4.1); the number of days from each season were determined by referring to the dates individual temperatures were recorded on. Under this definition autumn had a maximum temperature of 12.9°C and a minimum of 3.3°C.
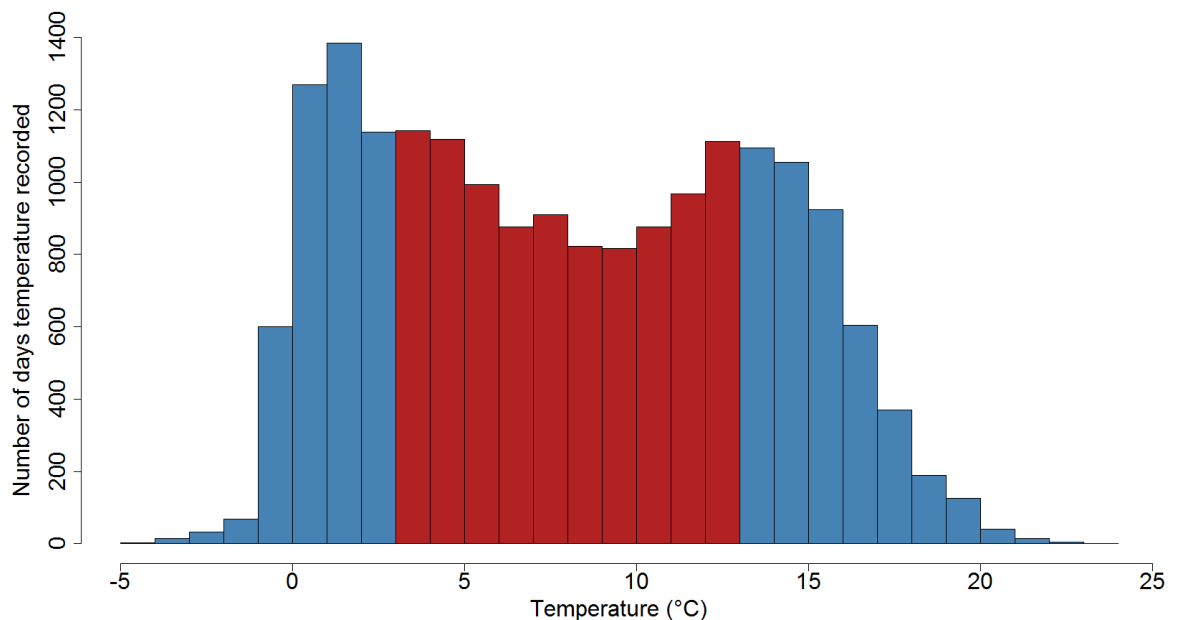
Figure 4.1: Histogram of temperatures recorded at 10 cm depth at the Mylnefield Met station, the range of temperatures used in incubations (12.9 to 3.3 °C) is highlighted in red.

The length of autumn was also estimated from the met station data. The data were inspected and the beginning of autumn defined as the date where temperatures first fell to or below 12.9 °C for 3 or more consecutive days. The end of autumn - beginning of winter was defined in the same way; the first date when temperatures fell to or below 3.3 °C for 3 or more days running. The number of days between these dates was the length of autumn for each year and gave a minimum length of 30 days, a maximum length of 127 days and a median length of 84 days. It was decided to use an incubation of 112 days; this included most of the variation in season length and allowed for a convenient sampling regime where the length of incubation doubled between each sampling event.

4.3.3. Organic matter amendments

A range of OM amendments were added to soil samples; these were designed to be a range of different qualities; a mixture of simple substrates (glucose, albumin and arginine – called simple mixture from now on), the water soluble fraction of green barley powder (freeze dried, powdered leaves from young *Holdum volgare* plants) (lgbp see below for further detail), green barley powder (gbp) and powdered barley straw (bs) (Figure 4.2 and summarized in Table 4.1). All were added so as to supply 3.2 mg of C per g of soil, with C contents of each substance obtained from either molecular formulae (glucose and arginine) or by elemental analysis (albumin, cold water soluble green barley powder, green barley powder and barley straw) carbon:nitrogen (C:N) ratios are shown in table 4.2. The decision was taken to

standardize the amount of substrate added by carbon content so that OM quality

would be determined by the nitrogen content.

Table 4.2: Percentage carbon and nitrogen contents along with C:N ratios of substrates added to samples.

Amendment	% C	% N	C:N ratio
Glucose	40	0	NA
Albumin	41.3	16	2.58:1
Arginine	47.2	13.6	35:1
Liquid green barley powder	0.9	0.4	2.25:1
Green barley powder	45.1	3.7	12.2:1
Barley straw powder	45.2	0.6	75.3:1

Constituent parts of the labile amendment were obtained from Acros Organics

(Arginine P/N 104991000, Albumin 400451000), weighed out and combined prior to

being mixed into soil samples. Green barley powder was obtained from Lifestream (24

Kawana Street, Northcote, Northshore 0627, NZ): for the semi-recalcitrant

amendment the powder was simply weighed out and mixed into the appropriate

samples. For the semi-labile amendment 4 g of green barley powder were added to

40 ml of distilled water, this was then shaken at 200 rpm for 30 minutes at room

temperature. The mixture was then centrifuged at 10, 000 rpm for 10 minutes and the

supernatant drawn off. The solution was then dried in an oven at 60 °C; the

dehydrated residue was then ground into a powder prior to being mixed into the soil

samples. The straw that made up the final amendment was collected from a field

adjacent to the Mylnefield Meteorological station. It was oven dried before being powdered in a ball mill, weighed out and mixed with the soil samples. Due to the small volume of each sample (approximately 140 g each) the organic amendments were mixed in by hand rather than using a homogeniser.

Table 4.1: Composition of substrates added to soil samples prior to incubation.

Treatment	Contents
control	Nothing added
simple mixture	1:1:1 mixture of glucose, albumin and arginine
lgbp	Dehydrated water soluble fraction of green barley powder
gbp	Green barley powder
bs	Powdered barley straw

Figure 4.2: Substrates added to soil samples (from left to right); mixture of simple substrates, lgbp, gbp and bs (further details of composition in Table 4.1)

4.3.4. Soil sampling

Soil was collected from the area next to the Mylnefield meteorological station at the James Hutton Institute, Dundee, in January 2011. Due to the low temperatures at this time (approximately 2 °C at 10 cm depth on the date of collection), the soil was

incubated at 10°C for 4 days prior to the beginning of the experiment. After 4 days at 10°C, all soil was sieved to pass a 4 mm mesh, with roots and above ground biomass removed along with stones and large fragments of particulate organic matter. Soil moisture was maintained at the level at which it was sampled.

4.3.5. Soil incubation

Samples in plastic boxes with ventilation holes punched in the top were placed in two separate incubators set at 12.9°C: one incubator was maintained at this temperature for the entire period of incubation, the temperature of the other incubator was decreased by 0.6°C every week for the whole experimental period (Figure 4.3). Each week, all samples were re-weighed and enough water added, using a spray bottle, to bring them back to their original weights. Destructive sampling then took place after 1, 3, 7, 14, 28, 56 and 112 days of incubation. Samples were incubated under these two contrasting temperature regimes to control for the effects of changing substrate availability over time.
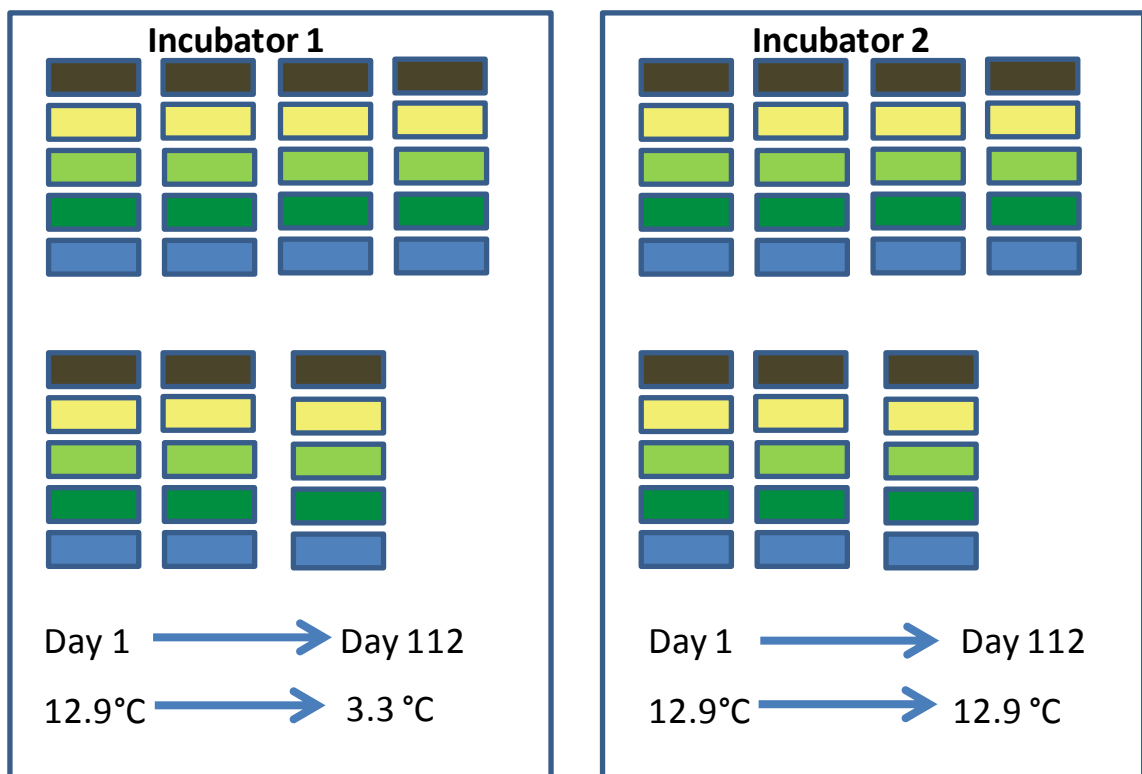
Figure 4.3:   Schematic of experimental set up showing 35 boxes of soil in each incubator (different colours represent different amendment types), incubation period and temperature regime also shown.

4.3.6. Chemical analysis

4.3.6.1. Enzyme assays

At each sampling event, a set of five samples – one of each amendment type – was assayed for protease and amidase activity using the protocol described in section 3.3.3, measuring rates of amidase activity involved measuring NH_4^+ concentrations therefore the controls for this assay doubled as measures of ammonification. Despite the fact that the amidase assay did not initially give measurable results in chapter 3 it was thought that, as with the addition of glucose in chapter 3, the organic amendment treatments described in 4.3.3 would kick-start amidase activity. Samples were also extracted and analysed for nitrate concentrations.

For samples under the changing temperature regime, five sample and three blank protease assays and three sample and one blank amidase assays were run for each amendment type. For samples under the stable temperature regime, three samples and one blank assays were run for both protease and amidase. In both cases three samples were extracted to measure nitrate concentrations.

4.3.6.2. Nitrate extractions

Nitrate (NO_3^-) extractions were carried out using the method described in Anderson and Ingram (1993) and results could be combined with NH_4^+ concentration results to give a measure of total mineral – N availability. Briefly, 5 g samples were weighed into labelled tubes and 10 ml of 0.5M potassium sulphate (K_2SO_4) was added to each, tubes were then capped and shaken at 200 rpm for 30 minutes. Samples were centrifuged at 10,000 rpm for 5 minutes and a 5 ml aliquot of each sample was frozen at -20°C for analysis at a later date.

Extracts were defrosted overnight and 0.5 ml of each pipetted into a clean tube; 1 ml of 5% salicylic acid (5g of salicylic acid dissolved in concentrated H_2SO_4) was added to each and the tubes, which were then carefully mixed on a vortex mixer and left to stand for 30 minutes. Next, 10 ml of 4M NaOH was added and each tube vortex mixed a second time and left to stand for 1 hour before absorbance was measured at 410 nm on the spectrophotometer (WPA Spectrawave S1000). Blanks were run using 0.5 ml of extractant (0.5 M K_2SO_4) and a standard curve was created using 50 µg ml^{-1} NO_3^--N potassium nitrate (KNO_3) solution diluted to 0, 2, 4, 6, 8 and 10 µg ml^{-1} NO_3^- -N. Nitrate concentrations were calculated from the absorbance measurements using Equation 4.1.

Equation 4.1:

$$NO_3^- - N(\mu g \ g^{-1} soil) = \frac{(measured \ concentration * total \ volume)}{dry \ weigt \ of \ sample}$$

4.3.6.3. Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) was carried out on samples collected after 1, 7 and 112 days of incubation to compare the size and structure of the microbial community with rates of enzyme activity. Samples for phospholipid fatty acid analysis (PLFA) were initially frozen at -20 °C and then freeze dried prior to extraction for analysis. Aliquots of approximately 1500 mg of freeze-dried soil were weighed into glass centrifuge tubes; 9.16 ml of extractant (extractant consisted of 80 ml of chloroform, 150 ml of methanol and 60 ml of citrate buffer) were added to each tube. The tubes were then vortex mixed and wrapped in aluminium foil. Samples were vortex mixed every 30 minutes for 2 hours and then centrifuged at 1500 rpm at 20 °C for 17 minutes. The supernatant was then decanted into clean borosilicate glass culture tubes with PTFE caps; a further 2.5 ml of extractant were added to each sample and they were again mixed every 30 minutes for 2 hours before being centrifuged as before. The supernatant solutions were combined and then 3.1 ml of chloroform and 3.1 ml of citrate buffer were added to each tube. The tubes were then mixed on a sample rotator for 15 minutes at 20 rpm and then left overnight in a cooled incubator at 4 °C.

The following morning, samples were centrifuged as before for 2 minutes. Then, using a vacuum aspirator, the upper, aqueous layer was removed and discarded. Next, the

lower, organic phase was removed using a Pasteur pipette and transferred to a clean glass tube and then evaporated to dryness using a centrifugal evaporator (Joan RC 1022) equipped with a vacuum pump. The samples were then capped and stored at -20 °C prior to lipid fractionation.

Lipids were separated into different fractions by solid phase extraction (SPE) using silica columns with a sorbent mass of 500 mg and a reservoir of 6 ml (Isolute columns from BIOTAGE, P/N 460 – 0050 – C). Each column was fitted with a one-way stopcock that was set to closed whilst 5 ml of chloroform was added to each column and then opened to allow the chloroform to drain through and "condition" the column. Taps were then closed again. Each desiccated sample was then re-suspended in 500 µl of chloroform, the tubes were mixed and using a clean Pasteur pipette the resulting mixture transferred to the appropriate column. The sample vials were washed twice with 500 µl of chloroform and the washings transferred to the correct columns. When all samples had been put in their columns the taps were opened and the samples loaded onto the silica. Next, 6 ml of chloroform was added to each column and as this drained through the neutral lipids were eluted. The glycolipids were then eluted using 6ml of acetone and finally 10 ml of methanol were used to elute the phospholipids. These were collected in a clean culture tube, evaporated to dryness using the centrifugal evaporator and then stored at -20 °C prior to methanolysis.

Samples were removed from the freezer and, once thawed, 200 µl of C19:0 internal standard (nonadecanoic acid, Sigma Aldrich, P/N 72332) was added to each sample. Next, 1 ml of a 1:1 mixture of methanol and toluene was added to each sample, samples were then vortex mixed and 1 ml of 0.2 M KOH was added and all samples

mixed again then incubated at 37 °C in a heated water bath for 15 minutes. After this methanolysis stage, the samples were removed from the water bath and allowed to cool. The cool samples then had 2 ml of a 4:1 mixture of iso-hexane and chloroform, 0.3 ml of 1 M acetic acid and 2 ml of milipore water added to them. The samples were then capped and mixed on the sample rotator at 20 rpm for 10 minutes.

Samples were then centrifuged at 1500 rpm at 20 °C for 2 minutes and, using a clean Pasteur pipette, the upper, organic phases were transferred to a clean glass tube. A further 2 ml of iso-hexane:chloroform were added to the tube containing the lower aqueous phase. All samples were vortex mixed and centrifuged again and the upper, organic phase transferred to the correct tube, these samples were again dried down using the centrifugal evaporator and frozen at -20 °C.

Prior to analysis by gas chromatography (GC), samples were thawed and 300 µl of 4:1 v:v iso-hexane: chloroform mixture was added to each. This was then mixed and transferred to a 350 µl flat bottom insert within a snap-cap GC vial using a clean Pasteur pipette.

Gas chromatography analysis was carried out by the GC lab at The James Hutton Institute's Aberdeen site. Analysis was carried out using an HP6890 GC, fitted with a flame ionisation detector and an HP7673 auto sampler. The GC was fitted with a capillary gas liquid chromatography column 50m x 0.20mm x 0.33µm (l:w:id) film thickness which was coated with 5%-Phenyl-methyl-polysilosane. The sequence of samples analysed is illustrated in Figure 4.4 and the Fatty Acid Methyl Ester (FAME) quality control standard was made up using the components in Table 4.3. Hewlett

Packard Chemstation software was used to control the GC and acquire the data and

concentrations calculated using Equation 4.2.

FAME QC standard
Sample 1
Sample 2
Sample 3

Sample 10
FAME QC standard
Sample 11
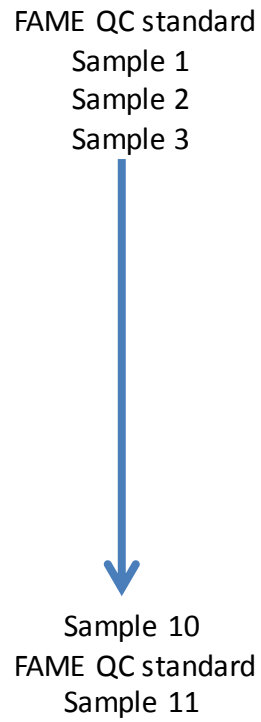
Figure 4.4: sample analysis protocol showing that a standard was analysed at the beginning and then once every 10 samples thereafter.

Table 4.3 Components of the FAME QC standard and their peaks, used to identify peaks found in the PLFA samples.

Component	Peak
Methyl laurate	C12:0
Methyl tridecanoate	C13:0
Methyl myristate	C14:0
Methyl petadecanoate	C15:0
Methyl palmitoleate	C16:1
Methyl palmitoleate	C16:0
Cis-16-heptadecenoic acid meth	C17:1
Methyl heptadecanoate	C17:0
Methyl linoleate	C18:2(9, 12)
Cis-9-oleic methyl ester	C18:1ω9
Methyl stearate	C18:0
Methyl cis-5, 8, 11, 14 eicosatet	C20:4 ω (5, 8, 11, 4)
Methyl cis-t, 8, 11, 14, 17 – eicosa	C20:5 ω 3
Methyl eicosenoate	C20:1 ω 9
Methyl arachidate	C20:0

Equation 4.2:

$$Concentraion = \frac{area\ of\ analyte}{area\ of\ internal\ standard} \times \frac{weigt\ of\ internal\ standard}{weigt\ of\ sample}$$

Results are then expressed in μg x-plfa/ g sample.

4.3.7. Data analysis

Rates of enzyme activity were determined as described in section 3.3.3. These were then plotted against both the length of incubation time and the temperature at the time samples were removed from the incubator to be assayed for activity, the same was done for nitrate concentrations, mineral-N concentrations and soil moisture. The effects of time, temperature and OM amendment type were assessed on all variables using ANOVAs (three-way for changing temperature regimes and two-way for stable temperature regimes). Total PLFA measurements were compared across time, temperature and OM type using two way ANOVAs and principal components analysis (PCA was carried out on the entire data set to assess changes in community structure). Bacterial: fungal ratios were also calculated and compared over time, temperature and OM amendment type. All ANOVAs and principle components analyses (PCAs) were carried out using the R statistical package, version 2.15.0 (R Development Core Team, 2010).

4.4. Results

4.4.1. Protease

Protease activity from each temperature regime was plotted over time (Figure 4.5), and the relationship between protease activity and temperature, time and amendment type was analysed by applying a two-way ANOVA to the results from each temperature regime separately (Table 4.4). Under both temperature regimes the type of organic matter amendment was found to have a statistically significant effect on protease activity rates ($p < 0.0001$ under a changing temperature regime and $p = 0.0013$ under a stable temperature regime). Under the stable temperature regime day was not significant ($p = 0.0772$) and the interaction between day and organic matter amendment type was also non-significant ($p = 0.0896$). However, under the changing temperature regime, both the effect of day and the interaction of day and organic matter amendment type were statistically highly significant ($p < 0.0001$ for both). A separate ANOVA was run to test the effect of temperature and the interaction between amendment type and temperature, and both were found to be highly significant ($p < 0.0001$ for both).

Figure 4.5: rates of protease activity from samples exposed to changing (left) and stable (right) temperature regimes after different lengths of incubation.

*Table 4.4: Results of ANOVAs applied to protease activity rates recorded under both temperature regimes; * represents interaction terms.*

Temperature regime	Variable	d.f.	F-value	p-value
Changing	Amendment type	4	33.678	<0.0001
	Temperature	1	97.721	<0.0001
	Days of incubation	1	2.866	0.09
	Amendment type*temperature	4	9.344	<0.0001
	Amendment type*Days	4	0.872	0.482
	Temperature*Days	1	13.616	0.0003
	Amendment type*temperature*Days	4	5.671	0.0002
Stable	Amendment type	4	4.856	0.0013
	Day	1	3.191	0.077
	Amendment type*Days	4	2.079	0.089

4.4.2. Amidase

Amidase activity rates were very low under both the stable and the changing temperature regimes (Figure 4.6; note units on y-axes are in ng g^{-1} soil rather than μg g^{-1} for protease). Under the changing temperature regime, Figure 4.6 shows a slight decrease in the rates of activity with increasing length of incubation time, although the data are very noisy and as such reliable patterns are difficult to extract. When temperature regimes are considered separately using two way ANOVAs, organic matter amendment type was shown to be highly significant under the changing temperature regime ($p < 0.0001$, Table 4.5). All other factors under the changing temperature regime were not significant. The significance of amendment type is likely to be largely attributable to the activity measured at day 7 in samples amended with the mixture of labile substrates (Figure 4.6). This is supported by the results of a Tukey's Honest Significant Differences (Tukey's HSD) test where samples amended with the simple mixture of substrates were found to have significantly different activities from those amended with green barley powder or the control samples ($p = 0.0053$ and < 0.0001, respectively). Samples amended with the smiple substrates mixture were also found to be different (approaching significance at the 5% level) from those amended with liquid green barley powder and straw ($p = 0.0517$ and 0.0527 respectively) (Figure 4.7). Under the stable temperature regime, amendment type and days of incubation were non-significant as individual variables but they did produce a significant interaction ($p = 0.0024$).
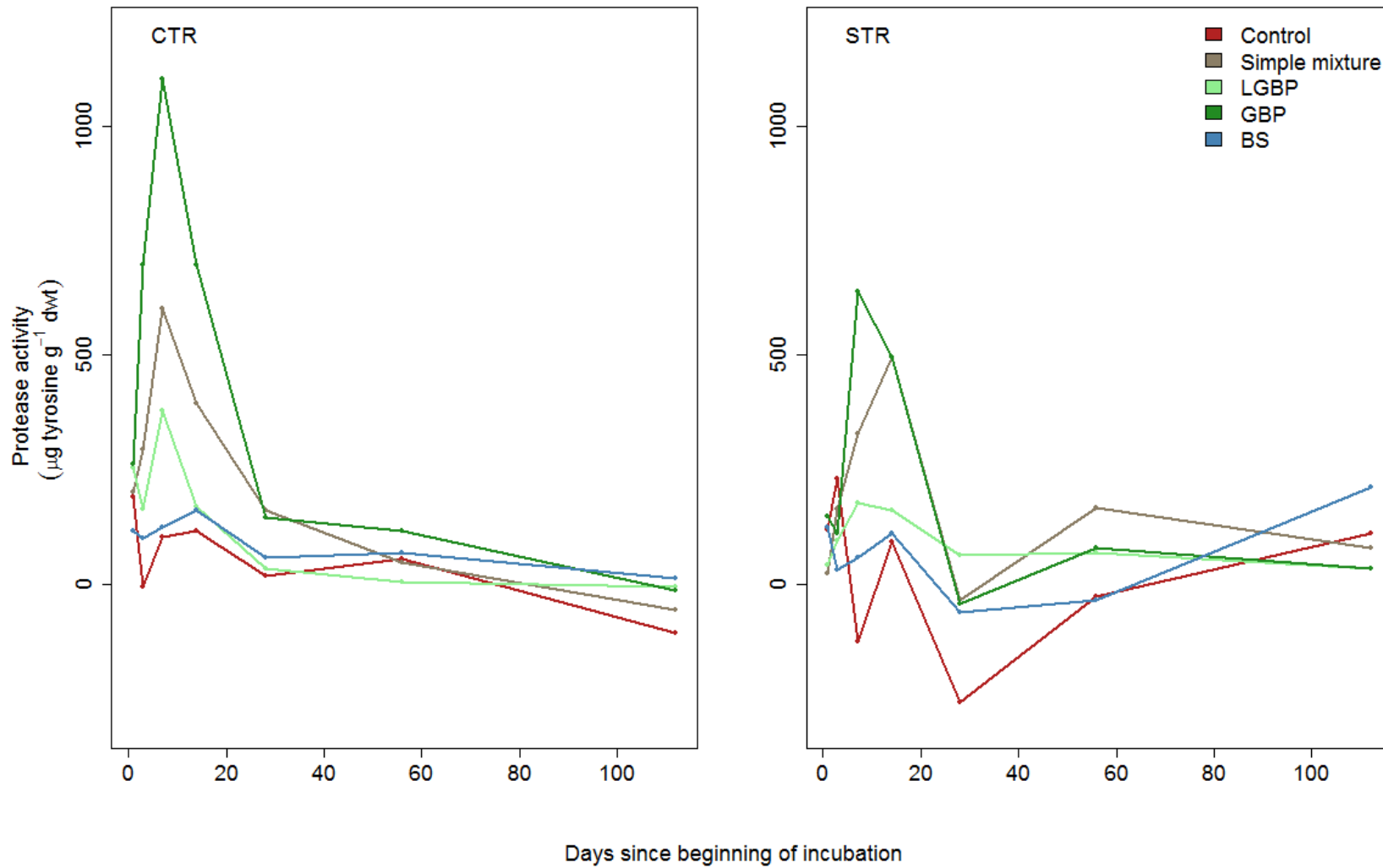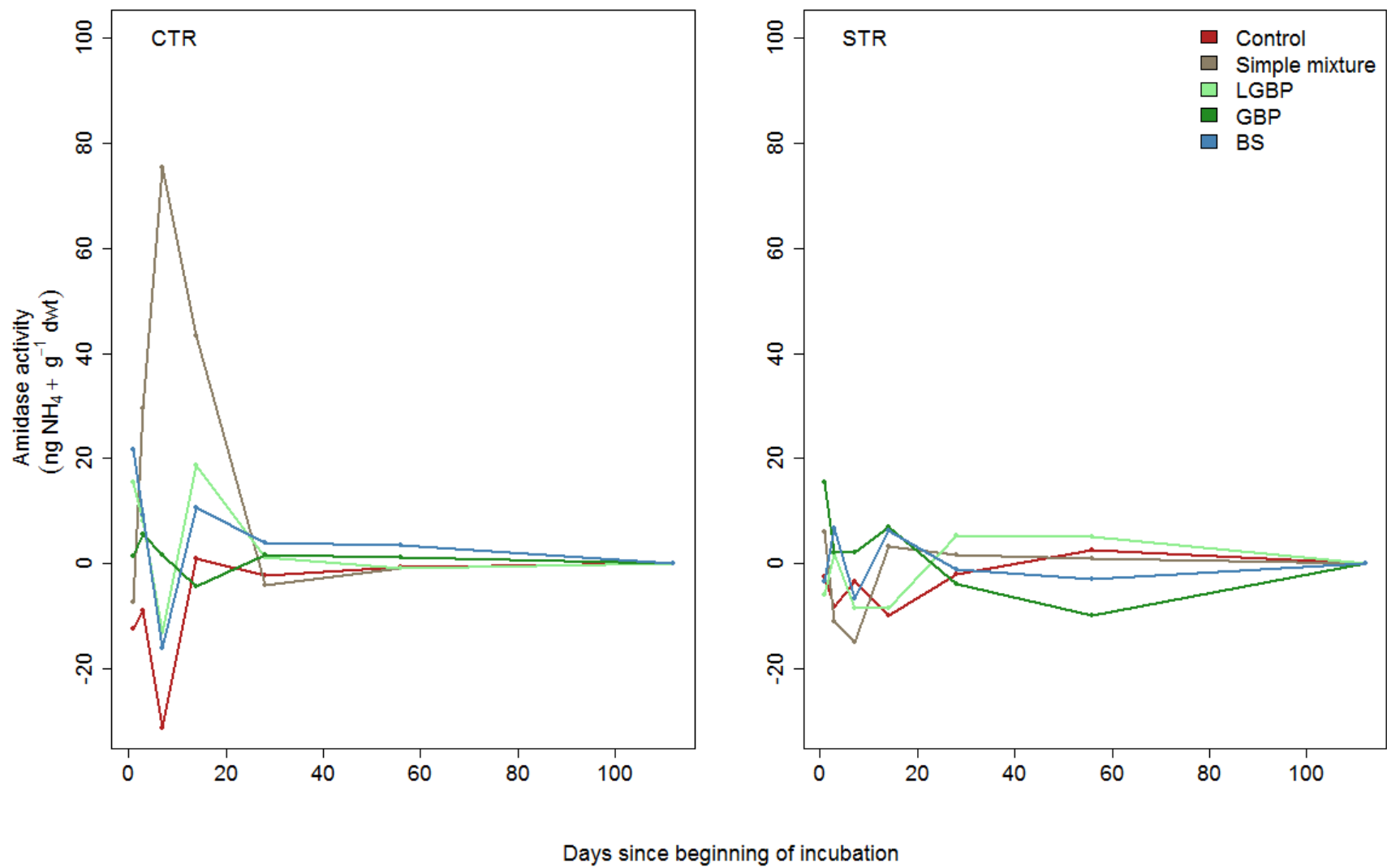
Figure 4.6: rates of amidase activity from samples exposed to changing (left) and stable (right) temperature regimes after different lengths of incubation.

Table 4.5: p – values, F – values and degrees of freedom (d.f.) for ANOVAs carried out using amidase activity data under a changing or a stable temperature regime.

Temperature regime	Variable	d.f.	F-value	P-value
Changing	Amendment type	4	8.105	<0.0001
	Temperature	1	1.330	0.252
	Days of incubation	1	0.003	0.9556
	Amendment type*temperature	4	2.102	0.0876
	Amendment type*Days	4	0.760	0.5540
	Temperature*Days	1	4.593	0.0350
	Amendment type*Temperature*Days	4	2.963	0.024
Stable	Amendment type	4	1.412	0.235
	Days of incubation	1	0.144	0.704
	Amendment type*Day	4	4.448	0.002

Figure 4.7: Tukey's honest significant difference confidence limits for the changing temperature regime amidase activities. Red bars show significant differences (at the 5% level), green bars show those differences that are approaching significance at the 5% level. Labels on y-axis are as follows C= control, S= simple mixture, LG = liquid green barley powder, G= green barley powder and BS= barley straw.

4.4.3. Nitrate concentrations

Nitrate concentrations generally increased over the 112 day incubation period (Figure 4.8). Two-way ANOVAs were used to investigate the significance of these apparent increases. Under th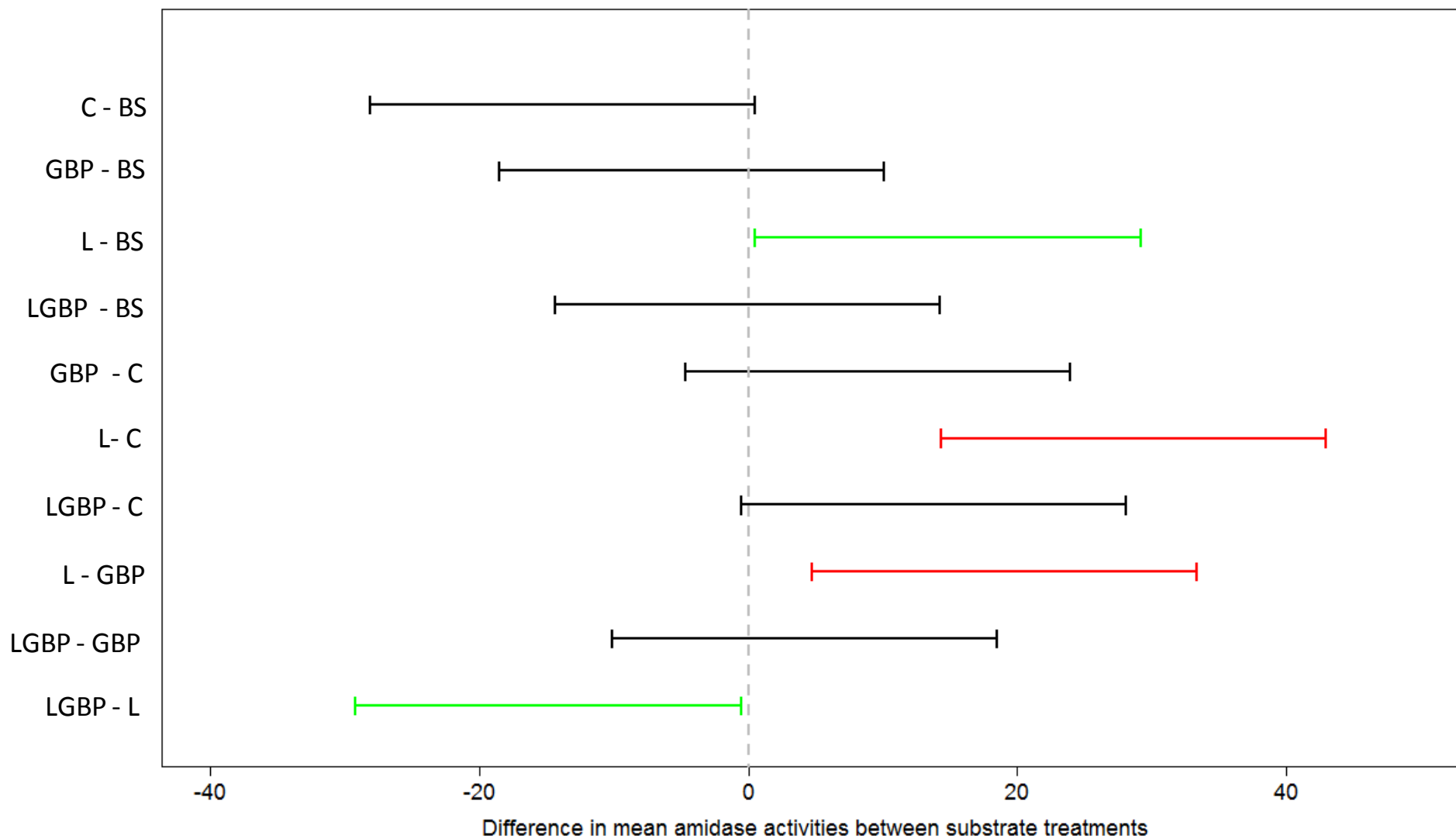e stable temperature regime, amendment type, incubation length and the interaction between the two were all highly statistically significant ($p < 0.0001$). Under the changing temperature regime, amendment type was highly statistically significant ($p < 0.0001$) and length of incubation and temperature were also statistically significant ($p = 0.0010$ and 0.0011 respectively). However, there were no significant interactions between amendment type and length of incubation or temperature under the changing temperature regime (Table 4.6). The same process was followed for total mineral-N concentration (obtained by adding together the NO_3^- concentrations with the NH_4^+ concentrations measured as control samples for amidase activity), the results were strikingly similar (Figure 4.8). Amendment type was the most important factor under the changing temperature regime ($p < 0.0001$) although the number of days after the beginning of incubation and the temperature that assays were carried out at were less significant ($p = 0.0673$ and 0.0636 respectively), there were no significant interactions (Table 4.7). Under the stable temperature regime both amendment type and number of days after the beginning of incubation were highly statistically significant ($p < 0.0001$) as is the interaction between the two ($p < 0.0001$) (Table 4.7).

Table 4.6: ANOVAs used to investigate differences in NO_3^- concentrations between days during the 112 day incubation period; all p-values quoted at the 95% significance level.

Temperature regime	Variable	d.f.	F-value	P-value
Changing	Amendment type	4	58.684	<0.0001
	Temperature	1	35.039	<0.0001
	Days of incubation	1	3.033	0.0852
	Amendment type*Temperature	4	3.862	0.0062
	Amendment type*Days	4	11.496	<0.0001
	Temperature* Days	1	72.758	<0.0001
	Amendment type* Temperature*Days	4	22.779	<0.0001
Stable	Amendment type	4	11.32	<0.0001
	Days of incubation	1	57.01	<0.0001
	Amendment type* Days	4	9.20	<0.0001

Table 4.7: ANOVAs used to investigate differences in mineral-N concentrations between days during the 112 day incubation period; all p-values quoted at the 95% significance level.

Temperature Regime	Variable	d.f.	F-value	P-value
Changing	Amendment type	4	8.588	0.0008
	Temperature	1	2.754	0.1177
	Day	1	8.542	0.104
	Amendment type*Temperature	4	0.055	0.9937
	Amendment type* Days	4	1.153	0.3698
	Temperature* Days	1	6.553	0.0217
	Amendment type* Temperature* Days	4	0.735	0.5820
Stable	Amendment type	4	9.164	0.0001
	Days of incubation	1	17.540	0.0003
	Amendment type* Days	4	0.861	0.5009
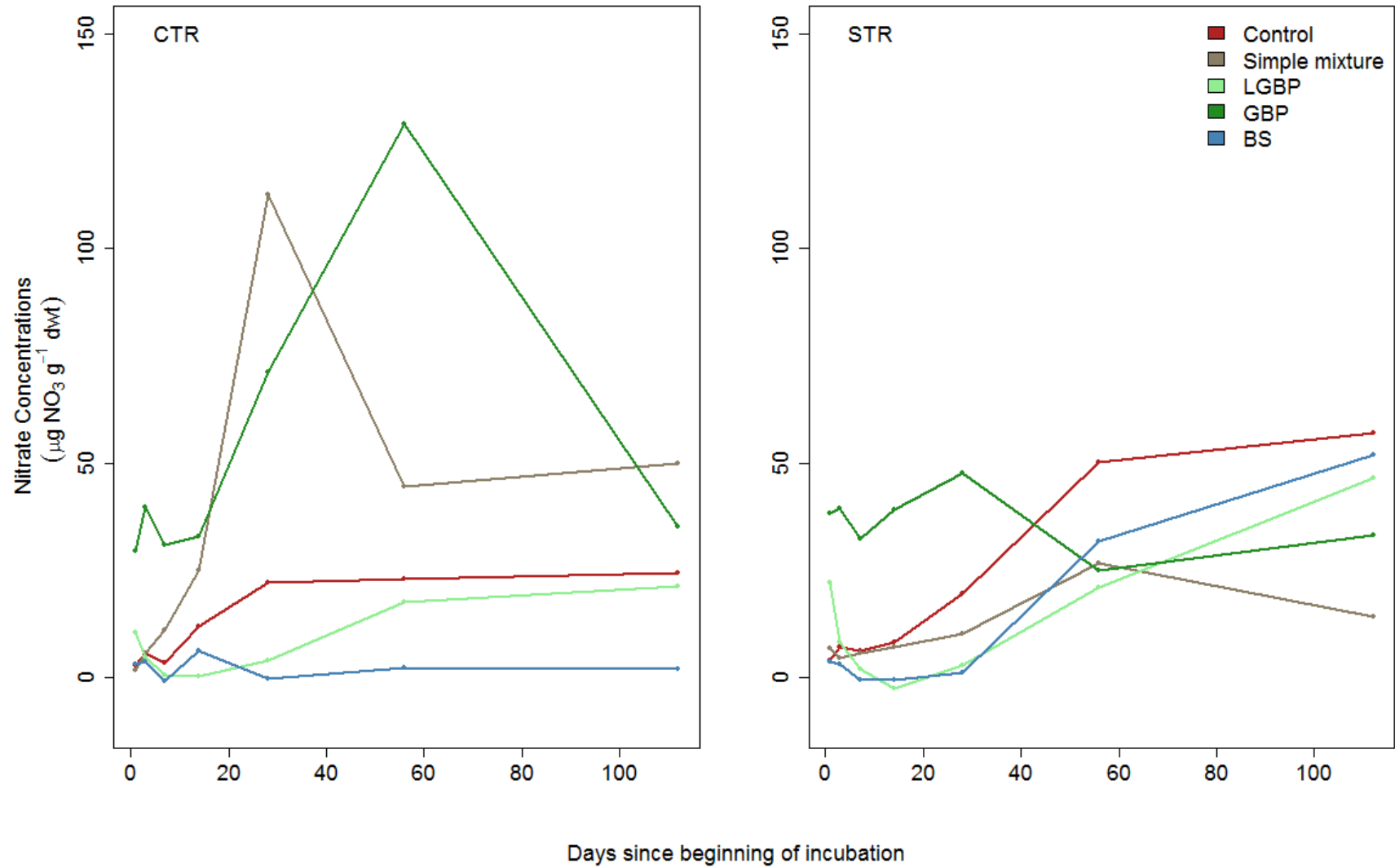
Figure 4.8: Plots showing the concentrations of Nitrate extracted from samples amended with different organic matter types after different lengths of incubation under a changing temperature (CTR) and stable temperature regime (STR).

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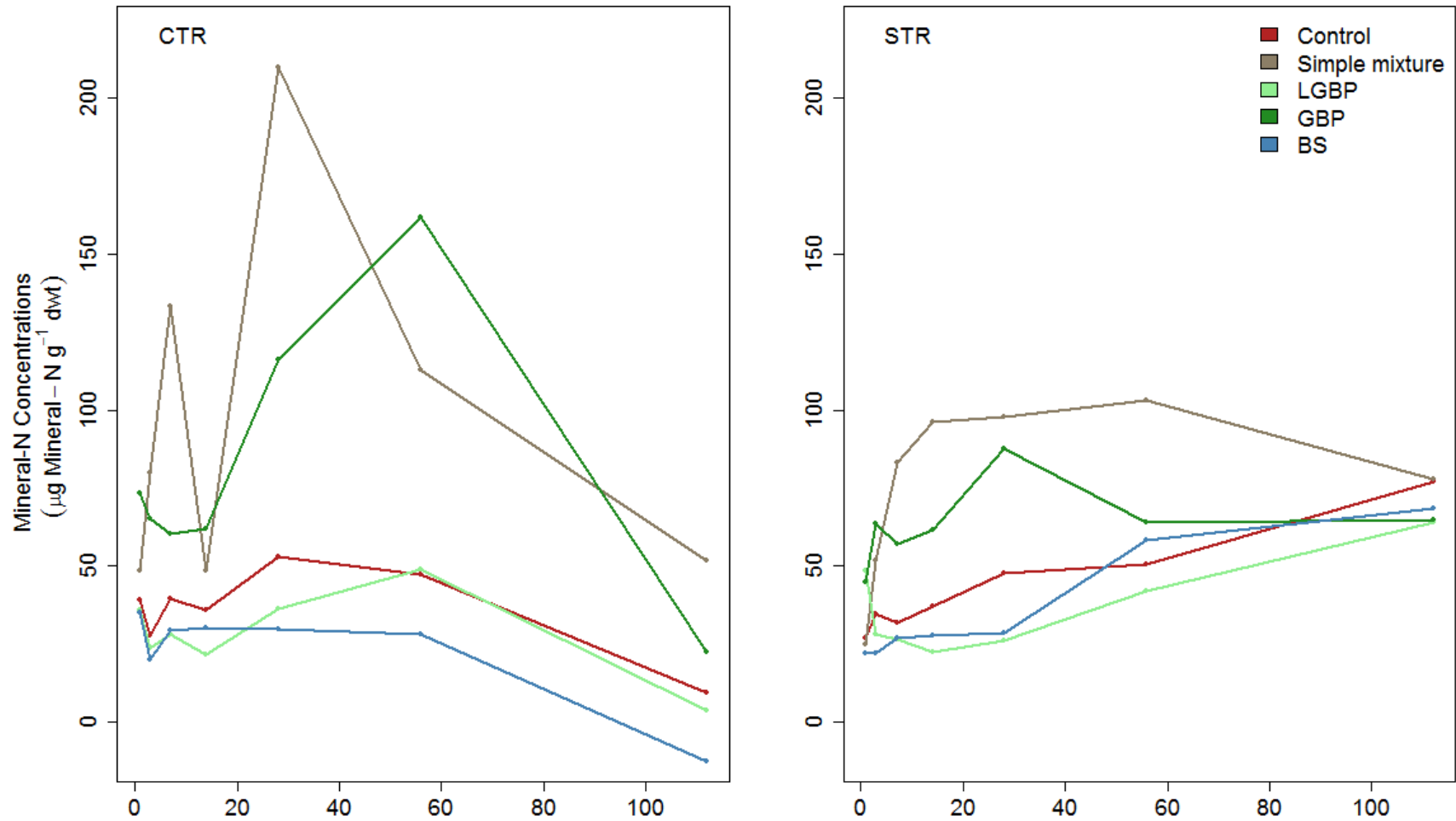
Figure 4.9: Plots showing the concentrations of mineral-N extracted from samples amended with different organic matter types after different lengths of incubation under a changing temperature (CTR) and stable temperature regime (STR).

4.4.4. Soil moisture

Soil moisture contents were also monitored throughout the 112 day experimental period, as it was thought that variation in this could represent a confounding factor. When the percentage water contents were plotted there is variation but it is difficult to tell if this was systematic or not (Figure 4.10). Again, two way ANOVAs were used to assess any pattern (Table 4.8) but no significant differences in soil moisture were found (Table 4.8).

Figure 4.10: moisture content of soils incubated under changing (CTR) or stable (STR) temperature regimes after different lengths of incubation.

Table 4.8: results of 2-way ANOVAs applied to soil moisture results from both temperature regimes

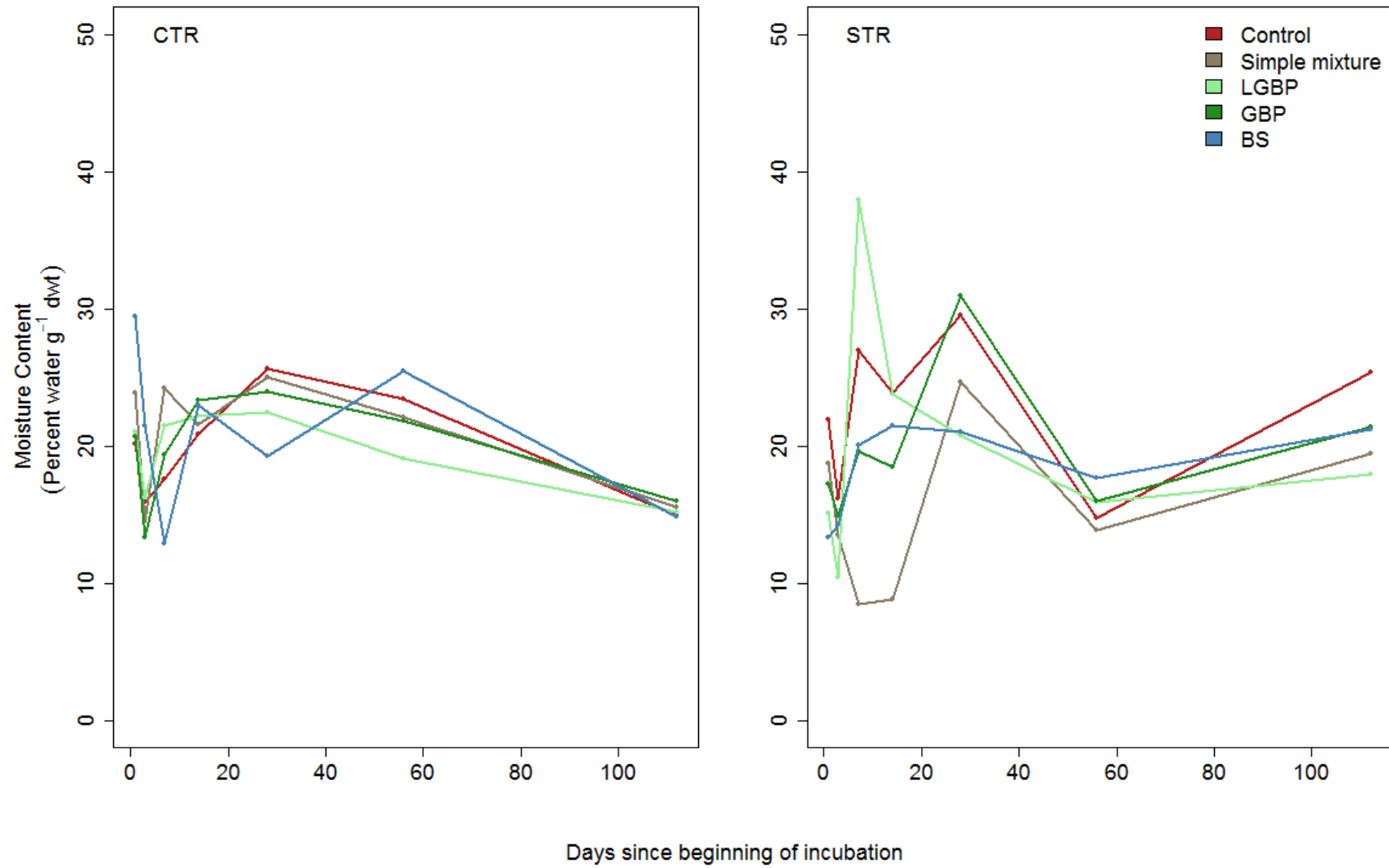| Temperature Regime	Variable	*d.f*	F-value	p-value
Changing	Amendment type	4	0.450	0.7710
	Temperature	1	0.156	0.6981
	Days of incubation	1	0.023	0.8811
	Amendment type*Temperature	4	0.297	0.8754
	Amendment type*Days	4	0.435	0.7811
	Temperature* Days	1	4.778	0.0451
	Amendment type* Temperature* Days	4	0.473	0.7552
Stable	Amendment type	4	1.196	0.337
	Days of incubation	1	0.351	0.559
	Amendment type* Days	4	0.264	0.898

4.4.5. PLFA results

Total PLFA results were used as a proxy for total microbial biomass. Generally the microbial biomass was lower at the end of the 112 days of incubation than at the beginning, but the highest levels were seen on day 7 of incubation (Figure 4.11). One-way ANOVA shows that there is no difference between the size of the microbial biomass in samples under the two temperature regimes (p = 0.731, Table 4.9). When data from both temperature regimes were considered together, amendment type was found to give significantly different total PLFA weights per gram of soil (p=0.0013). The number of days of incubation also had an effect on the size of the microbial population but this was not significant (p=0.0591) there was no significant interaction between these two variables (p=0.1229).

Table 4.9: ANOVA results for total PLFA weights per gram of soil.

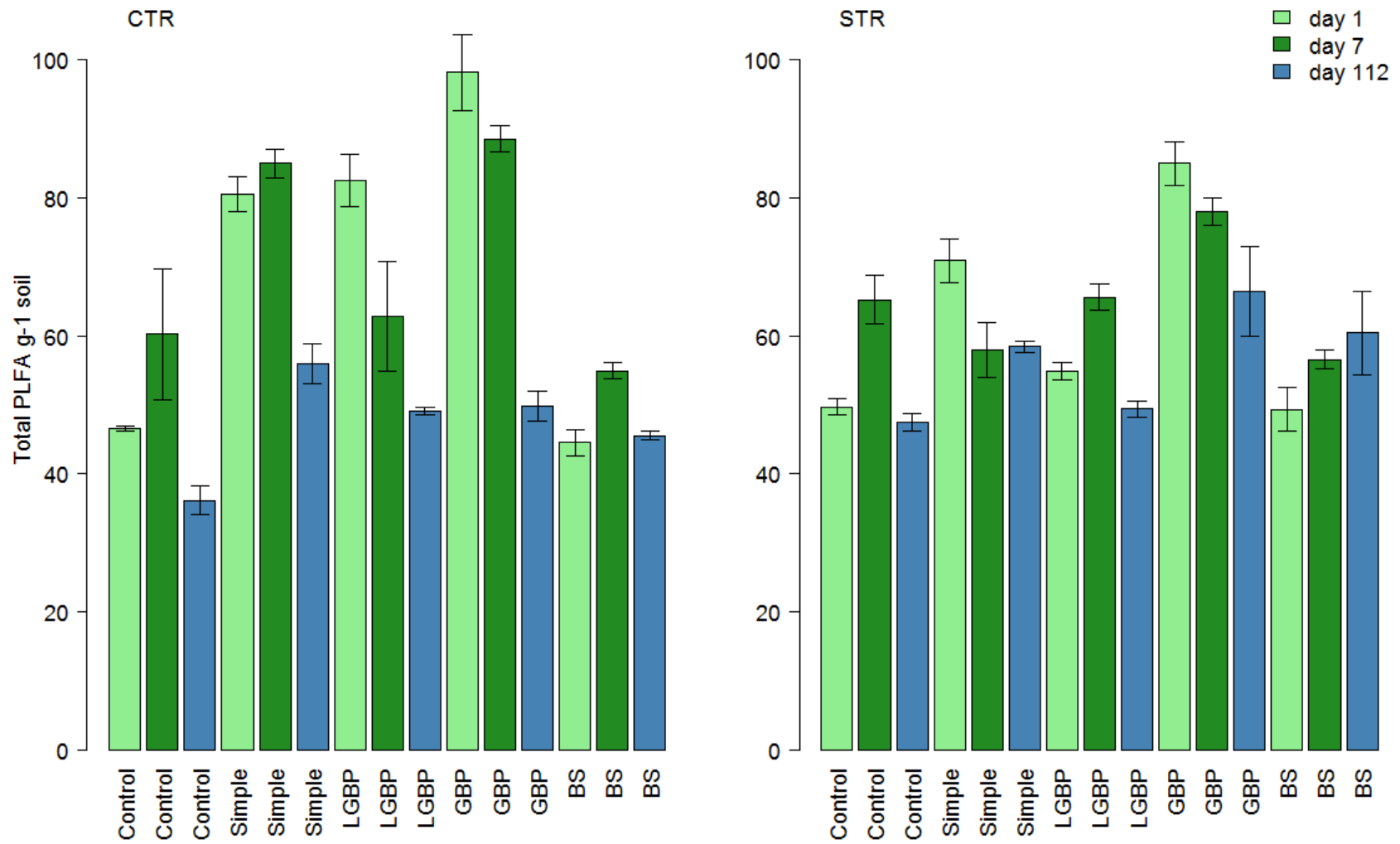| Variable	d.f	F – value	p - value
Temperature regime	1	0.119	0.731
Days of incubation	1	3.665	0.0591
Amendment type	4	4.917	0.0013
Days of incubation * amendment type	4	1.875	0.1229

Figure 4.11: Average total PLFA values (as a proxy for total microbial biomass) for days 1, 7 and 112 under both temperature regimes for each type of OM amendment, error bars = +/- 1 standard error.

PCA carried out on the whole dataset results in 43 principal components (PCs); the first two of which explain 57.72% of the variation in the data (Figure 4.12). Figure 4.12a suggests that the microbial community structure is similar at the end of 112 days of incubation to the beginning of the incubation. Figure 4.12 suggests that the temperature regime the samples were subjected to has no significant effect on community structure and that organic matter amendment type was also inconsequential for community structure. Bacterial : fungal ratios were calculated for each sample using the PLFAs listed in table 4.10. With the exception of samples amended with straw, all samples under both temperature regimes show small increases in the bacterial : fungal ratio over time (Figure 4.13). Those samples amended with straw under both temperature regimes show slight increases in the bacterial : fungal ratio by the end of the 112 day incubation, suggesting the community has changed slightly to deal with the type of organic matter available in the soil.

Table 4.10: Specific PLFA's used to calculate bacterial : fungal ratios in samples and their groupings.

Taxonomic group	Specific PLFA's	Reference
Gram positive bacteria	i15:0, a15:0, i16:0, i17:0, a17:0	Fierer et al., 2003; Potthoff et al., 2006; Moore-Kucera and Dick, 2008
Gram negative bacteria	16:1ω7t, cy17:0, cy19:0, 18:1ω7	Fierer et al., 2003; Potthoff et al., 2006; Moore-Kucera and Dick, 2008
Actinomycetes	10Me16:0, 10Me17:0, 10Me18:0	Fierer et al., 2003; Moore-Kucera and Dick, 2008
Fungi	16:0, 16:1ω5, 16:1ω7c	Olsson, 1999
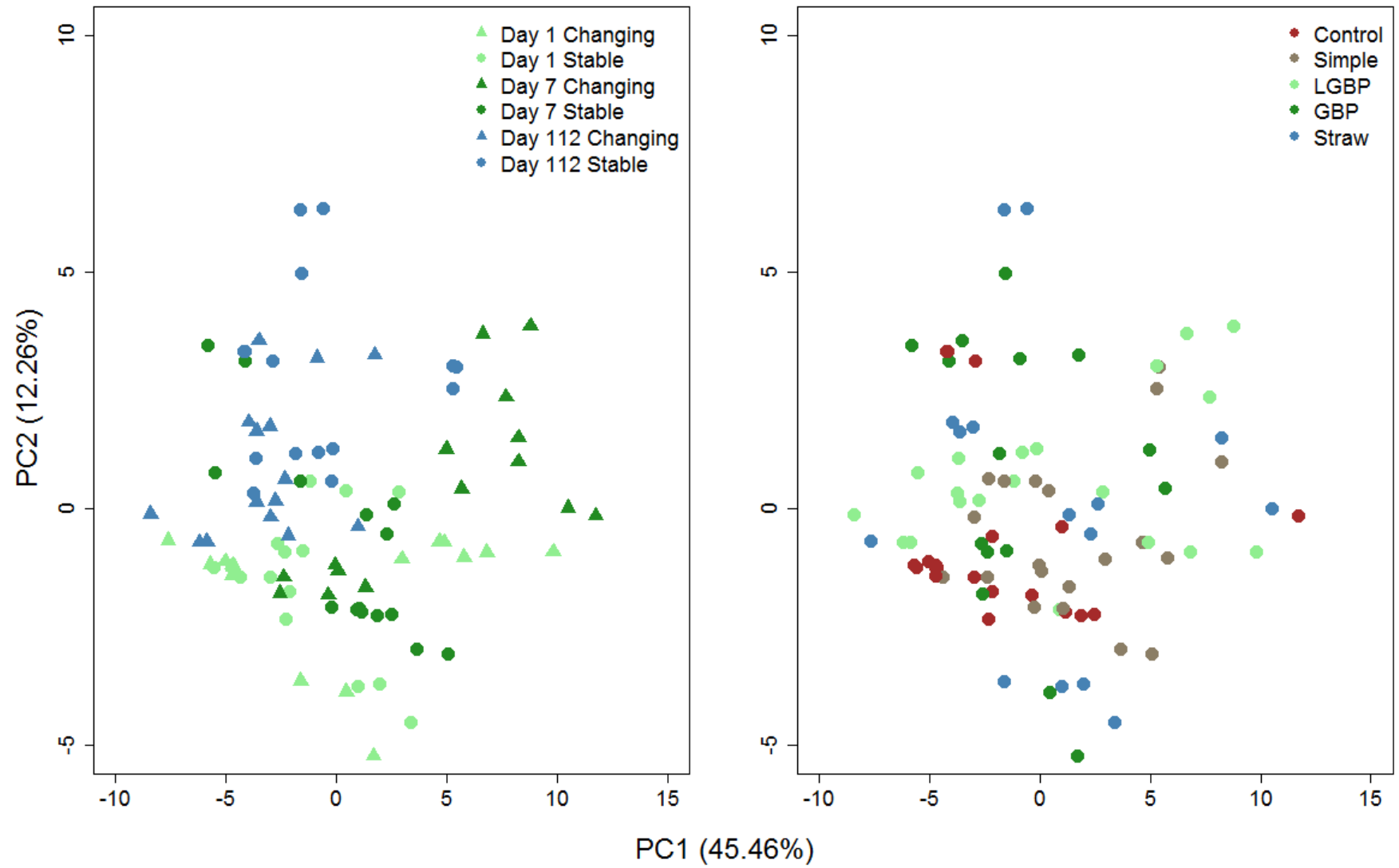
Figure 4.12: plots of the scores for PC1 against PC2 showing the distribution of samples taken on different days (colours in 9a), samples kept under different temperature regimes (symbols in 9a) and samples amended with different types of
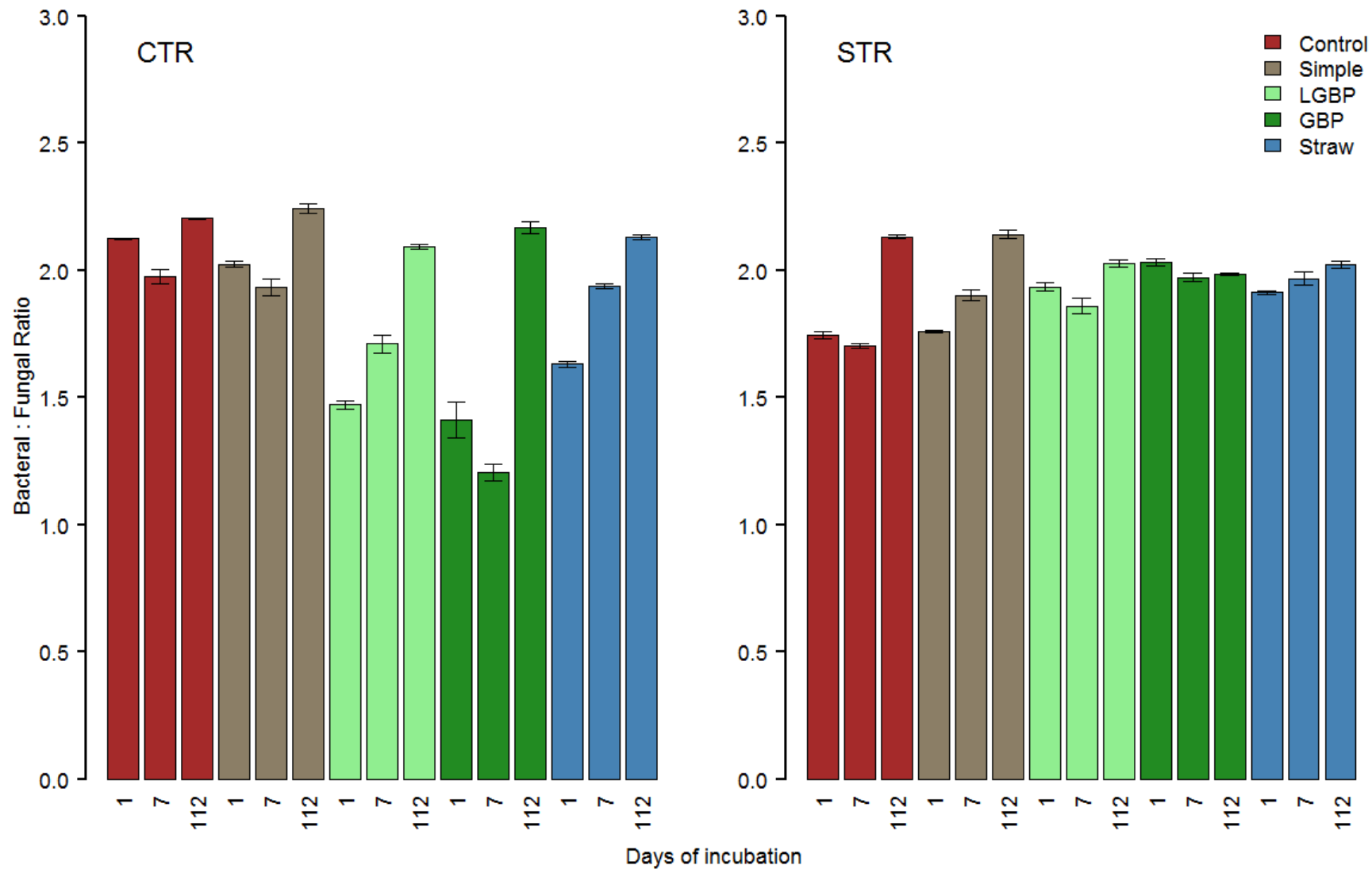
Figure 4.13: Bacterial : Fungal ratios (calculated as described in section 4.4.5.) for samples under different temperature regimes, amended with different types of organic matter after 1, 7 or 112 days of incubation.

4.5. Discussion

Enzymes sometimes showed negative rates of activity as these were determined by calculating the difference between activity measured in the sample and the activity measured in a control. If the activity in the sample is very low then the activity in the control may be marginally higher and so the difference is negative. Despite this, these results show that it is possible to find robust, measurable differences in rates of mineralization reactions over this narrow (9.6 °C) temperature range. Based on the results in Chapter 2 – where significant increases in soil temperatures, particularly during the coldest months of the year, were demonstrated – these results suggest that mineralization in this soil is likely to increase with warming, even at cooler times in the year.

Changes in activity rate of enzymes involved in N-mineralization at cooler points in the year could have a range of knock-on consequences for the soil system. Increased rates of N-mineralization, associated with increased soil temperatures, are likely to lead to an increase in the availability of inorganic forms of N, such as NH_4^+ and NO_3^- (Cookson et al., 2002; Agehara and Warncke, 2005). Some field studies in other, non-grassland, environments have suggested that N-mineralization is relatively insensitive to temperature change and is more sensitive to soil moisture change (e.g. Beier et al., 2008), though the work here does not test this as efforts were made to keep soil moisture levels constant throughout incubation. Increased N-mineralization driven by temperature increases during winter months could lead to leaching of NO_3^- as this is the period in the year when plants and many microbes are likely to be least active (Freppaz et al., 2007). Empirical evidence of how plants are likely to respond to

changes in soil temperature is limited and suggests that plants from different environments may respond variably (Bassirirad, 2000; Birgander et al., 2012).

This experiment was designed to do two things. First was to try to disentangle the effects of time and temperature on the rates of reactions taking place in incubated soil. The second was to assess the effect of different types of OM (and therefore OM quality) on the rates of soil enzyme activities.

4.5.1. Disentangling the effects of time and temperature

In order to resolve the effect of changing incubation temperature from the effect of differing length of incubation period, the entire experiment was replicated at a stable temperature for the whole 112 days of incubation allowing the determination of enzyme activity rates, mineral-N pools and size and structure of microbial biomass from samples of the same soil identically treated with the exception of changing temperatures.

Difference in protease activity rates between samples incubated at a constant temperature and samples incubated at a changing temperature show that temperature is a stronger driver of activity than length of incubation time (Table 4.3). However, when the size of the microbial biomass was considered, incubation temperature had no effect but length of incubation time had a small effect (Table 4.8). These results show that the functioning of the soil but not the structure of the microbial community was affected by temperature change. As discussed in section 1.4.3, some previous studies have suggested that changes in rates of soil processes such as respiration or N-mineralization are directly as a result of changes in the

microbial community (Luo et al., 2001; Lipson et al., 2002; Bradford, Davies, et al., 2008; Bárcenas-Moreno et al., 2009; Rousk et al., 2012). Various routes by which changes in the microbial community could be affecting the rates of soil functioning have been suggested including changes in overall size (Lipson et al., 2002), changes in structure (Avrahami et al., 2003; Allison and Martiny, 2008; Bárcenas-Moreno et al., 2009; Kaiser et al., 2010) or physiological changes (Schimel et al., 2007; Bradford, Davies, et al., 2008; Rousk et al., 2012) in response to a range of changes in soil conditions. The results presented here find no correlation between the structure of the microbial community and soil functioning (here the rates of enzyme activity). This insensitivity of the microbial biomass or community structure to temperature change, but apparent plasticity of function, suggests that either the activity of extracellular enzymes extant in the soil changes (Allison, 2006) or the synthesis and secretion of enzymes by the microbial community changes (Sollins et al., 1996; Tscherko et al., 2004; Burns et al., 2013) in response to temperature change. The overall response of the samples analysed here is likely to be as a result of a combination of both increased activity of enzymes at higher temperatures (due to thermodynamic constraints discussed in section 1.4.1) and a change in the rate of synthesis and secretion of enzymes with temperature as a result of factors such as substrate availability (Moorhead and Sinsabaugh, 2000; Marx et al., 2005).

4.5.2. A question of quality

Substrate availability or depletion is an issue, particularly for long-term soil heating experiments in the field, which can lead to a misinterpretation of the temperature sensitivity of organic matter decomposition (Kirschbaum, 2006). By using a decreasing

temperature range the objective was to avoid this issue and to ensure that the most labile substrates (glucose, albumin and arginine) would not be entirely used up prior to the final sampling day. It is unclear whether this was entirely successful given the very low enzyme activities measured after 112 days of incubation, although it is possible this was due to low temperatures under the changing temperature regime as activity was still detectable in samples from the stable temperature regime.

The organic amendments used here were chosen to be a series of substrates of decreasing quality, where the mixture of simple substrates amendment was thought to be the highest quality and the barley straw the lowest (most recalcitrant, as per Figure 4.1). Usually when researchers consider OM quality, they do so by measuring respiration rates (Fang et al., 2005; Kirschbaum, 2006; Conant, Drijber, et al., 2008; Conant, Steinweg, et al., 2008; Melillo et al., 2011); high quality substrates should give high rates of respiration, and vice versa. However, in this case, by examining OM quality in relation to enzyme activity, samples amended with high quality OM ought to give lower activity rates than those amended with lower quality OM (in accordance with q-theory sections 1.4.1 and 4.1). Given this, these results suggest that the LGBP is higher quality than the simple mixture of glucose, albumin and arginine. The protease activity rates are lower in those samples amended with LGBP than the labile amendment under both temperature regimes. This is because the LGBP consisted of readily soluble molecules such as sugars and amino acids, both of which can be utilized by microbes (and indeed plants) without the need for prior digestion (Näsholm et al., 1998, 2009; Jones and Kielland, 2002; Hill et al., 2011). In comparison, the simple substrates mixture contains albumin, a more complex molecule likely to require some

break down prior to use by most microbes. This observation does not hold true for amidase, however, and is likely due to reasons of stoichiometry discussed in Chapter 3 (Section 3.5.3.2.). Briefly, the economics of enzyme production suggest that enzymes should only be manufactured by microbes when nutrients are scarce (Koch, 1985; Allison and Vitousek, 2005; Wallenstein and Weintraub, 2008). In this case, soil N becomes limiting when very labile C (glucose here) is added thus triggering the production – and therefore increased activity – of amidase to provide N to enable use of the added C (Fraser et al., 2012).

This association between decreasing quality being associated with increasing enzyme activity holds for the GBP amendment, as the samples amended with this show the highest rates of protease activity. As with LGBP, there is no apparent effect of GBP amendment on rates of amidase activity. There is only a very small effect on the rates of protease activity when samples are amended with barley straw (recalcitrant substrate). Given the preceding arguments, it might well be assumed that barley straw is in fact a very high quality substrate. However, it is more likely that the straw amendment does not provide a real drive for the microbial population to produce enzymes to break down the complex molecules due to stoichiometric constraints in the same way as amidase activity is not measurable without the addition of labile C.

4.6. Conclusion

Taken collectively these results show that soil processes are affected more strongly by temperature change than the soil microbial community. Although the overall size of the microbial community changed slightly over the experimental period, the structure of the community did not change significantly. This shows that the functioning of the soil (in this case protease activity) can be varied without the need for significant changes in the size of the microbial community or even changing the structure of this population. The type of OM amendment applied to the samples also affected the rates of soil processes but not the structure of the microbial community. Rates of enzyme activity were higher in samples amended with complex OM although there was no evidence of changes in temperature having a greater effect on these samples. Again no changes in the structure of the microbial community were found in response to the different amendments showing that the microbial community is flexible enough to deal with both substrate and temperature change without need for population change. This experiment benefits from highly controlled laboratory conditions and low sample masses, so homogeneity of temperature and other factors could be achieved. This is not the case in the undisturbed soil system. It is important to find out what effect elevated temperatures are likely to have on real soil systems which are more heterogeneous in structure and have complex inputs.

Chapter 5: The effect of *in situ* heating on soil enzyme activities in the field.

5.1. Introduction

The previous two chapters have examined the temperature responses of enzymes associated with soil-N transformations under controlled laboratory conditions using small sample sizes. Such experiments are useful for assessing the fundamental nature of these reactions, and can be used to quantify the temperature response of OM of different quality under highly controlled conditions, but they are inherently limited in scope (Kirschbaum, 1995, 2006). It is not easy to simulate natural variation, such as seasonal temperature or precipitation patterns, which are factors likely to have a substantial impact on results of soil nutrient cycling experiments and their interpretation (Kirschbaum, 2000, 2006; Ågren and Bosatta, 2002). This chapter will present results from a controlled *field* experiment using heating cables to assess the effect of a systematic change in soil temperatures all year round on the reactions previously analysed in the laboratory. Soil temperatures were increased and this warming treatment was designed to track variations in the ambient temperature, with all other environmental factors allowed to vary naturally.

A mechanistic understanding of how soil-N transformations respond to change in climate is essential for understanding the knock-on consequences to other soil processes. These are likely to include C-cycling and sequestration as well as the timing and supply of plant- and microbially-available N.

Much previous work has focused on how changing climate is likely to affect forest ecosystems, and given the large amount of C stored in these systems a high level of understanding is necessary. Work to date has suggested that increased temperature resulting in greater concentrations of plant available–N may result in greater plant N uptake and therefore increased C-sequestration as a result of increased plant growth (Thomas et al., 2010; Melillo et al., 2011). If this results due to increased soil temperatures, it remains unclear how long increased rates of plant growth will be sustained, or whether they will vary among species, and what effects, if any, changes in litter quantity and/or quality will have on the decomposition processes (Thomas et al., 2010; Melillo et al., 2011).

Fewer studies have considered the effects of changing temperatures on temperate grassland systems; these systems are likely to be susceptible to change as they are often used for agricultural purposes and can receive N inputs not only as N-deposition from the atmosphere (Goulding et al., 1998; Phoenix et al., 2003; Zavaleta et al., 2003) but also as a result of grazing animals or as inorganic or organic fertilizers (Scholefield et al., 1991; Jarvis, 1993; Frank et al., 2000).

Those who have studied temperate grassland systems have often focused on measuring gross-N transformations, such as the size of organic and inorganic N pools before and after different treatments, or the flow from NH_4^+ to NO_3^- (Holland et al., 1992; Hamilton and Frank, 2001; Cookson et al., 2002; Roux et al., 2003). Of the studies measuring enzyme activity in grassland systems, several explicitly assessed the effects of grazing on these processes (Frank et al., 1994; Hamilton and Frank, 2001; Roux et al., 2003). The results show changes in N-use under grazed swards as well as

changes in the availability of labile C due to root exudates (Bardgett et al., 1999). The effect of plant community composition on N-cycling is also a common variable examined in these systems (Wheatley et al., 1990; Tracy and Frank, 1998; Liu et al., 2010). Plant species composition affects the levels of soil nutrient (including-N) use and soil-N retention, although this was not found to increase with increasing functional diversity (Hooper and Vitousek, 1998). Exotic plant invasions have also been found to change the availability of N to the plant community, setting up positive feedbacks which further alter the plant community structure (Evans et al., 2001; Ehrenfeld, 2003).

As stated in section 1.4, very few documented studies have quantified the direct effect of soil warming on soil enzyme activities (Allison and Treseder, 2008; Bell and Henry, 2011; Brzostek and Finzi, 2012; Weedon et al., 2012; Burns et al., 2013). To the best of the author's knowledge, this is the first detailed, year round assessment of the direct effect of heating on soil enzyme activities in the field. This experiment deployed buried heating cables to examine the effect of increased soil temperature on N-transformations examined in the lab. Because the heating grids were highly controlled, it was possible to capture the seasonal variation in soil-N transformations not only at the ambient temperature, but also at the artificially elevated soil temperatures throughout the whole year. This experiment also examined the effect on N-cycling, at both current and elevated temperatures. In addition the effect of the removal of natural vegetation was studied by leaving some plots to lie fallow.

5.2. Hypotheses

1. Rates of mineralization reactions vary throughout the year, with temperature as a primary control (subject to other limiting factors, e.g. soil water content).

2. Rates of reactions are faster, year round, in plots subject to heating to 3°C above ambient.

3. Plots clipped to be free of vegetation show smaller rates of activity, year round, than those which are unclipped, due to more limited rhizosphere interactions.

4. There is an interactive effect of heating and clipping. Those plots which are clipped and unheated show the lowest rates of activity and those plots which are unclipped and heated show the highest rates of activity.

These hypotheses will primarily be tested by assessing the difference in rates of enzyme activities between plots exposed to different treatments. Additional information about microbial community size and structure, plant above and below ground biomass and size of the earthworm population will be collected to account for any differences found between treatments.

5.3. Materials and Methods

5.3.1. Field site

The field site was the same area as for the soil sampling for use in the previous two chapters; an area of undisturbed grassland adjacent to the Mylnefield Meterological Station. The plots were positioned approximately 21 m from the thermometers used to record daily temperatures at 10, 20, 30, 50 and 100 cm depths in the soil, and the Stephenson screen housing thermometers used to monitor daily maximum and minimum air temperatures. The vegetation was mainly grasses (including *Poa annua* and *Lolium perenne*) with some clover (*Trifolium* spp) and other perennials including dandelions (*Taraxacum officinale*) and ragwort (*Jacobaea vulgaris*), among others. Prior management of the site consisted of regular grass cutting during the growing season only.

5.3.2. Experimental set up

In late April 2011, sixteen 1 m² 10 cm deep holes were dug by hand in a 4 x 4 grid pattern at the field site (Figure 5.1), a heating grid (Figure 5.2) was installed in each hole and the turf replaced on top. A sprinkler was then placed in the middle of the 4 x 4 grid and the plots watered between 9 am and 5 pm on fair days for the following 2 weeks to ensure that the blocks of turf bedded back in properly.

Approximately 1 month after the heating grids were installed, eight out of 16 heating grids were switched on. At this time, eight of the plots were also clipped, using a hand-held strimmer, to remove above-ground vegetation creating a 4 x 4 fully factorial

design of heating and clipping treatments. All clippings were collected and weighed into labelled paper bags; these were dried at 60°C for two weeks before being re-weighed. Clipping was carried out continuously throughout the experiment to keep these plots vegetation-free, and the plots that were left with above-ground vegetation were clipped down to approximately 10 cm sward height in mid-November 2011 to keep the vegetated plots manageable. Sampling began approximately 1 month after the switch on of the heating and the initial clipping.
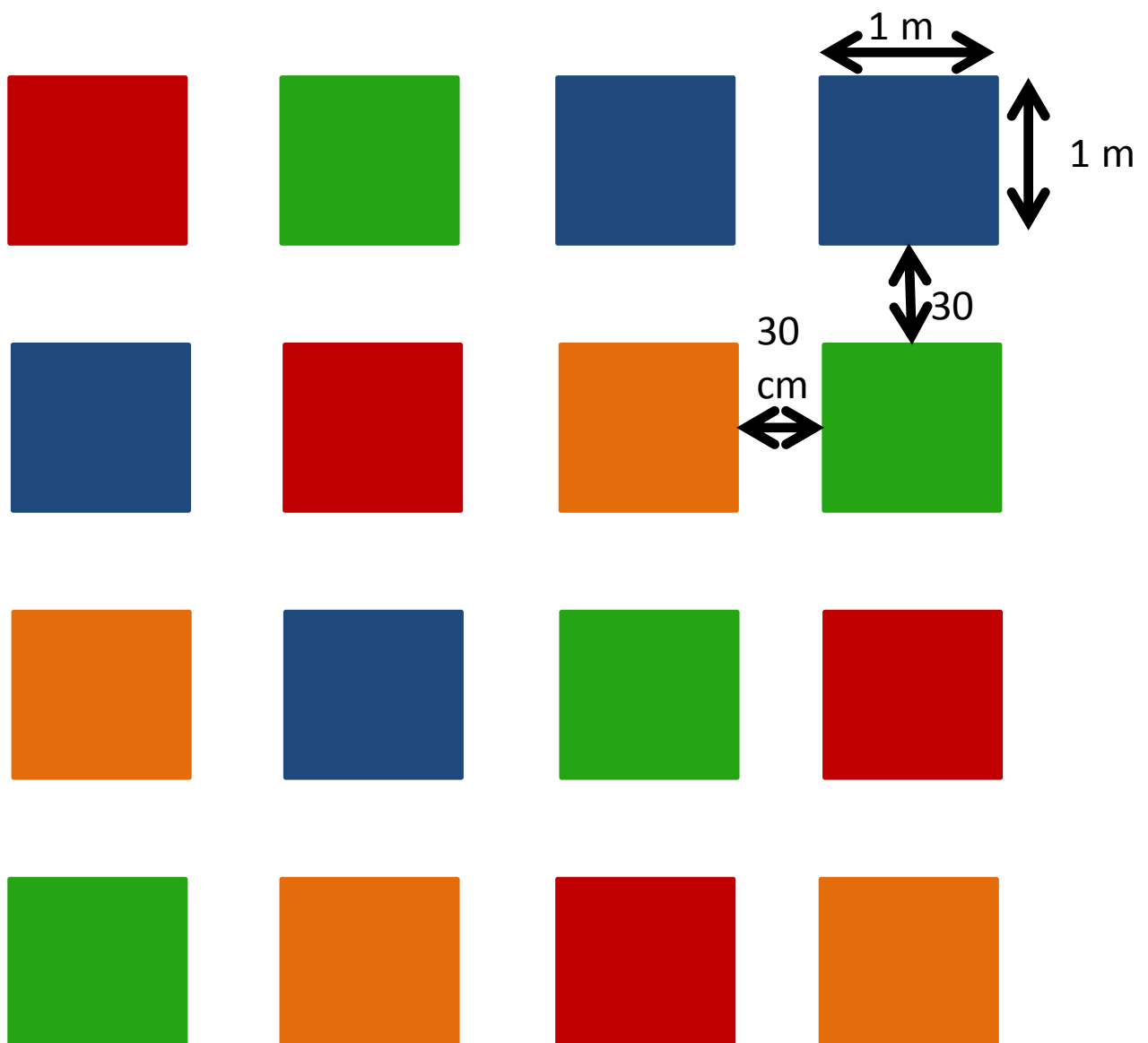

Figure 5.1: Field plot lay out, red = heated unclipped, orange = heated clipped, green = unheated unclipped and blue = unheated clipped.
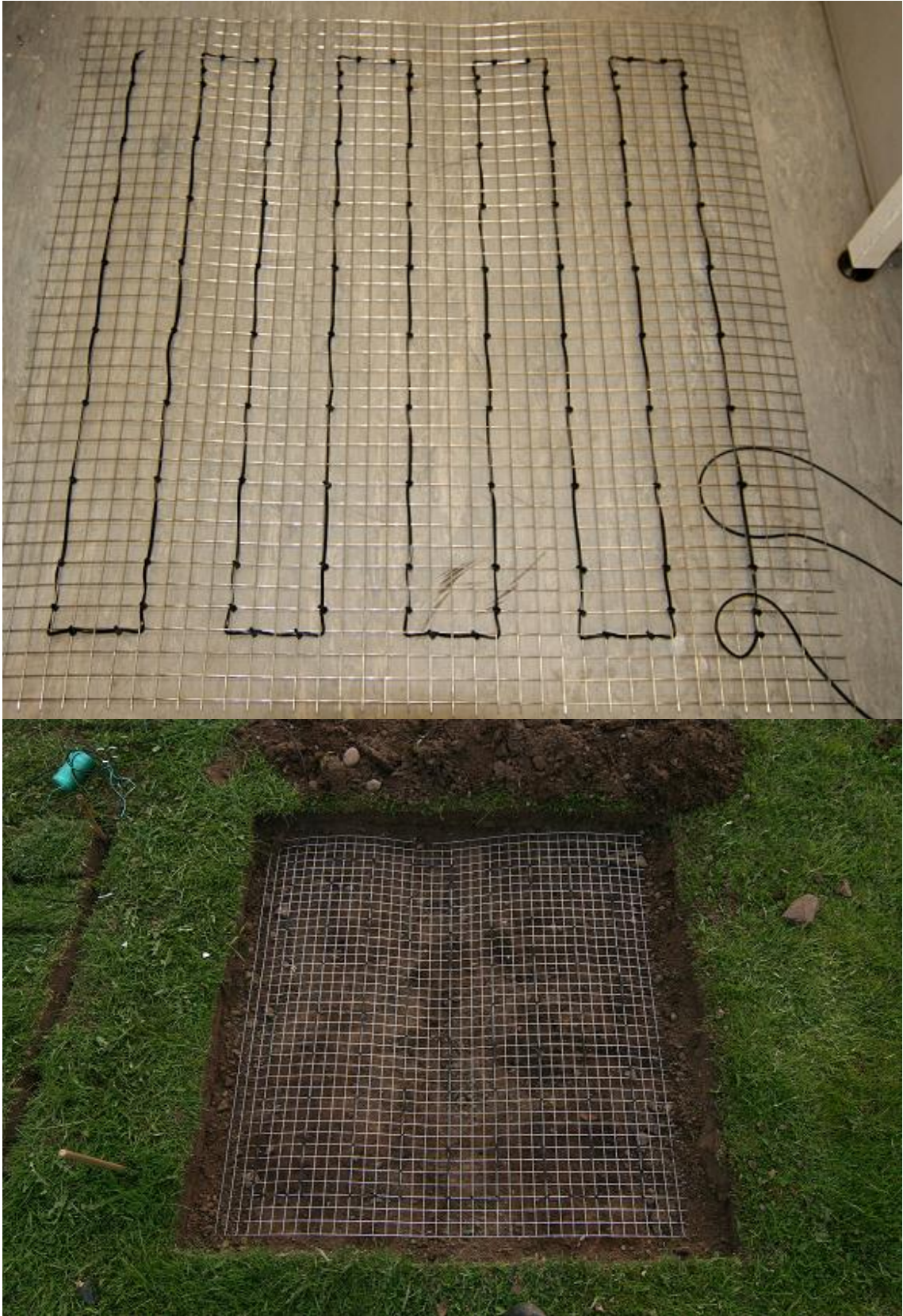
Figure 5.2: Stainless steel 2.5 cm diameter grids threaded with K-type thermocouple cables at 10 cm intervals and how each grid was positioned in each of the 16 plots.

5.3.3. Heating system

Once all sixteen grids had been buried the eight powered grids were switched on supplied with power via two Toroidal transformers (RS components, P/N 671-9028) rated at 160 VA, with one primary winding, 230 volts AC 50Hz and two secondary winding, each supplying 80 VA 30 volts/ 2.6 amps per winding. Each winding provided power for two grids. Individual grid supplies were fused (value 1.5 amps) and wired to their own indicator lights.

The heating control system consisted of a Novus differential temperature controller, model No. N321 (Novus Ltd www.novusautomation.co.uk). This controlled the temperature by measuring the difference between two NTC thermistors, one buried in a heated plot and the other in a non-heated plot these were buried at approximately 9.5 cm depth so as to be just above but not touching the heating grids. A 24 volt DC signal was switched on to enable a timer when the temperature difference dropped below 3°C.

A symmetrical timer (ABB model No. CT-TGD) provided the pulse requirements for switching on and off the solid state relay that provided the heating power to the grids. Depending on seasonal weather conditions, the pulse duty factor could be increased or decreased to provide the electrical supply to the grids needed to maintain a 3°C difference.

During the year-long experiment one ibutton data logger (iButtons are manufactured by Maxim Integrated www.maximintegrated.com but purchased from HomeChip www.homechip.com) was buried between 5 and 7 cm depth in each plot to record the

soil temperature every 4 hours. These had no bearing on the control system but did allow continuous monitoring of the system performance until almost universal failure of the loggers occurred in April 2012 apparently due to moisture ingress.

5.3.4. Soil sampling

Samples were taken from each of the 16 plots, every two weeks, starting on the 13th of July 2011 and ending on the 11th of July 2012. Samples were taken using 2 cm diameter x 10 cm deep cores. From each plot 6 replicate cores were taken at a set of randomly generated co-ordinates from a 39 x 39 grid. The cores were pushed into the soil to a depth of 10 cm and pulled out with a plug of soil inside; the cores were then extruded from the plastic corer using a piece of wooden dowel. The cores were collected in labelled bags and taken back to the laboratory where they were sieved to pass a 4 mm mesh. During sieving, all roots, above-ground plant biomass and stones were removed. All visible invertebrates were also removed. Cores from the same plot were bulked and thoroughly mixed prior to being weighed out into plastic tubes for analysis.

5.3.5. Biomass measurements

5.3.5.1. Roots and shoots

During sieving all the roots and above ground biomass were removed from the soil samples. These were split into labelled envelopes (roots in one, shoots in another) and dried at 60°C for 48 hours to determine dry weights.

5.3.5.2. Invertebrate biomass

As of the 28th of February, 2012 all invertebrates found during sieving of soil cores and sorting of biomass were retained and stored in 100% ethanol rather than being discarded as previously. Invertebrates were counted and the volumes of earthworms estimated. Earthworm volumes were estimated by measuring the length of a fragment. Then by taking 3 measures of the diameter at different points along the length, the diameters were averaged and this was used to calculate the total volume of earthworm collected per plot per day.

5.3.6. Enzyme assays

Assays were carried out for protease (Ladd and Butler, 1972), urease (Kandler and Gerber, 1988) and amidase activity (Frankenberger and Tatatabai, 1979) at each sampling event [exception: no amidase activities were reported for the 16th of November 2011 due to lack of uranyl acetate with which to stop the reaction]. Assays were carried out as previously described (section 3.3.3.). At each sampling event 5 samples and 3 controls were carried out for both protease and urease, while for amidase only 3 samples and 1 control were carried out for each sampling event due to cost and time considerations.

Assays were carried out either at ambient soil temperatures or at ambient plus 3°C, depending on which plot the sample was from; these temperatures were recorded at the beginning of sampling (i.e. 5.30 am on the sampling day).

5.3.7. Nitrate concentrations

Nitrate concentrations were determined as described in Anderson and Ingram (1993) (further detail can be found in section 4.3.6.2). Three replicate samples were extracted per plot per sampling event if there was sufficient soil. Samples were extracted, centrifuged and then pipetted into clean tubes and frozen at -20°C prior to analysis at a convenient time.

5.3.8. Microbial community analysis

Analysis of the microbial community size and structure was carried out using Phospholipid Fatty Acid Analysis (PLFA) (same protocol as described in section 4.3.6.3). Briefly, soil was collected for each plot on each sampling day and frozen, wrapped in aluminium foil, at -20°C until the end of the experimental year. All samples were then transferred into aluminium foil boxes and freeze dried after which they were transferred into labelled zip-lock plastic bags. Due to constraints of time and cost, only samples from the first and last sampling days (13th July 2011 and 11th July 2012 respectively) were extracted and analysed; all other samples were returned to -20°C storage. The results of the PLFA analysis were handled in the same way as described in section 4.3.7 to evaluate the size and structure of the microbial community and whether this had changed over time.

5.3.9. Statistical analysis

The efficiency of the heating system was assessed by calculating mean daily temperatures within plots (from 4 hourly data collected using the ibutton loggers) and comparing between the treatments using 2-way ANOVA. Two-way ANOVA were also

used to assess the effect of heating and clipping on the dry weight of above and below ground plant biomass.

Linear regression was used to assess the effect of soil temperature and soil moisture content on the activity of each enzyme. The effects of all measured variables on each of the enzyme activities was modelled using Generalized Estimating Equations (GEEs), all predictor variables were included in the initial models, for subsequent iterations predictors were excluded on the basis of non-significance in the preceding iteration. The relative goodness of fit for models of each enzyme activity were assessed using the corrected Akaike Information Criterion (AICc), a reduction of 3 in the AICc is taken to show a significant improvement in model fit.

Principle Components Analysis (PCA) was used to analyse data obtained from PLFA as is common in the literature (e.g. Fostegård et al., 1993; Bárcenas-Moreno et al., 2009; Rousk et al., 2012), this proved to be relatively uninformative so bacterial : fungal rations, and the relative sizes of particular bacterial populations were compared using 2 way ANOVAs.

5.4. Results

5.4.1. Efficacy of treatments

5.4.1.1. Heating

Within-plot temperatures were recorded every 4 hours using iButton data loggers buried between 5 and 7 cm below the soil surface so as to be just above, but not

touching, the heating cables. Figure 5.3 shows the mean daily temperatures from 4th of August 2011 until the 12th of April 2012, with sampling days represented by the green points. The heating cables were capable of maintaining a 3°C difference between heated and unheated soil in plots where the grass cover was maintained (Figure 5.3a). However, in the clipped plots where the grass was removed, the difference between the heated and unheated plots was less clear (Figure 5.3b). The mean daily temperatures of the heated unclipped plots and the unheated unclipped plots were compared using a Student's t-test and the difference was found to be highly statistically significant ($t = 28.10$, $df = 17$, $p < 0.0001$); the mean difference was 3.30 °C, with a 95% confidence interval of 3.06 to 3.60. When the same test was carried out using data from plots where the vegetation had been removed (heated clipped and unheated clipped) no statistically significant effect of heating was found ($t = 1.2041$, $df = 17$, $p = 0.2451$) with the mean difference being only 0.6601 °C (95% confidence interval of -0.4966 to 1.8169).

Daily variation in temperature between clipped and unclipped plots was different at all times of year (Figure 5.4); those plots that were clipped to be free of vegetation had greater variation than those where the vegetation was left in place.

The effect of heating on the dry weight of biomass or roots collected at each sampling event (Figure 5.5) was assessed using a two-way ANOVA. No effect of heating regime was found for either root or above-ground biomass dry weight ($p = 0.939$ and 0.208 respectively).

5.4.1.2. Clipping

Two-way ANOVA was used to look at the effects of clipping and heating on the average above ground biomass and roots removed from sample cores. Clipping had significant effects on both above ground biomass and root dry weights ($p > 0.001$ in both cases), there was also a slightly significant interaction between heating and clipping on root dry weight ($p = 0.041$).
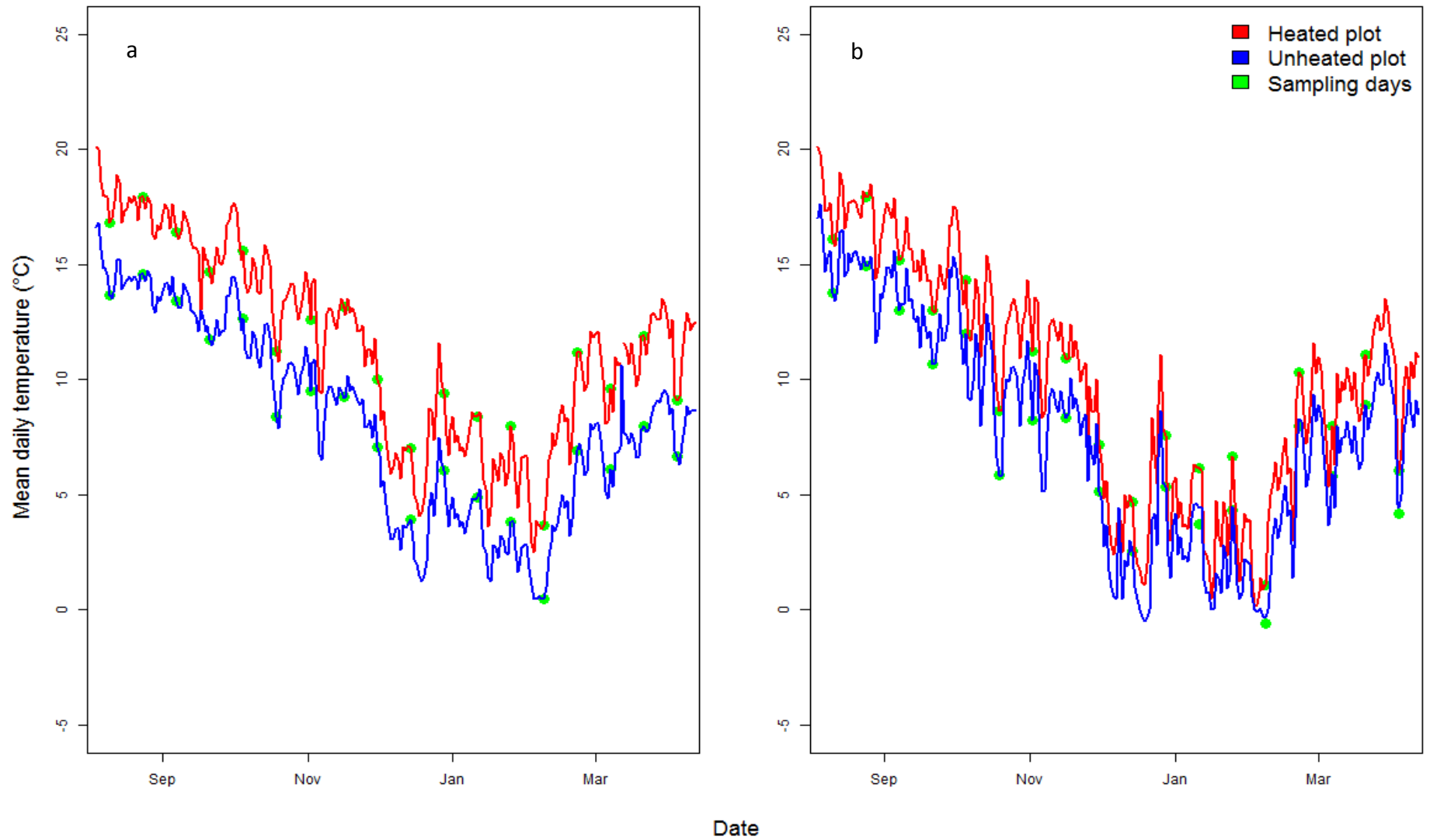
Figure 5.3: Mean daily temperatures recorded at 5-7 cm in a) unclipped or b) clipped plots, red lines indicated that the plot was heated and blue lines that the plot was unheated, the green points are the dates where samples were taken from each plot.
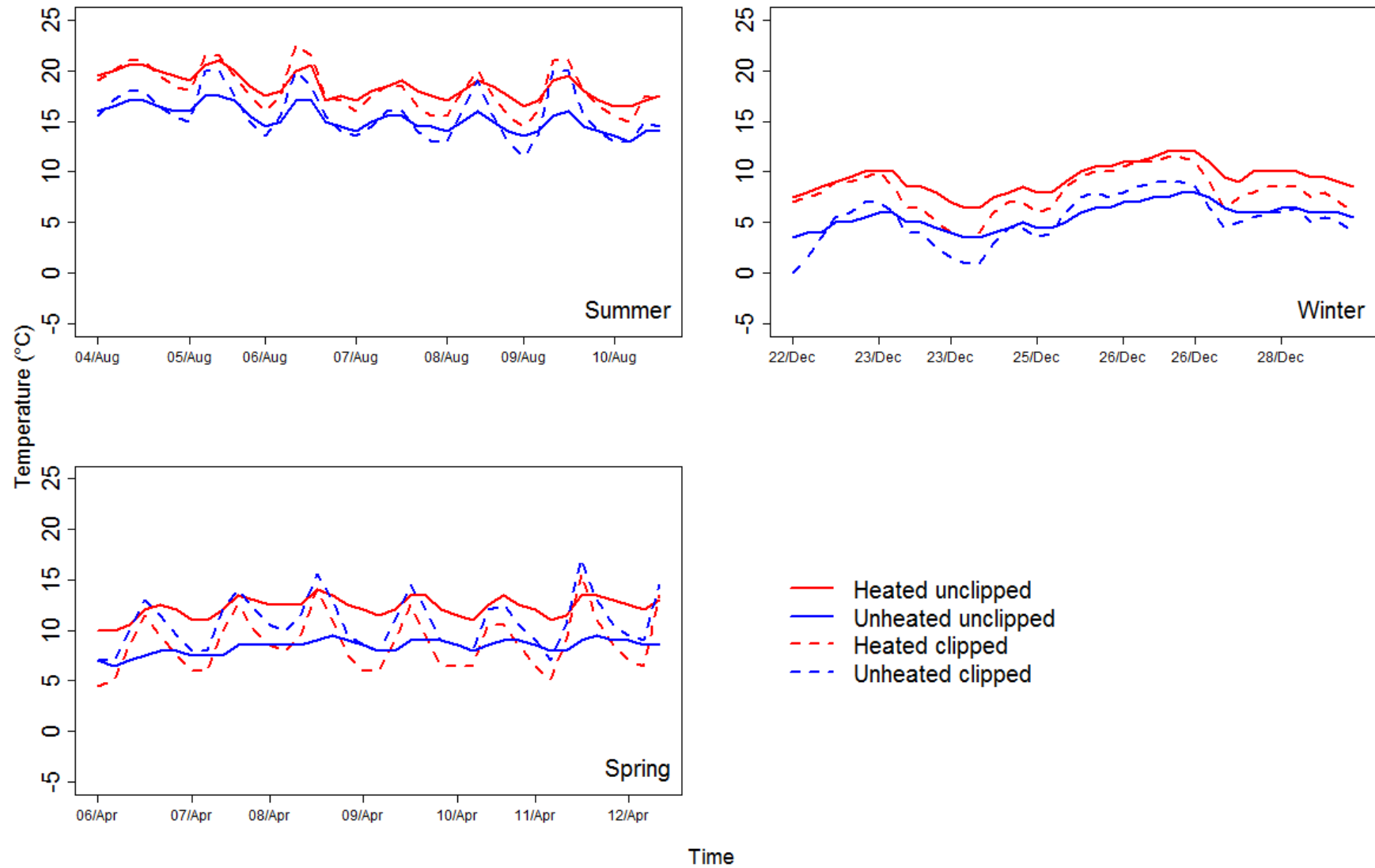
Figure 5 4: Graphs showing the variability of temperatures recorded in individual plots at different points in the year depending on whether the plot was heated (red) or unheated (blue) and if the plot was clipped (dashed lines) or unclipped (solid lines).
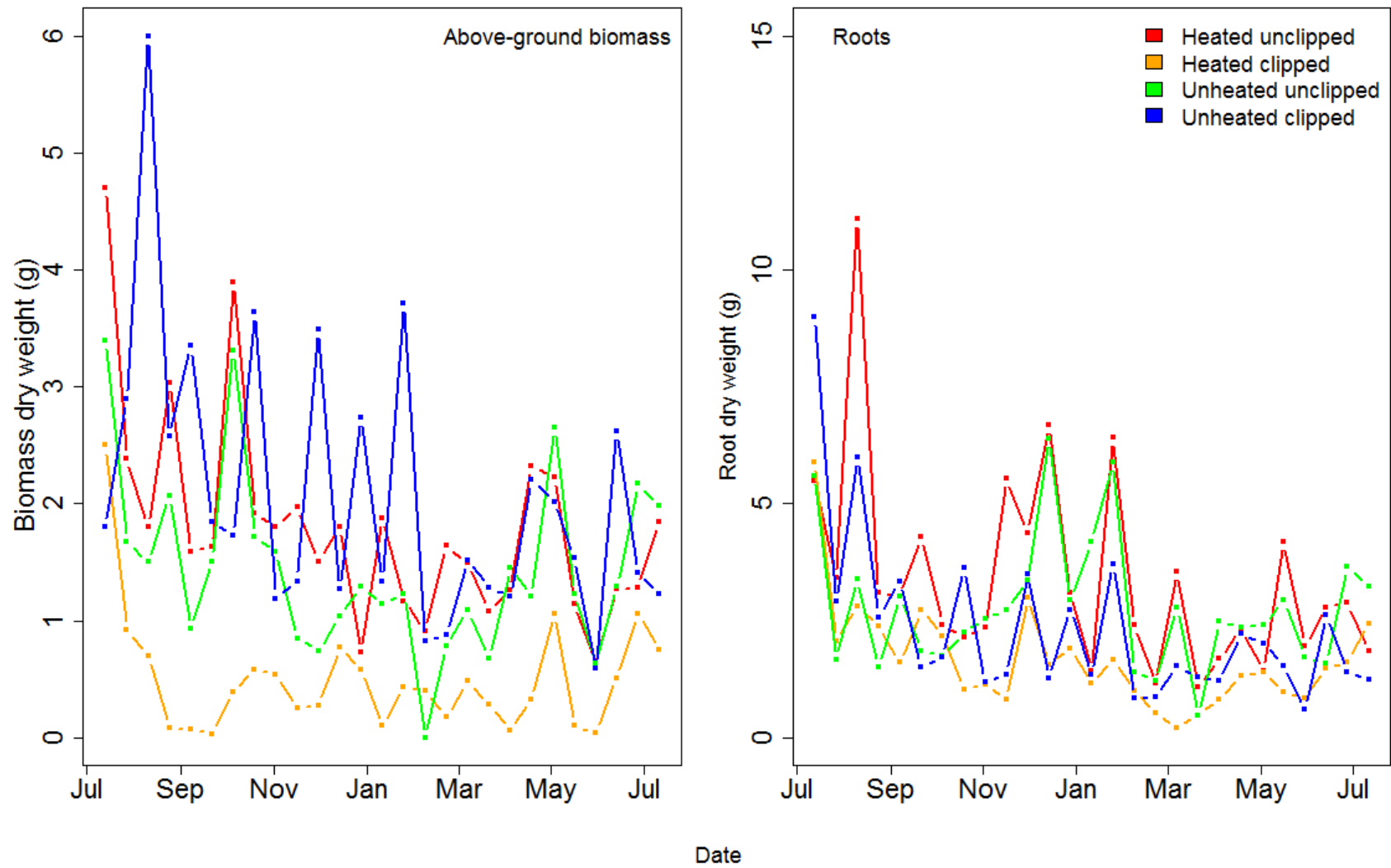
Figure 5.5: Mean dry weight of above ground biomass and roots from cores taken at each sampling event from each plot treatment type.

4

5.4.2. Enzyme activities

5.4.2.1. Protease

Initial interrogation of mean protease activity shows a strong association with field temperature (Figure 5.6a); linear regression shows that assay temperature explains approximately 47% (p<0.0001) of the variation in the data. Mean protease activity was also analysed with reference to soil moisture content; although an association was found between protease activity and the percentage soil moisture (Figure 5.6b), linear regression showed that soil moisture accounted for only *ca.* 13% of the variation in the protease activity data (p<0.0001).
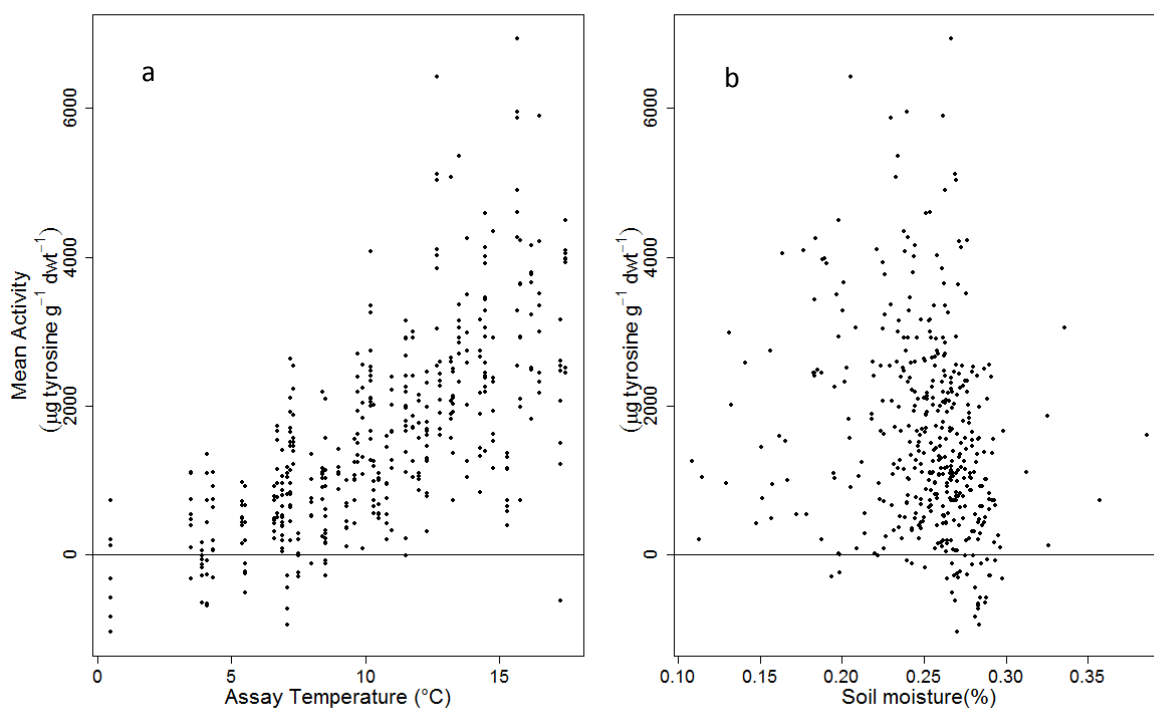
Figure 5.6: Mean protease activity in relation to assay temperature (a) and percentage soil moisture content (b).

Protease activities follow a predictable pattern during the course of the year; the initial period from mid-July to early October shows relatively stable activity, which then decreases between October and February reaching a minimum measured rate of activity on February the 11th 2011. Protease activity then increases again from March to July before reaching levels similar to those at the beginning of the experimental period (Figure 5.7). Analysis of these data was carried out using Generalized Estimating Equations (GEEs) and models were compared using the corrected Akaike's Information Criterion (AICc) statistic; models and AICcs are shown in Table 5.1.

Due to the large amount of data it is possible to include all the measured predictor variables and still to decrease the value of the AICc statistic and therefore increase the goodness-of-fit of the model. When the fitted values for each field plot are plotted (Figure 7) there is a clear difference between those plots that were heated and those that were not ($p < 0.0001$). There is also evidence of an effect of vegetation cover, where those plots that have above-ground vegetation have higher rates of protease activity than bare plots. In the model results this effect of plant cover is not reflected in the clipping regime but in the absolute weight of above ground biomass collected from the sample cores ($p = 0.037$). The model also shows a possible interactive effect of heating and clipping regime with the group that the plot is associated with (heated, clipped etc.) having a significant effect on protease activity rates ($p < 0.00001$). Date and assay temperature are also significant in the model ($p < 0.0001$ in both cases).

Table 5.1: Model parameters and goodness of fit measures for protease activity GEE's.

Model name	Parameters included	AICc
Model.one	Heating regime, clipping regime, field temperature, date	30381
Model.two	Heating regime, clipping regime, moisture content, root weight, biomass weight, field temperature, group	30305
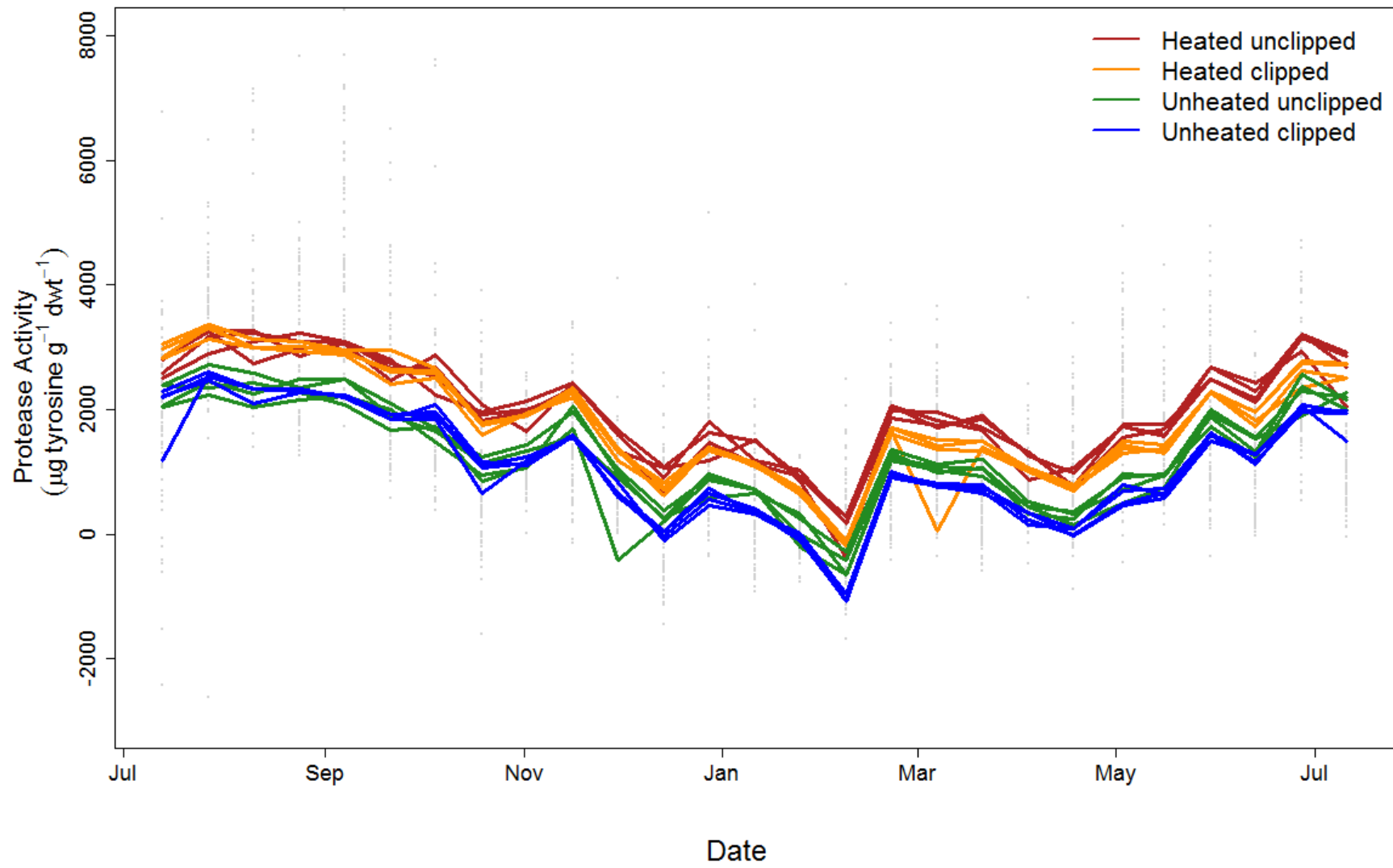
Figure 5.7: Fits of protease GEE's for each plot treatment type; grey points represent raw measured protease activities.

5.4.2.2. Urease

Initial analysis of mean urease activity shows strong associations with both field temperature and percentage soil moisture (Figure 5.8; p<0.0001 and 0.001, respectively). In both cases, however, relatively little of the variation in the urease activity can be accounted for by the predictor variables (R^2 values = 0.1285 and 0.0244 respectively).
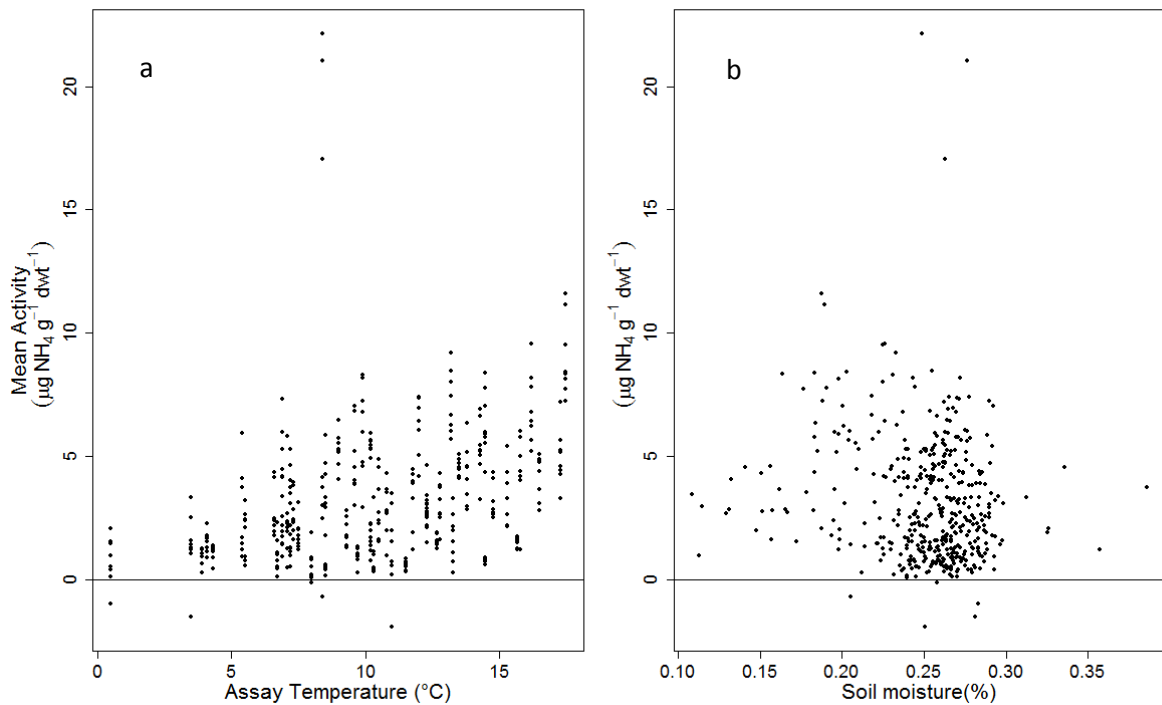
Figure 5.8: Mean urease activity in relation to assay temperature (a) and percentage soil moisture (b).

Urease activity generally decreased between July and December 2011, reaching a minimum during February 2012; thereafter it then began to increase again from February to July 2012 but never regained the levels measured at the beginning of the experiment in July 2011 (Figure 5.9). These data were analysed using GEEs and, again, due to the large volume of data, it was possible to include all the measured predictor variables and still to reduce the value of the AICc (model.one compared to model.three in Table 5.2). However, it was found that the value of the AICc could be further reduced by removing plot grouping from the model as a predictor variable (model.two in Table 2). When the fitted results were plotted (Figure 5.9) it was found that, as for protease activity, those plots that were heated and unclipped had the greatest rates of urease activity at all times of year, and those plots that were unheated and clipped had the lowest rates of urease activity at all times of year. Unlike protease activity, however, there was no clear difference between the urease activity rates in heated clipped plots and unheated unclipped plots. When each of the parameters in the model are inspected, heating regime was found to be statistically significant (p = 0.0319) but clipping regime was not (p = 0.1691); the absolute above-ground biomass is also statistically significant (p = 0.0160). The model also shows that assay temperature, sampling date and soil moisture content are all statistically significant (p = 0.0002, 0.0002 and 0.0009 respectively).

Table 5.2: Model parameters and goodness of fit measures for urease activity GEEs.

| Model name	Parameters included	AICc
Model.one	Heating regime, clipping regime, field temperature and date	5398
Model.two	Heating regime, clipping regime, moisture content, root weight, biomass weight, field temperature and date	5380
Model.three	Heating regime, clipping regime, moisture content, root weight, biomass weight, field temperature, date and group	5382
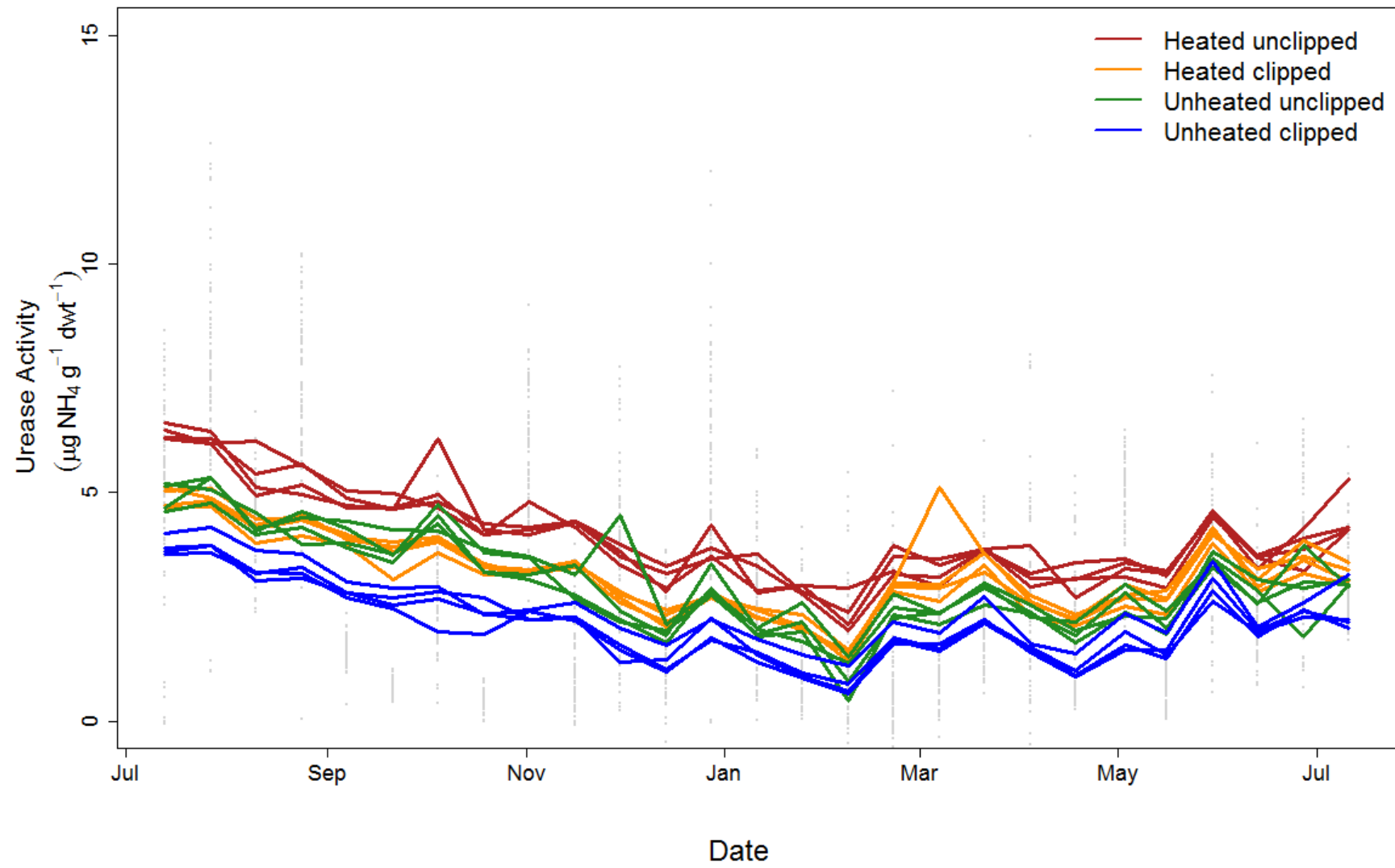
Figure 5.9: GEE fits for urease activity rates for each treatment type; grey points represent raw measured urease activities.

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5.4.2.3. Amidase

Initial analysis of mean amidase activity rates shows no association with either field temperature or percentage soil moisture (p=0.75 and 0.65 respectively, Figure 5.10).
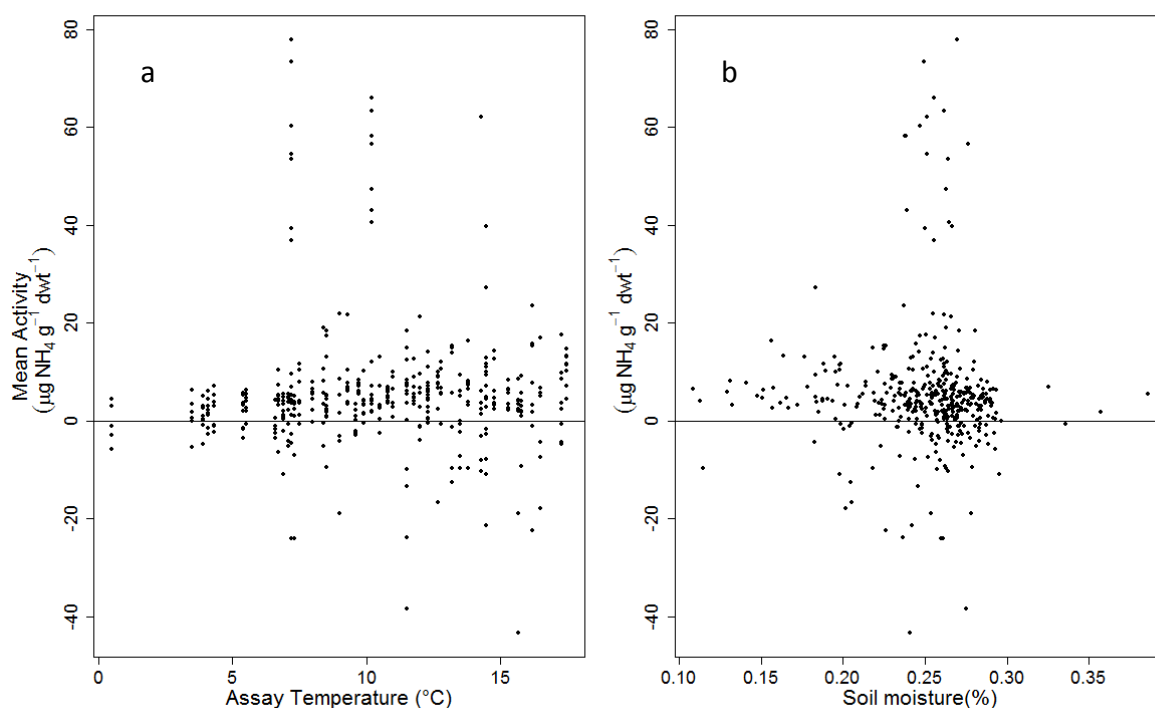
Figure 5.10: Mean amidase activity in relation to assay temperature (a) and percentage soil moisture content (b).

For the initial 5 months of the experiment (from July to November 2011) there was very little or no amidase activity, and from December 2011 to April 2012 amidase activities increased and then decreased again between May and July (Figure 5.11). Amidase activity in all experimental plots was very low and often the variation in the measurement makes it difficult to detect a clear pattern (Figure 5.11); thus there was very little pattern in amidase activity other than in the spring, where the activity in

plots under all the treatments generally increased until late spring or early summer where measures of activity fell back to their previous levels. Despite the large volume of data available, the model with the lowest AICc value was the one with the fewest parameters (model.one in Table 5.3). When the model is examined more closely, only the assay temperature and the sampling date were found to be statistically significant (p < 0.0001 in both cases) but when any of the other parameters are removed the AICc of the model increases again (model.five in Table 5.3).

Table 5.3: Model parameters and goodness of fit measures for amidase activity GEEs.

| Model name	Parameters included	AICc
Model.one	Heating regime, root weight, biomass weight, field temperature and date	6444
Model.two	Heating regime, moisture content, root weight, biomass weight, field temperature and date	6445
Model.three	Heating regime, clipping regime, moisture content, root weight, biomass weight, field temperature and date	6446
Model.four	Heating regime, clipping regime, moisture content, root weight, biomass weight, field temperature, date and groups	6447
Model.five	Field temperature and date	6445
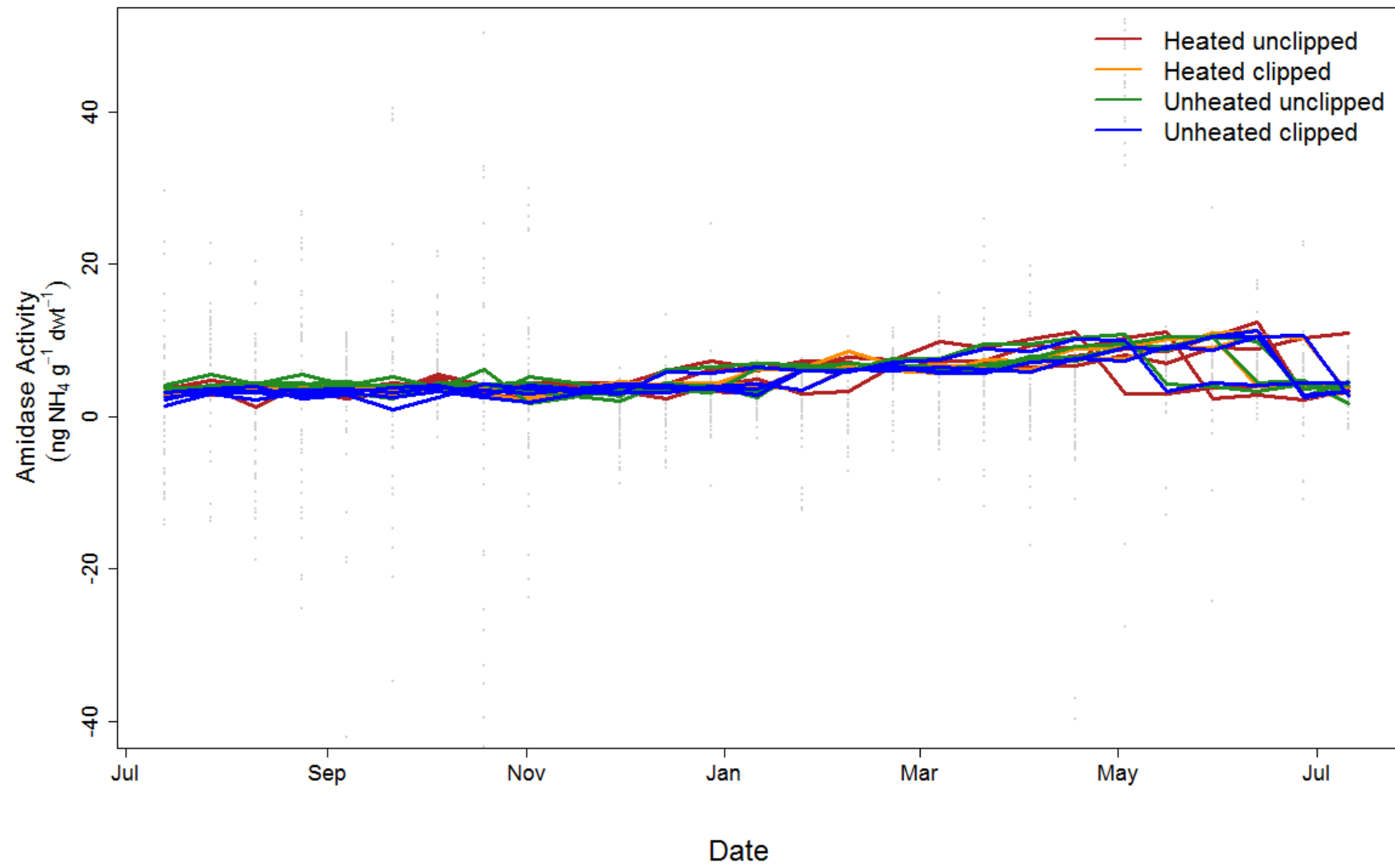
Figure 5.11: GEE fits for amidase activity rates for all treatment types; grey points represent raw measurements of amidase activity.

5.4.3. Nitrate Concentrations

For logistical reasons the data set for nitrate concentration is only *ca.* 75% complete. When nitrate and ammonium concentrations were plotted against assay temperature and percentage soil moisture, there were no apparent relationships; this was confirmed using linear regression (Table 5.4)

Overall N-mineralization (NO_3^- and NH_4^+ concentrations combined) show a much more variable pattern than the rates of enzyme activity. When mean inorganic-N concentrations for each treatment are plotted in conjunction with daily precipitation measurements made at the Mylnefield Meteorological Station, it is apparent that rain events preceding soil collection influenced the measureable inorganic-N content of the soil (Figure 5.12). There was no difference between the concentrations of inorganic-N extracted from different treatment regimes (p = 0.201).

Table 5.4: significance of relationships between nitrate and ammonium concentrations and assay temperatures or % soil moisture content as measured by linear regression.

	Assay temperature	% soil moisture
Nitrate	p = 0.331	p = 0.288
Ammonium	p = 0.16	p = 0.874
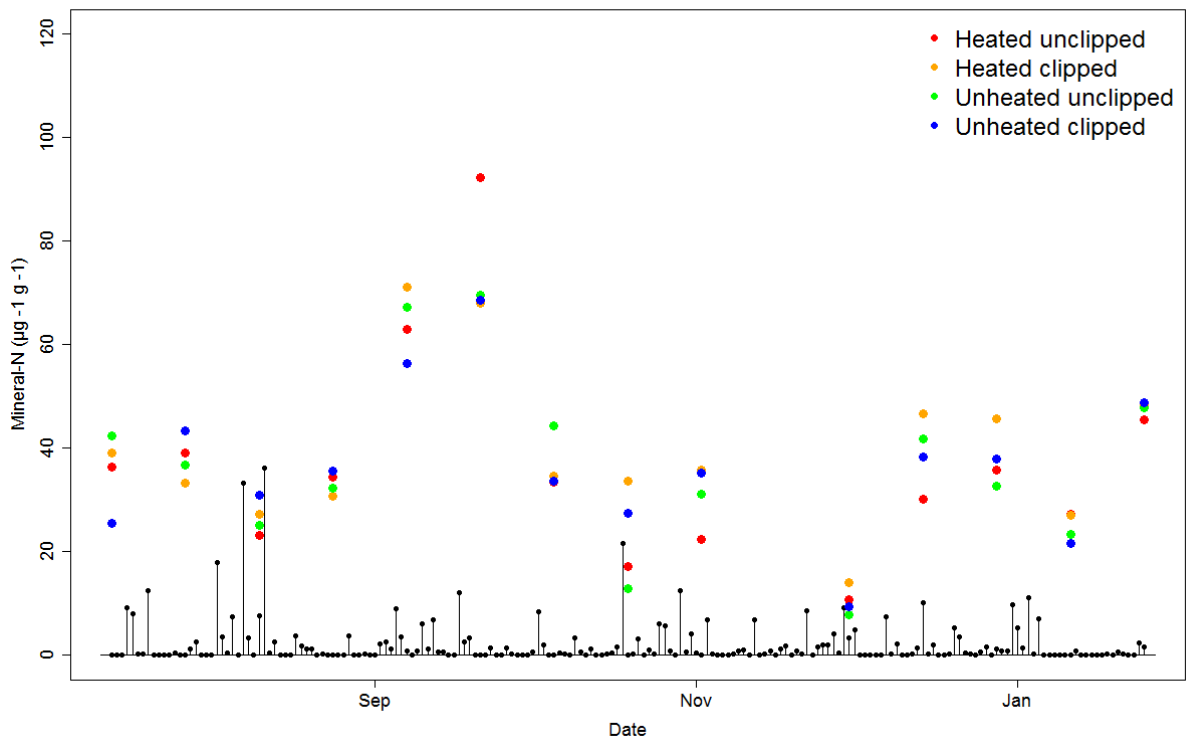
Figure 5.12: mean inorganic-N concentrations plotted over time compared to daily precipitation values recorded at the Mylnefield Meteorological Station. Values missing for the 16th of November 2011 due to missing NH_4^+ values.

Those samples which were exposed to rain in the 7 days prior to being extracted, to measure inorganic-N concentrations ("wet" samples) on the whole have lower inorganic-N concentrations than those samples where there had been no rain in the 7 days preceding sampling ("dry" samples) (Figure 5.13). The exception was in spring where "wet" samples had consistently higher concentrations than "dry" samples; however the error bars are large showing that these concentrations were highly variable. There was also some suggestion that clipped "wet" samples had higher inorganic-N concentrations than unclipped particularly in the autumn.
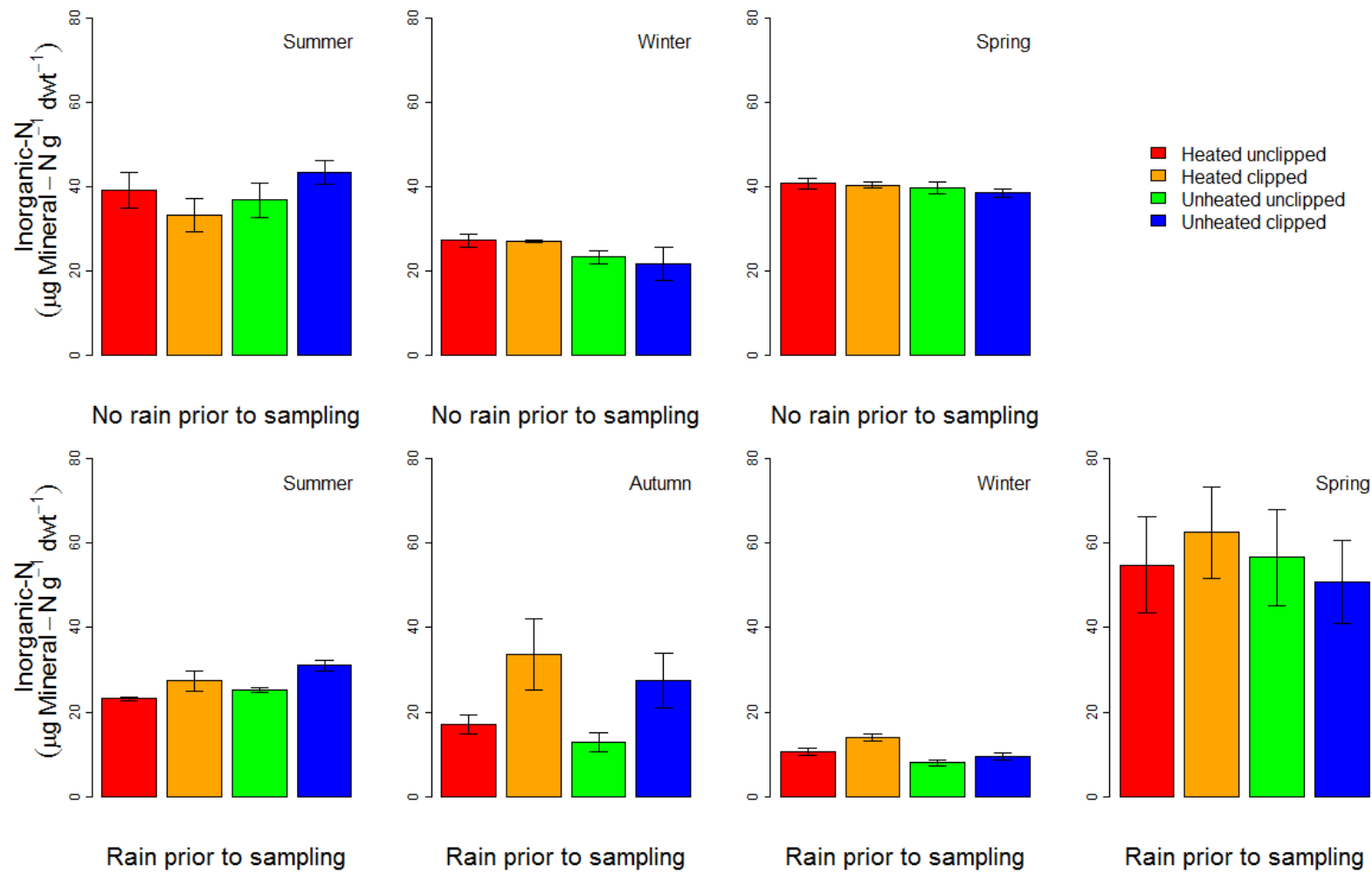
Figure 5.13: Bar charts showing the mean concentrations of inorganic-N (combination of NH_4^+ and NO_3^- concentrations) for samples taken after a 7 day period of dry conditions (upper row) or a period of 7 days with no rain (lower row). Error bars = +/- 1 standard error, n=4.

5.4.4. Soil Moisture

The percentage soil moisture, when plotted across the year, shows a steady increase between August and December, the soil then remained wet until early February after which it dried until early July after which it showed signs of getting wetter (Figure 5.14). During the period February to June there were two distinct points where conditions were very dry; one in late March and one in late May (Figure 5.14). Percentage soil moisture data were analysed using a 2-way ANOVA; the results show that heating has no statiscally significant effect on soil moisture content ($p = 0.1121$) but that clipping has a slight effect on moisture content ($p = 0.0639$). The interaction of the two was found to be statistically significant ($p = 0.0053$), using a post-hoc Tukeys Honest Significant Difference test (Tukeys HSD) the difference was found to be between plots which were both heated and clipped compared to those which were heated and unclipped and between plots which were heated and clipped and those which were unheated and clipped.
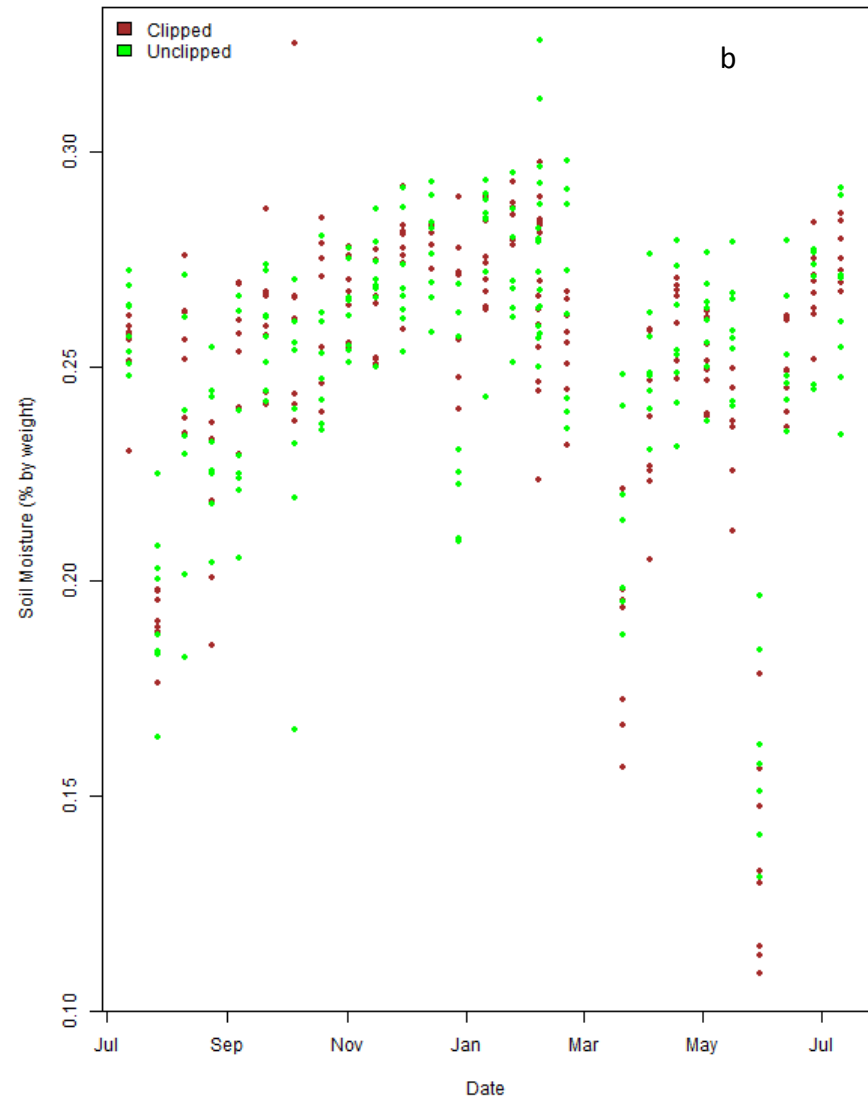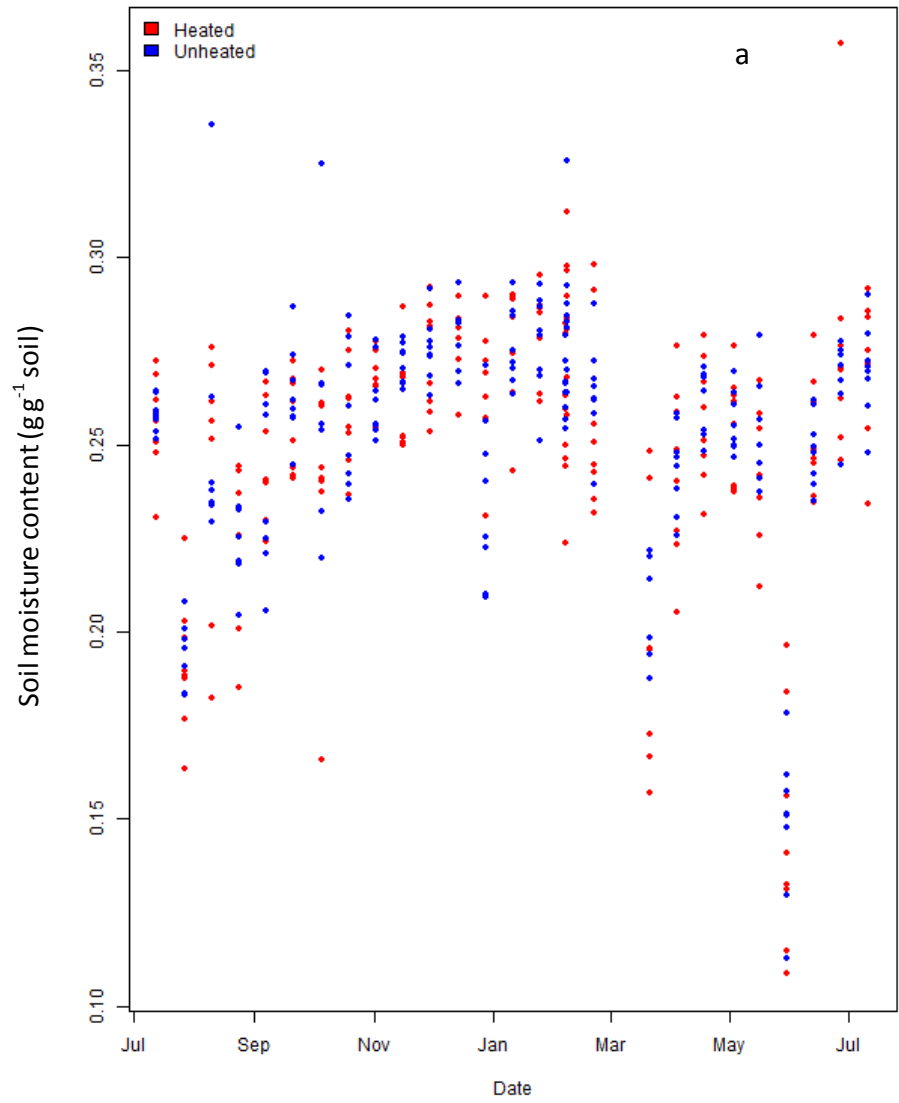
Figure 5.14: Percentage soil moisture content measured at each sampling event shows changes over time but no differences between heated and unheated plots (a) or clipped and unclipped plots (b).

5.4.5. Microbial Community

5.4.5.1. Microbial biomass

As previously stated, only samples collected on the 13th of July 2011 (the beginning) and the 11th of July 2012 (the end) were analysed to assess the size and structure of the microbial community. As an initial analysis, the total weight of PLFA extracted from each sample (total PLFA $\mu g\ g^{-1}$ soil) was calculated as a proxy for total microbial biomass. This shows that in 75% of the experimental plots the total microbial biomass appeared to decrease over the course of the year. However, because of financial and time constraints only one sample from each plot was extracted and as such it is not possible to state whether this apparent change was statistically significant. Of those few plots where the total microbial biomass increased over the year, 3 out of 4 were unheated clipped plots (the fourth was heated unclipped).

In order to resolve any treatment effect on total microbial biomass further; data were analysed using a general linear model (glm). Heating and clipping were found to be non-statistically significant ($p = 0.9819$ and 0.8896 respectively), there was also no interaction between the two treatments ($p = 0.8593$). However, there was a statistically significant effect of sampling time on total PLFA ($p = 0.0363$); this is shown graphically in figure 5.15.
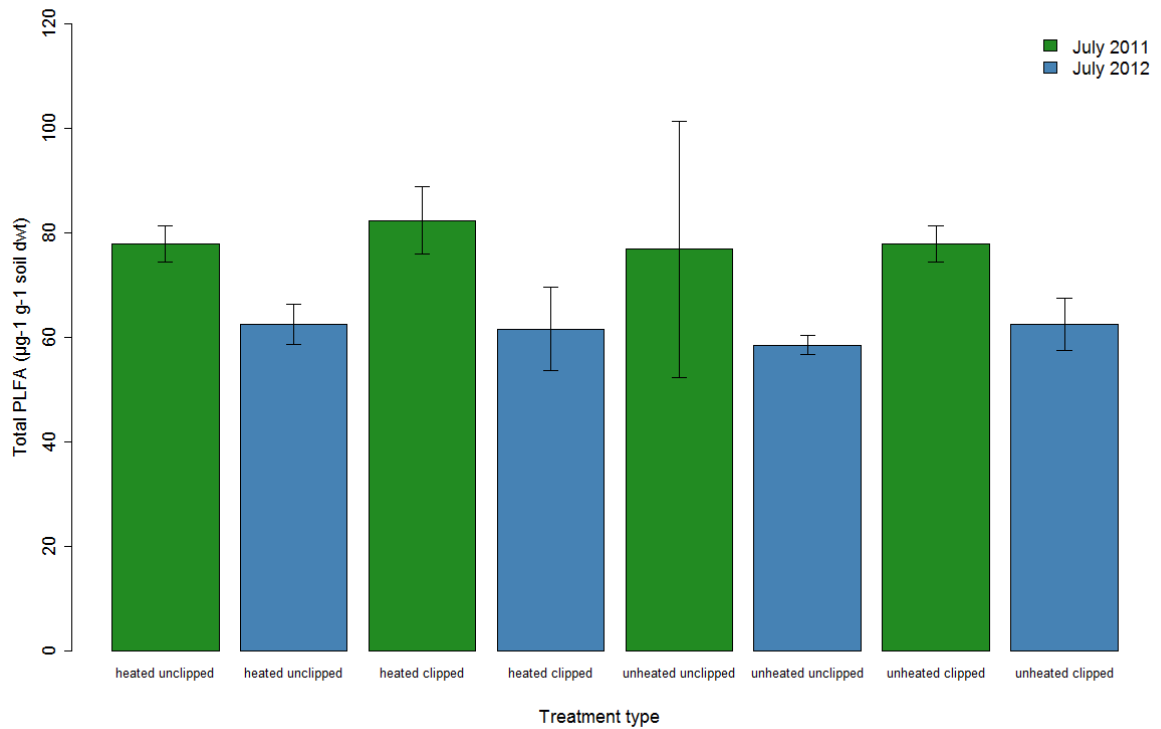
Figure 5.15: Total PLFA as a proxy for total microbial biomass on the first (13th July 2011; green) and last (11th July 2012; blue) sampling days for each plot type, error bars = +/- 1 standard error, n=4, with the exception of heated unclipped 2012 where n=3.

5.4.5.2. Microbial community structure

Microbial community structure was assessed using principal components analysis (PCA); this resulted in 31 Principal Components (PCs), the first of which alone accounted for 73.08% of the variation. When the loadings and scores derived from the PCA were plotted for the first two PCs there were no obvious changes in community structure between the beginning and the end of the experimental year (Figure 5.16a). The same is true when plots are distinguished by their temperature and clipping regime (Figure 5.16b).

Bacterial: fungal ratios were calculated for each sample as described in section 4.4.5. When these are considered for each plot there are no obvious patterns, although

there are some plots with sizeable differences between the start and the end (for example plots 5, 6 and 10). When bacterial: fungal ratios are grouped by treatment there are not only no significant differences over the duration of the experiment but also no differences between the four different treatments (Figure 5.16).

When markers for particular groups of microbes are considered (Figure 5.17) the absolute sizes ($\mu g\ g^{-1}$) of the Gram negative bacteria and fungi populations are greater than those of the gram positive bacteria. There appeared to be an effect of time on each of these populations under all plot treatments although these are only significant for plots which are either heated and clipped or unheated and unclipped (Figure 5.17b & c).
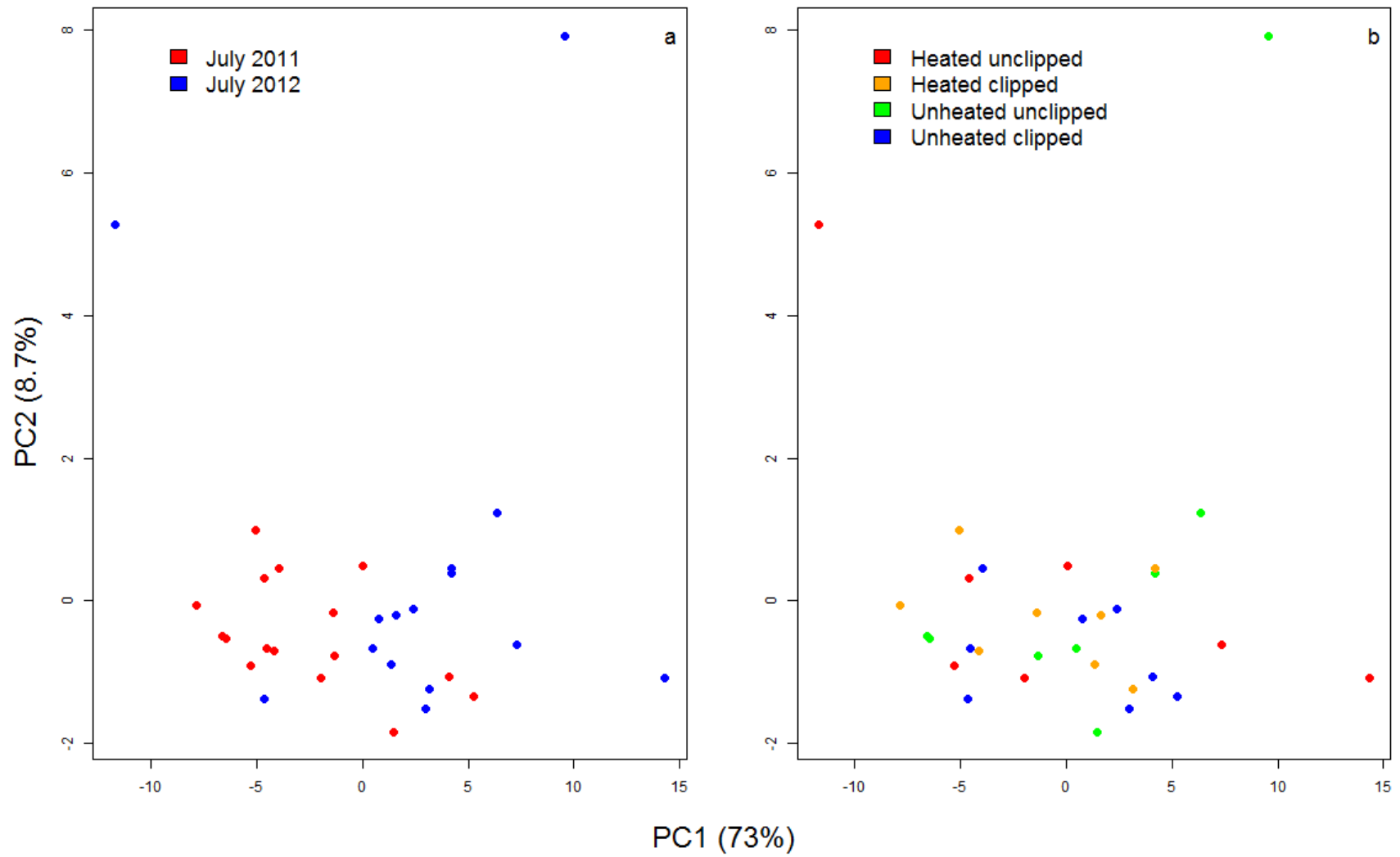
Figure 5.15: Plots of scores from PLFA analysis showing the effects of time (left hand plot) and the effects of heating and clipping treatments (right hand plot).

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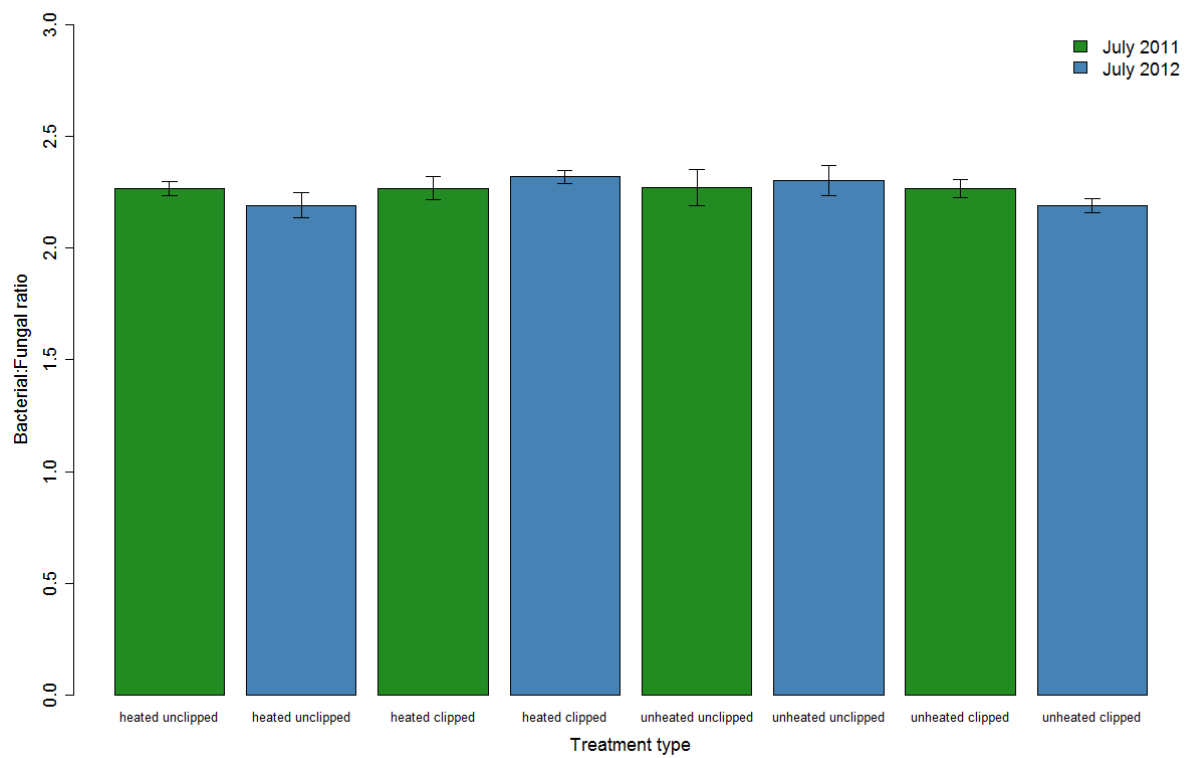
Figure 5.16: Bacterial: fungal ratios at the beginning (13th July 2011; green) and end (11th July 2012; blue) of the experimental year for each treatment type, error bars = +/- 1 standard error, n=4 with the exception of heated unclipped 2012 where n = 3.
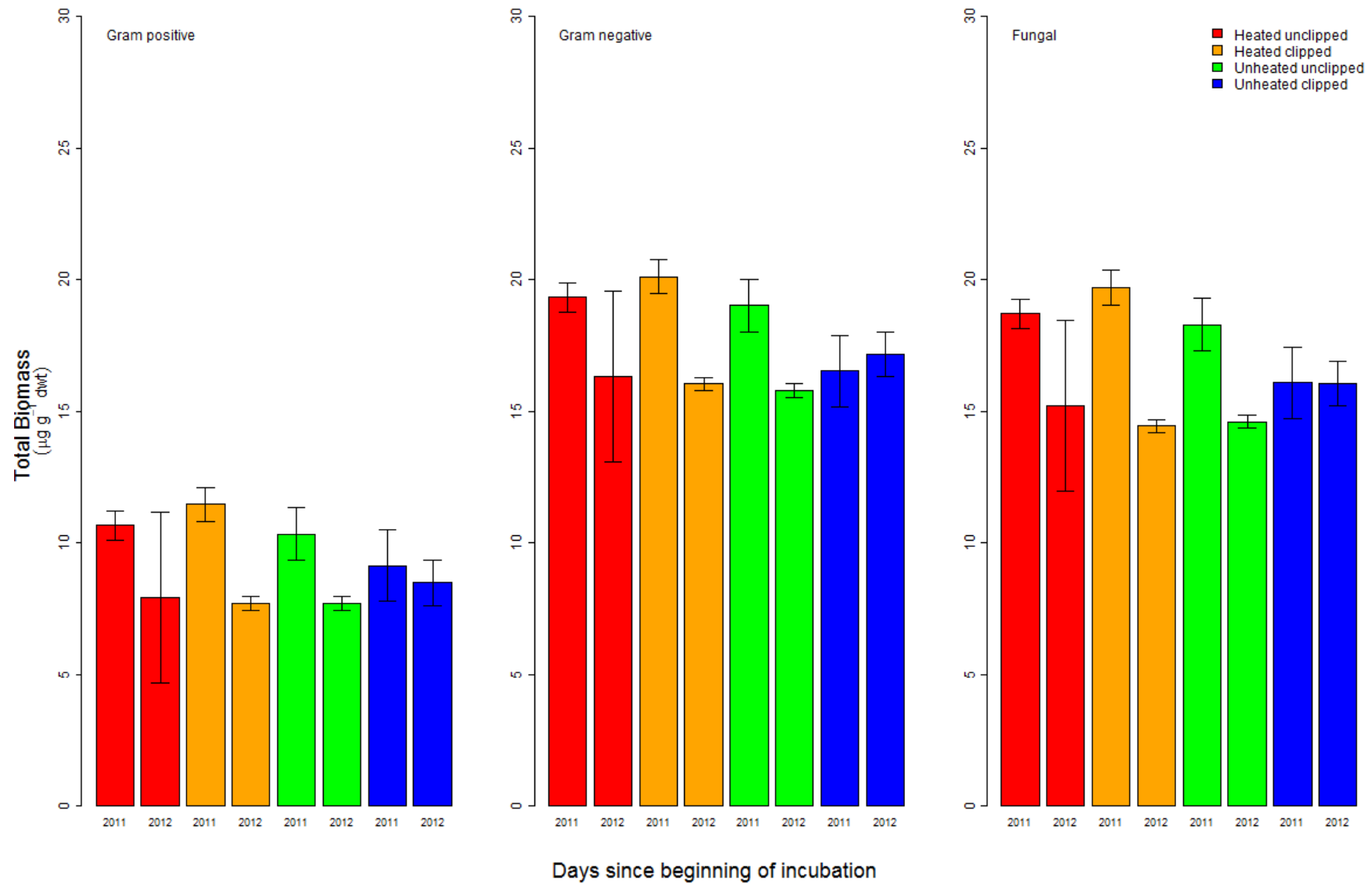
Figure 5.17: size of populations of particular groups of microbes; Gram positive bacteria (a), Gram negative bacteria (b) and fungi (c), error bars = +/- 1 standard error, n = 4, except in the 2012 heated unclipped group where n = 3

5.4.6. Biomass

5.4.6.1. Plants

Regular removal of vegetation from the clipped plots allowed estimation of annual biomass production under heated and unheated conditions. Those plots which were clipped and left unheated appeared to have a greater amount of biomass removed from them over the year than those plots which were clipped but also heated; this was not found to be statistically significant, however, using a paired t-test (t = -0.8269, p = 0.4689). When cumulative biomass production for these plots was calculated (Figure 5.18), the unheated plots are split into two obvious pairs; groups two and thirteen have the greatest cumulative biomass, while plots seven and nine have smaller overall weights. The heated plots also split into two groups – although this is less obvious than for unheated plots. Plots ten and sixteen tend to have greater biomass than plots eight and three although the biomass of plot eight became more similar to that of ten and sixteen towards the end of the experiment (5.18).

5.4.6.2. Invertebrates

The volume of earthworms (mm^3) for each plot treatment over the period February to July is shown in Figure 5.19. Use of a one-way ANOVA revealed no significant impact of heating or clipping regime on the volume of earthworm retrieved from sample cores (p=0.78).
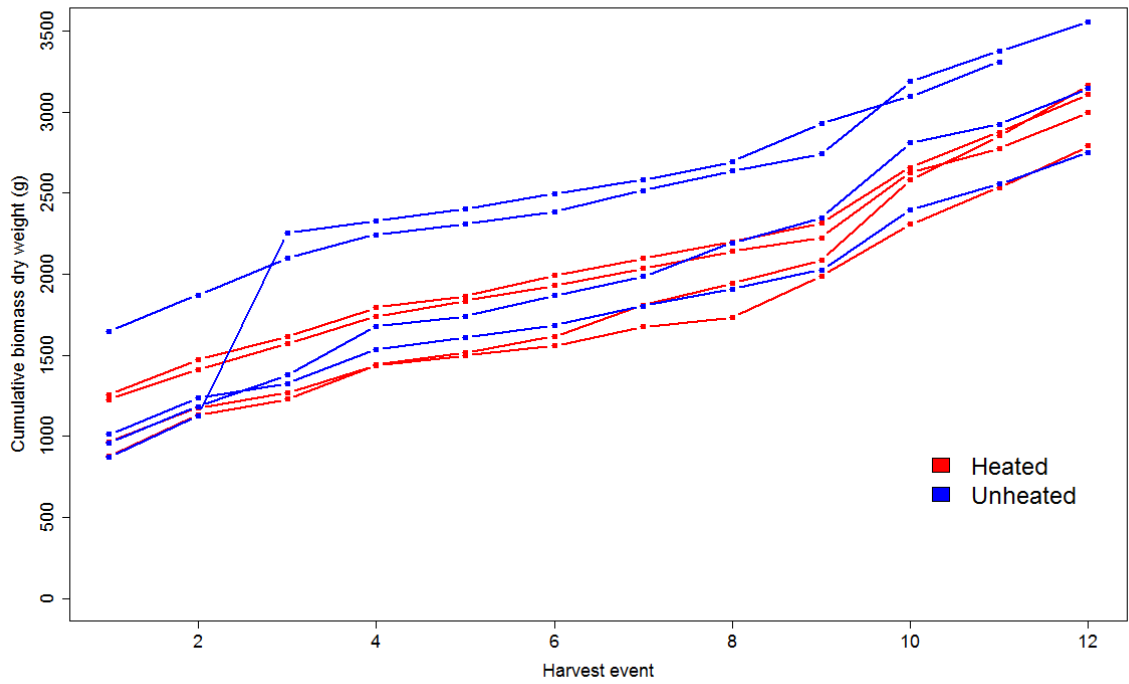
Figure 5.18: the effect of heating on cumulative biomass production.
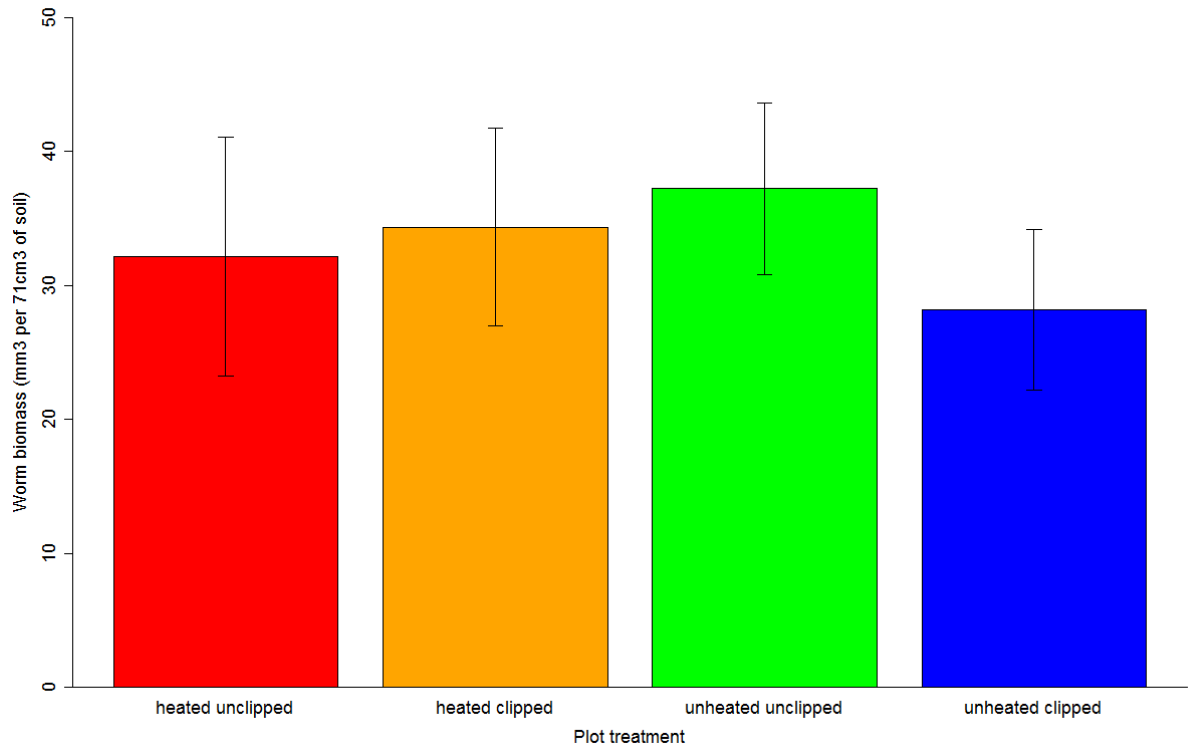
Figure 5.19: The effect of heating and clipping on the volume of earthworm found in sample cores; error bars = +/- 1 standard error, n = 4.

5.5 Discussion

5.5.1 Warming method

Previous soil warming experiments in the field have used various methods to achieve target temperature changes. Most of these have involved warming from above using ceramic heaters (Shaw and Harte, 2001; Dijkstra et al., 2010; Turner and Henry, 2010), plastic tents or open top chambers (OTCs) (Hartley et al., 1999; Weedon et al., 2012) or heating cables laid on the soil surface (Ineson et al., 1998b; Strömgren and Linder, 2002; Dawes et al., 2011). Further studies have exploited natural temperature ranges by transplanting intact soil blocks or cores to different parts of the landscape (Johnsson et al., 1987; Ineson et al., 1998a; Sjögersten and Wookey, 2002). These methods all provided some degree of temperature change but often resulted in changes in other factors which could potentially affect the plant or microbial communities and therefore the responses of the soil system as a whole. For example, ceramic heaters supply increased infrared radiation to plots which can result in changes in plant phenology (Sherry et al., 2007; Rollinson and Kaye, 2012), and plastic tents or OTCs can change the amount of rain or snow that reaches temperature manipulation plots (Allison and Treseder, 2008). Although many of these things may occur alongside temperature change as a result of climate change, they all have the potential to confound experimental results, thus making interpretation difficult. The present study benefits from the use of heating cables buried at 10 cm depth in the soil, which achieved a consistent ~ 3 °C difference in temperature year round in those plots where overlying vegetation was maintained. There was no evidence that this temperature difference affected the moisture content or the above- and below-

ground plant biomass, suggesting that differences in N-cycling reactions between heated and unheated plots were due to changes in soil temperatures alone.

5.5.2 Enzyme Activity

5.5.2.1 Heating effects

The results of these enzyme activity assays show that both protease and urease are highly temperature-sensitive (Figures 7 and 8). This is consistent with results obtained previously in the laboratory (Chapters 3 and 4), suggesting that the enzyme activity results from laboratory incubations are a reasonable predictor of enzyme activities from field warming experiments. However, field and laboratory results differ substantially in magnitude of activity, so although the response to temperature is the same for soil incubated in the laboratory or heated in the field the absolute activity differs. The obvious difference between the two sets of assays in that field samples were collected then assayed within two days where as lab samples were incubated for 7 or 14 days. It is possible that increased incubation period results in a gradual inactivation of extracellular enzymes possibly due to a depletion of useable substrate in samples or metabolism by other enzymes. For both protease and urease activity this suggests that soil, with or without vegetation, will experience an increase in substrate degradation if soil temperatures continue to change in line with those changes detailed in Chapter 2. This may have a variety of implications for wider soil metabolic activity; increased rates of protease activity will result in a greater pool of low molecular weight organic N sources. There is a growing body of recent research showing that low molecular weight dissolved organic nitrogen (DON), such as amino acids and oligopeptides, is a viable and often important source of N for both plants

and microbes in a variety of ecosystems (Näsholm et al., 1998, 2000, 2009; Jones and Kielland, 2002; Schimel and Bennett, 2004; Hill et al., 2011). Increases in protease activity potentially result in a larger pool of organic-N available to both plants and microbes; this could lead to an increase in the ability of ecosystems to sequester carbon (Li et al., 1994; Schlesinger and Andrews, 2000; Neff et al., 2002; Reich et al., 2006; Kardol et al., 2010; Melillo et al., 2011).

As previously stated, there have been very few studies that investigated the direct effects of soil warming on soil enzyme activity (but see Allison and Treseder, 2008; Sardans et al., 2008; Bell and Henry, 2011; Brzostek and Finzi, 2012; Weedon et al., 2012; Burns et al., 2013). These studies generally sampled very few points in time, often only during one season (Allison and Treseder, 2008; Bell and Henry, 2011; Weedon et al., 2012). The only study that measured protease activity in response to warming in the field has the advantage that data was collected on rates of enzyme activity over a wide range of sites all involved in long-term warming studies (Brzostek and Finzi, 2012). However, these were only measured at one point in time for each site and were measured at one temperature over all sites (21°C). The work presented here is unique as it not only looks directly at the effect of soil warming on rates of enzyme activity at field temperature but it does so in all seasons at relatively high temporal resolution.

5.5.2.2 Vegetation effects

There is also an effect of above-ground plant biomass although this varies in magnitude between enzyme assays suggesting that urease activity is more closely associated with the plant community than protease activity (Figures 8 and 10). It is

possible that this reflects a difference between the conditions that trigger activity or synthesis of the different enzymes. The mechanisms that stimulate enzyme activity are still a matter of some debate; it is unclear whether enzyme synthesis, and therefore activity, is stimulated by i) a rate limiting concentration of target nutrients (Koch, 1985; Sinsabaugh and Moorhead, 1994) or ii) the availability of decomposable substrate (Harder and Dijkhuizen, 1983; Shackle et al., 2000). It is possible that the reaction of urease activity to the heated unclipped and the unheated clipped treatments may reflect a similar response of urease to heating and substrate availability. This is not the case for protease activity, however, where the effects of heating completely override the presence or absence of vegetation; this apparent irrelevance of plants for the rates of protease activity was also detected by Jan et al., (2009) in a similar grassland situation. Amidase activity, as previously discussed, seems to be limited by the availability of labile C (Fraser et al., 2012), as when labile C is supplied amidase activity can be measured but it cannot prior to the addition of labile C. During this experiment the change in stoichiometry associated with the increased rates of plant growth during the spring of 2012 resulted in measurable rates of amidase activity which had not been present in the preceding 7 – 8 months. Increased availability of soil–N as a result of global change is predicted to result in increased plant growth (Melillo et al., 2002, 2011; Butler et al., 2012); these results suggest there would be an increased likelihood of NH_4^+ production as a result of increased amidase activity. This not only increases the potential for N-immobilization by the microbial community but also for increased nitrification, potentially resulting in increased rates of NO_3^- leaching from soils (Aber et al., 1989) as well as denitrification with the potential for release of N_2 or N_2O to the atmosphere (Kaiser et al., 1998).

5.5.3 Inorganic Nitrogen

As stated in section 5.4.3. the data set for NO_3^- concentration is only approximately 75% complete; most of the missing data are from the later 6 months of the experiment, some due to time constraints and others to lack of soil (6 cores were taken from each plot on each day but priority was given to performing the enzyme assays rather than extracting NO_3^-). For this reason overall nitrogen mineralization (NO_3^- and NH_4^+ combined) was only analysed for the period July 2011 to January 2012. Neither heating nor clipping were found to have statistically significant effects on total mineral-N, NO_3^- or NH_4^+ concentrations. This at first seems counterintuitive; unclipped plots might be expected to have lower mineral-N concentrations than those where vegetation was largely removed due to higher rates of plant uptake. This pattern was not found, possible reasons for this are that the lack of vegetation on clipped plots either means that lower levels of N are mineralized by the microbial community in the first instance (Clarholm, 1985), or that increased amounts of mineral-N are leached out of the soil due to increased infiltration by rain (Arora & Juo, 1982; Johnsson et al., 1987; Wyland et al., 1996; Di & Cameron, 2002). However, both the observed moisture content of the soil on sampling days throughout the year, and the lack of difference betwee the microbial communities measured between different treatments would seem to discount both of these possibilities. To clarify this response to the imposed treatments further work could include measuring the N-content of plant tissue, or N-uptake of plants and monitoring of leachate from the plots.

5.5.4 Microbial Community

An understanding of the microbial community is of primary importance as the majority of processes underway in the soil system are mediated in some way or another by the microorganisms. In this study it was only possible to measure the size and structure of the microbial population at the beginning and the end of the experiment. The PLFA measurement showed a decrease in the size of the microbial community over the course of the year (this was true of all treatment types) and there was no significant difference between treatments. The PCA showed no evidence of a change in the structure of the community over the year, although there was a minor change in the bacterial: fungal ratio (although not statistically significant in any of the treatment types). Between the beginning and the end of sampling the amount of PLFA markers for gram negative bacteria and fungi decreased significantly in plots that were heated clipped and unheated unclipped, showing that treatment has had an effect on specific parts of the microbial community although reasons for this are unclear. This does reflect, however, some of the differences in urease activity and possible differences in inorganic-N concentrations. It is possible that if the frequency of PLFA sampling were to be increased it might have resolved a seasonal change in microbial community structure, as has been found by other studies (Bardgett et al., 1999; Lipson et al., 2002; Lipson and Schmidt, 2004; Schimel et al., 2004; Cregger et al., 2012), however, these seasonal changes can take several years to manifest (Habekost et al., 2008) despite laboratory studies often showing almost immediate changes in size and/ or structure of the microbial community in response to disturbance (Lundquist et al., 1999; Jackson et al., 2003). This is not true of all studies, however, where even several years of

warming have little or no consistent effect on the size and composition of the microbial community (Weedon et al., 2012), indicating that soil microbes are capable of highly dynamic activity requiring little variation in community structure. Similar results have been found previously, where changes in nitrogenase activity could be explained by changes in only a few individual PLFAs or DNA fragments (Patra et al., 2005, 2006) or when different plant species are found to have little effect on the overall microbial community but do have some effect on particular sub-groups within the whole (Wheatley et al., 1990).

5.6 Conclusions

The work presented here revealed seasonal variation in enzyme activity, the pattern of which was preserved even with the imposed warming and clipping regimes. However, no change in microbial community structure was measured. This contrasts with previous studies, where seasonal changes in the microbial community are cited as explanations of changes in N-transformations over the course of a year (Bardgett et al., 1999; Schimel et al., 2004), but supports the results of other workers who have observed highly variable enzyme activities based only on very minor changes in the microbial community (Patra et al., 2005, 2006). Further work on the size and structure of the microbial community in conjunction with measurements of enzyme activity is needed to further understand exactly how the two are interconnected to determine ecosystem function. Other environmental variables associated with climate change, in particular precipitation and resulting changes to soil moisture fluctuations, also need to be considered. Whilst no direct impact of soil moisture was found in this study, the confounding impacts of variable temperature may have masked differences. This

experiment has also revealed that in this temperate grassland system the measured enzyme activities are strongly driven by soil temperature, and to a lesser extent affected by vegetation, but that gross measures of N-cycling, such as N-mineralization or nitrification, appear to be more related to precipitation than to either soil temperature or vegetation.

Chapter 6: Concluding Remarks

6.1 Addressing the aims

This thesis has used enzyme analysis during both laboratory and field incubation experiments to assess the effect of realistic temperatures on the rates of reactions important to soil N mineralization. Work was also carried out to assess how soil temperatures have been responding to global change, specifically to calculate trends in soil temperature over recent decades to address a specific gap in the literature.

The main aims of this thesis stated initially were:

1. To determine long term trends in soil temperatures from a variety of sites and to compare to these trends in air/ surface temperatures.

2. To determine the effect of temperatures regularly experienced in the field on the measured enzyme activity of the soil in the lab.

3. To assess the effect of differing organic matter quality on enzyme activity and how this combines with the effects of temperature to influence N-cycling in this soil.

4. To measure the effects of seasonal temperatures and elevated temperatures (as a simulation of climate change) on N-mineralization in the field.

The work in each of the preceding four chapters was designed to address one or more of the four aims listed above, which were defined in recognition of the gaps in the literature identified in Chapter 1. The next few pages will briefly recap how each aim was addressed, what the outcomes were and a short discussion of how these results

fit into the literature. This will be followed by a short discussion of how value could be added to these in the future.

6.1.1 Determining long-term trends in soil temperature

Chapter 2 addresses the gap in the literature relating to how soil temperature has changed over past decades (Figure 1.1). This lack of data is problematic as it is required to properly parameterize ecosystem/global nutrient circulation models (Gu et al., 2004; Pendall et al., 2004; Jones, McConnell, et al., 2005; Wolkovich et al., 2012). Chapter 2 makes use of periodic regression to analyse five long term soil and air temperature data sets to assess trends over the past 45 – 50 years. The results show that soil temperatures are changing as quickly, if not faster, than air temperatures and that there are seasonal differences between the trends in soil and air temperatures. The most important difference is likely to be that spring soil temperatures are increasing almost as rapidly as those in winter whereas in air winter temperatures are increasing at almost twice the rate of spring temperatures. Most current nutrient cycling models and many experimental studies that consider the effects of temperature use the mean annual temperature (MAT) or similar (e.g. (Bahn et al., 2008, 2010; Bradford, Fierer, et al., 2008; Mahecha et al., 2010), however, often it is not clear whether this is an air temperature mean or a soil temperature mean (Bradford, Fierer, et al., 2008; Conant et al., 2011) or even whether reference to any field measured temperatures has been made (Dalias et al., 2001; Weintraub and Schimel, 2003; Zhu and Cheng, 2011). Using a mean temperature also takes no account of the overall seasonal variability of temperature in the system. This means that current models could potentially under- or over-estimate efflux of greenhouse

gases , such as CO_2, N_2O and CH_4 (Gu et al., 2004) from the soil system over the year resulting in misleading predictions of future nutrient cycling rates and their effects on the environment. Inclusion of more detail in these models might make them increasingly useful for understanding consequences of activities such as fertilizer application (Cookson et al., 2002; Mallory and Griffin, 2007; Meals et al., 2010), leaving fields fallow or growing catch crops (Martinez and Guiraud, 1990; Thorup-Kristensen et al., 2003; Beaudoin et al., 2005) or changes in land use or land cover over the longer term (Pienkowski et al., 1998; Scanlon et al., 2005). The datasets used for this analysis also show that soil temperatures (particularly from the maritime sites) never get much hotter than 25 °C despite the fact that many incubation experiments examining the effects of temperature on reaction rates in soil use temperatures as high as 40 °C.

6.1.2 The effects of realistic temperatures on laboratory enzyme assays

Both Chapters 3 and 4 assess N-mineralization enzyme activities in soil incubated in the laboratory; this work benefits from access to a high quality, long term data set of soil temperatures collected nearby. This has enabled very accurate estimation of regularly experienced field soil temperatures and their variability (section 3.3.2 and Figure 3.2). The work in chapters 3 and 4 differs from other assessments of soil enzyme activities and kinetics because assays were conducted at each incubation temperature rather than one common, often very high, temperature (e.g. Ladd and Butler, 1972; Frankenberger and Tabatabai, 1979; Kandeler and Gerber, 1988). Despite this the estimates of activation activity (E_a) sit comfortably within those found in the literature (section 3.4.4.2).

Conventionally, assays of soil enzyme activity are measured under idealized pH, substrate and temperature conditions (Alef and Nannipieri, 1995). This means that laboratory experiments generally consist of the incubation of samples at a range of temperatures for a set period of time. These are then assayed for enzyme activity by creating a soil slurry with a buffer adjusted to idealized pH and the addition of substrate at a known and non-limiting concentration. All the samples are then incubated at one, common temperature (often the pre-determined optimum which usually exceeds any temperature likely to be experienced in the field) for the duration of the assay. This, in effect, measures the size of the enzyme pool that is produced at any one particular temperature rather than the ability of the enzyme to work at that temperature. In the work presented here, samples were assayed (at idealized pH and non-limiting substrate concentrations) at the temperature at which they had previously been incubated, thereby measuring the ability of the extant enzyme pool to work at these temperatures. This is the ability of the extant enzyme pool to work at these temperatures. This adjustment to the widely used protocol is likely to give results more akin to what actually occurs in the field, although use of field pH would likely improve the accuracy of this further (Rejsek et al., 2008). This approach is likely to be more useful when it comes to drawing conclusions about the effect of temperature change on soil nutrient cycling as it does not simply consider the effect of enzyme pool size but how active that pool, regardless of size, is at realistic temperatures.

6.1.3 The importance of organic matter

Organic matter availability can be measured in several different ways. In the work presented here OM availability is addressed by examining amounts of OM entering the soil system (by removing vegetation from field plots, Chapter 5) and by varying the quality of substrates added to samples (Chapter 4).

In Chapter 5 the importance of plant biomass (both above-ground and root mass) was assessed thereby indirectly giving information about the effect of OM inputs from plants over the course of the year. The removal of all above ground biomass had no effect on the rates of enzyme activity, although the amount of biomass removed with the fortnightly cores was of some small importance (section 6.2.2). The reasons for this are unclear but could be that there is enough OM in the soil due to incomplete exclusion of plants for the microbial community to make use of and a year is not long enough to deplete the more labile OM pools. It is also possible that clipping the vegetation back every 1 – 2 weeks acted like grazing and encouraged root exudation of labile compounds (Holland et al., 1996; Mawdsley and Bardgett, 1997; Bardgett et al., 1998; Ayres et al., 2007; Harrison and Bardgett, 2008), which seems to be consistent across a wide range of plants with differing life histories (Medina-Roldán and Bardgett, 2011). It is also possible that in plots where vegetation was maintained, substrates for enzymes to work on were simply scarcer due to competition from the plant community for organic as well as inorganic substrates (Näsholm et al., 1998, 2009; Jones et al., 2005; Hill et al., 2011). When the results in Chapter 5 are considered as a whole, none of the experimental manipulations resulted in an overall decrease in rates of enzyme activity overtime; when temperatures returned to summer values in 2012

the activities were comparable to those measured in 2011. This is in contrast to previous studies of field temperature manipulation and its effect on soil respiration. Several studies have found that increased temperature is initially associated with increased rates of respiration (Raich and Schlesinger, 1992; Rustad et al., 2001; Fang et al., 2005; Hartley et al., 2007, 2008). Over time these rates are often observed to decrease until they resemble rates prior to temperature manipulation (Jarvis and Linder, 2000; Oechel et al., 2000; Melillo et al., 2002; Eliasson et al., 2005). This has led to speculation about whether soil microbes acclimate to increased temperatures by reducing their respiration rates (Bradford, Fierer, et al., 2008; Allison et al., 2010; Crowther and Bradford, 2013) as is seen in plants (Atkin and Tjoelker, 2003; Atkin et al., 2005) or whether this decrease is a symptom of substrate depletion within heated plots due to faster rates of substrate use as indicated by the increased respiration rates (Kirschbaum, 2000, 2006; Conant, Steinweg, et al., 2008). To the best of the author's knowledge, even though enzymes are often cited as a mechanism by which acclimation could occur (due to conformational constraints and flexibility found in enzymes from high and low temperature environments (Hochachka and Somero, 2002; Bradford, Fierer, et al., 2008)), there are no temperature manipulation studies where enzyme activity has been measured which show this same phenomenon. This may, in part, be because there are few long-term detailed studies of soil enzyme activity in response to temperature manipulation in the field (Burns et al., 2013). The results presented here show no evidence of acclimation to a uniform temperature increase of 3°C after 1 year of heating.

The theory of organic matter quality described earlier (section 1.4.1) suggests that the lower the quality of the OM the greater the number of enzymatic steps that are required to mineralize OM (Bosatta and Ågren, 1999). This was partially borne out in the results presented in Chapter 4 with increasing enzyme activity found as follows LGBP (liquid fraction of green barley powder) < simple substrates mixture < GBP (green barley powder – substrate definitions in Table 4.1). However, both the control samples (not supplied with additional OM) and the samples amended with barley straw exhibited very low activity. This is likely due to mechanisms triggering enzyme synthesis and secretion by the microbial community (Allison and Vitousek, 2005; Burns et al., 2013) as proposed in Chapter 4. Another aspect of the thermodynamic approach to OM quality is that the lower the quality of the substrate the more its decomposition ought to be affected by low temperatures (the enzyme activity in the samples amended with low quality OM should be proportionally lower at low temperatures), but no evidence was found to support this in Chapter 4

6.1.4.1 Response of N-mineralization to elevated temperatures in the field

Chapter 5 deals with how elevated field temperatures affect N-mineralization; a year round elevation of 3°C had no effect on either NH_4^+ or NO_3^- concentrations and gross mineralization appears to be more strongly associated with precipitation patterns (Figure 5.12). However, when individual reactions are considered the temperature change becomes a statistically significant driver of reaction rate with both protease and urease activity showing strong responses to elevated temperature (Figures 5.7 and 5.9, respectively). The response of protease is clearer than that of urease suggesting that the step that will be most severely impacted by elevated soil

temperatures due to climate change is the depolymerisation of organic molecules into either smaller organic compounds or inorganic nutrients which is thought to be the rate limiting step in N-mineralization (Wallenstein and Weintraub, 2008). This increase in protease activity with increased temperature was also found in a study that looked at the direct effect of soil warming on protease activity in the field at only one point in time (Brzostek et al., 2012) but not in one examining the effect of increased temperatures at different points in the year (Sardans et al., 2008). This illustrates the requirement for further work in this area. These changes in rates of soil processes in response to elevated soil temperatures are not reflected in the size and/or structure of the microbial community which only changed slightly over time (slight decrease in overall community size, and minor changes in amount of gram negative and fungal markers in two treatments). Given the importance of the microbial community in mediating the majority of reactions occurring in the soil system (Nannipieri et al., 2002, 2003) one might intuitively expect dramatic changes in soil process rates to be accompanied by similarly striking alterations to the soil microbial community. Some studies have indeed found that changes in soil process rates occur concomitantly with changes in microbial markers in response to a range of different disturbances including composition of the plant community (Westover et al., 1997; Lukow et al., 2000; Kourtev et al., 2003), changes in pH (Frostegård et al., 1993; Lauber et al., 2009) and temperature (Chin et al., 1999; Conrad et al., 2009). However, many of these studies have taken place under tightly controlled laboratory conditions. Studies measuring the response of soil processes and the microbial community have often presented a picture of a system of complex interactions which show no concomitant changes in rates of processes and microbial community (Bossio et al., 1998; Marschner et al.,

2001; Blume et al., 2002; Elgersma et al., 2011), suggesting high levels of functional redundancy within the soil community.

6.1.4.2 Seasonality of temperature responses in the field

The work in Chapter 5 is unique as it looks at the effect of elevated soil temperature year round, taking multiple samples during each season. Of the few studies that have examined soil enzyme activity under altered temperature regimes in the field, most are based only a limited number of sampling events (Allison and Treseder, 2008; Sardans et al., 2008; Brzostek et al., 2012; Weedon et al., 2012; McDaniel et al., 2013; Zhou et al., 2013) and often only over one season (Brzostek et al., 2012; Weedon et al., 2012; Zhou et al., 2013). The work presented in Chapter 5 shows that the effect of elevated temperature on enzyme activity is maintained throughout all seasons, suggesting that temperature is a very strong driver of activity under these conditions. The response of enzyme activity rates to temperature, and on the whole to clipping, treatments are consistent over time. This suggests that only temperature was driving rates of activity, which is contrary to some previous work that has suggested that rates of soil processes change seasonally as a result of changes in the microbial community and its activity (Bardgett et al., 1999; Lipson et al., 2002; Bell et al., 2009; Kaiser et al., 2010). In this study, the size and structure of the microbial community was only measured at the beginning and the end of the experiment (June 2011 and 2012). As such there are no measures of seasonal changes in microbial community. It is possible that if these measurements were made in autumn, winter and spring as well as those made in summer, seasonal changes would be found. If this were the case and the structure of the microbial community varied seasonally, further evidence would add to

the theory that changes in the microbial community will have no effect on the rates of soil processes due to high levels of functional redundancy in the soil system (Marschner et al., 2003; Nannipieri et al., 2003; Allison and Martiny, 2008; Rousk et al., 2012).

6.2 Future work

6.2.1. The link between soil extracellular enzymes and soil microbial community activity.

The results of this thesis not only address the aims of determining how temperature in the laboratory and in the field affects the rates of enzyme activity and how the rates of enzyme activity respond to different types of OM, but it also asks questions about the link between the soil microbial community and the processes taking place in soil. The soil microbial community is thought to mediate between 70 and 80% of the reactions occurring in soil (Nannipieri et al., 2003) and is likely to be the primary source of extracellular enzymes found in the soil (with another potential source being plant roots). However, this work found no relationship between the size of the microbial biomass and the rates of enzyme activity in the field, though in the laboratory there was a reduction in the size of the microbial biomass over time, as well as a reduction in enzyme activity. However, in both cases there was no significant change in the structure of the microbial community over time. Many studies use enzyme activity, often extracellular enzyme activity, as a surrogate measure for microbial community activity (Bandick and Dick, 1999; Tscherko et al., 2004; Sinsabaugh et al., 2005; Jin and Evans, 2007; Bell et al., 2009; Brockett et al., 2012). However, the actual link between the activity of extracellular soil enzymes and the activity of the microbial community

remains unclear. Extracellular enzymes are fully capable of maintaining their activity in the absence of any living cells, and, as such, decoupling of extracellular activity and microbial activity would seem logical. This might even be expected in particular at lower temperatures as rates of microbial activity will be low (Nicolardot et al., 1994; Kirschbaum, 1995; Bosatta and Ågren, 1999) and rates of degradation of extracellular enzymes are also likely to be lower (simply due to thermodynamic constraints). Further work on this linkage could involve irradiation (provided the radio-sensitivity of the soil has been tested previously (McNamara et al., 2003), other sterilisation methods could include air drying or repeated chloroform fumigation) of samples to remove living biomass; the rates of extracellular enzymes recorded in irradiated samples could then be compared with those recorded in non-irradiated samples. If this was done for a wide selection of soil types under the same management (or differing management if the set of samples was larger) it would be possible to estimate a scaling parameter which would enable a better estimation of microbial community activity by measuring extracellular enzyme activity. This could be done for a range of different temperatures/times of year to assess the coupling of the rates of enzyme activity and rates of microbial activity. This could be assessed further by coupling with PLFA or genetic analysis.

6.2.2 Monitoring of soil temperature trends

As previously stated, current literature focuses on trends in surface and air temperatures. Whilst these are obviously linked with temperatures and trends in the soil system, Chapter 2 shows that there are seasonal differences between trends in the two systems. Monitoring trends in soil temperatures at a large number of sites spread

across the world would enable predictions of how plant growth might respond to future temperatures or how changes in temperature will affect the ability of the soil system to maintain stores of C, N, and CH_4. This may be possible through use of soil temperature records collected by FLUXNET (http://fluxnet.ornl.gov/) or the International Soil Moisture Network (http://www.ipf.tuwien.ac.at/insitu/), although these records may not cover such a long period of time or as many soil depths as those used in Chapter 2. However, they are widely spread across at least three continents (Europe, North America and Australia) and also raise the possibility of incorporating variability in soil moisture records. This would help to parameterize more accurately models of regional or global nutrient cycling and net primary productivity.

Current models of net primary productivity (NPP) do have climate input parameters which include temperature but these tend to be monthly average temperatures (or mean annual temperatures (section 6.1). It is usually unclear as to whether these are air or soil temperatures, but given the paucity of soil temperature studies (figure 1.1) it is more likely that they are air temperatures. This is normally the case for models of nutrient cycling too, even much of the experimental work examining the effect of temperature on soil processes uses MAT when determining laboratory incubation regimes although again it is usually unclear whether this is based on a soil or an air measurement (section 6.1). The discrepancies between air and soil seasonal trends coupled with the dearth of experimental work relating to different seasons (particularly with reference to soil enzyme activity) suggests that predictive models that do exist (for example, CENTURY, RothC and DOLY) are often parameterized on what is at least incomplete information. As such, further work on how soil processes

are affected by changing climate during different seasons (particularly during winter) is warranted. The work presented here focused on temperature effects both in the laboratory and in the field. Further studies need to consider a wider range of soil types, the impacts of land management practices such as fertilisers, compaction or plant species and possible impacts from changes in precipitation patterns predicted with climate change.

6.2.3. Complexity

The difficulty with examining how soil processes will respond to future climate is complexity. Multiple climatic factors are likely to change over coming decades (temperature, precipitation and atmospheric CO_2 concentrations). These changes will, in turn, affect both the soil and plant systems which are so closely associated that it can be difficult to know which is driving activity in the other (Giovannetti et al., 2004; Eskelinen et al., 2009). There are areas where further experimental work would be useful such as the link between extracellular enzyme activity and microbial community activity (section 6.2.1), or the effect of changes in atmospheric chemistry on the composition of plant litter in the future and how that will affect decomposition (Luo et al., 2001, 2004; Norby et al., 2001; Reich et al., 2006). However, there are also several time consuming, expensive, long term experiments running presently, for example the Long Term Ecological Research Network (LTER Network) run (mainly) in the United States of America, which have, and are continuing to collect large quantities of data examining changes in climate and their effects on soil (and plant) processes. Application of appropriate statistical techniques, such as critical path analysis (Dale et al., 1989; Hunt and Gee, 2002), structural equations (Chamizo et al., 2012) or additive

Bayesian network modelling (Lewis and McCormick, 2012) to data collected over the long-term by these types of experimental networks would shed further light on how processes in the soil system are likely to be affected by changes in climate, either directly (through increased temperatures or changed soil moisture), or indirectly (through changes in plant community or land management).

6.3 Overall Conclusions

The first set of results presented in this thesis show clearly that soil temperatures have changed significantly over the past 50 years. Given the large body of knowledge about how surface and air temperatures have changed over a similar period of time this is not terribly surprising. However, the work presented here has highlighted the difference between the patterns of change in soil and air.

The results presented in this thesis demonstrate that enzymes involved in N-mineralization are highly temperature sensitive both in the laboratory and in the field. This strongly suggests that as soil temperature increases over coming years, N-availability in the soil system will increase, potentially affecting the growth and proliferation of both the plant and the microbial community, altering the potential for NO_3^- leaching and the efflux of NO_2 from the soil system as well as denitrification via, for example, nitrous oxide release.

An attempt was also made to address the question of organic matter quality; the results showed evidence of increased protease activity with OM complexity, although no evidence was found for increased temperature sensitivity of OM with decreasing quality. Taken collectively, the results presented here show that soil temperature is

likely to change further, which will affect the activity of enzymes involved in the mineralization of nitrogen in soil. Previous work has suggested that climate change might affect the quality of OM in the future, but these results suggest that this may not promote increased C-sequestration in soil because increased temperatures will promote the activity of enzymes designed to break down OM.

7. References

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen Saturation in Northern Forest Ecosystems. BioScience 39, 378–386.

Accoe, F., Boeckx, P., Busschaert, J., Hofman, G., Van Cleemput, O., 2004. Gross N transformation rates and net N mineralisation rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. Soil Biology and Biochemistry 36, 2075–2087.

Adams, M.B., 2003. Ecological issues related to N deposition to natural ecosystems: research needs. Environment International 29, 189–199.

Agehara, S., Warncke, D.D., 2005. Soil Moisture and Temperature Effects on Nitrogen Release from Organic Nitrogen Sources. Soil Science Society of America Journal 69, 1844.

Ågren, G.I., Bosatta, E., 1998. Theoretical Ecosystem Ecology: Understanding Element Cycles. Cambridge University Press.

Ågren, G.I., Bosatta, E., 2002. Reconciling differences in predictions of temperature response of soil organic matter. Soil Biology and Biochemistry 34, 129–132.

Ågren, G.I., Bosatta, E., Magill, A.H., 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128, 94–98.

Ågren, G.I., Bosatta, N., 1987. Theoretical Analysis of the Long-Term Dynamics of Carbon and Nitrogen in Soils. Ecology 68, 1181.

Alef, K., Nannipieri, P., 1995. Methods in applied soil microbiology and biochemistry. Academic Press.

Allison, S.D., 2006. Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. Biogeochemistry 81, 361–373.

Allison, S.D., Martiny, J.B.H., 2008. Colloquium Paper: Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512–11519.

Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14, 2898–2909.

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37, 937–944.

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340.

Andersen, M., and Jensen, L., 2001. Low soil temperature effects on short-term gross N mineralisation–immobilisation turnover after incorporation of a green manure. Soil Biology and Biochemistry 33, 511–521.

Anderson, J.M., Ingram, J.S.I., 1993. Tropical soil biology and fertility: a handbook of methods. C.A.B. International.

Andrews, J.A., Matamala, R., Westover, K.M., Schlesinger, W.H., 2000. Temperature effects on the diversity of soil heterotrophs and the $\delta^{13}C$ of soil-respired CO_2. Soil Biology and Biochemistry 32, 699–706.

Arora, Y., Juo, A.S.R., 1982. Leaching of Fertilizer Ions in a Kaolinitic Ultisol in the High Rainfall Tropics: Leaching of Nitrate in Field Plots under Cropping and Bare Fallow. Soil Science Society of America Journal 46, 1212–1218.

Atkin, O.K., Bruhn, D., Hurry, V.M., Tjoelker, M.G., 2005. Evans Review No. 2: The hot and the cold: unravelling the variable response of plant respiration to temperature. Functional Plant Biology 32, 87–105.

Atkin, O.K., Tjoelker, M.G., 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science 8, 343–351.

Avrahami, S., Liesack, W., Conrad, R., 2003. Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. Environmental Microbiology 5, 691–705.

Ayres, E., Dromph, K.M., Cook, R., Ostle, N., Bardgett, R.D., 2007. The influence of below-ground herbivory and defoliation of a legume on nitrogen transfer to neighbouring plants. Functional Ecology 21, 256–263.

Bahn, M., Reichstein, M., Davidson, E.A., Grünzweig, J., Jung, M., Carbone, M.S., Epron, D., Misson, L., Nouvellon, Y., Roupsard, O., Savage, K., Trumbore, S.E., Gimeno, C., Curiel Yuste, J., Tang, J., Vargas, R., Janssens, I.A., 2010. Soil respiration at mean annual temperature predicts annual total across vegetation types and biomes. Biogeosciences 7, 2147–2157.

Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drösler, M., Williams, M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M., Kumar, S., Newesely, C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S., Tenhunen, J., Wohlfahrt, G., Cernusca, A., 2008. Soil Respiration in European Grasslands in Relation to Climate and Assimilate Supply. Ecosystems 11, 1352–67.

Baldocchi, D.D., Black, T.A., Curtis, P.S., Falge, E., Fuentes, J.D., Granier, A., Gu, L., Knohl, A., Pilegaard, K., Schmid, H.P., Valentini, R., Wilson, K., Wofsy, S., Xu, L., Yamamoto, S., 2005. Predicting the onset of net carbon uptake by deciduous forests with soil temperature and climate data: a synthesis of FLUXNET data. International Journal of Biometeorology 49, 377–387.

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biology and Biochemistry 56, 60–68.

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biology and Biochemistry 31, 1471–1479.

Bárcenas-Moreno, G., Gómez-Brandón, M., Rousk, J., Bååth, E., 2009. Adaptation of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. Global Change Biology 15, 2950–2957.

Bardgett, R.D., Lovell, R.D., Hobbs, P.J., Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry 31, 1021–1030.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30, 1867–1878.

Baron, J.S., Hall, E.K., Nolan, B.T., Finlay, J.C., Bernhardt, E.S., Harrison, J.A., Chan, F., Boyer, E.W., 2012. The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. Biogeochemistry 1–22.

Barrett, J.E., Burke, I.C., 2000. Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. Soil Biology and Biochemistry 32, 1707–1716.

Bassirirad, H., 2000. Kinetics of nutrient uptake by roots: responses to global change. New Phytologist 147, 155–169.

Batjes, N. h., 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47, 151–163.

Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005. Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices, soils and crop rotations. Agriculture, Ecosystems & Environment 111, 292–310.

Beier, C., Emmett, B.A., Peñuelas, J., Schmidt, I.K., Tietema, A., Estiarte, M., Gundersen, P., Llorens, L., Riis-Nielsen, T., Sowerby, A., Gorissen, A., 2008. Carbon and nitrogen cycles

in European ecosystems respond differently to global warming. Science of the Total Environment 407, 692–697.

Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T., Zak, J.C., 2009. Linking Microbial Community Structure and Function to Seasonal Differences in Soil Moisture and Temperature in a Chihuahuan Desert Grassland. Microbial Ecology 58, 827–842.

Bell, T.H., Henry, H.A.L., 2011. Fine scale variability in soil extracellular enzyme activity is insensitive to rain events and temperature in a mesic system. Pedobiologia 54, 141–146.

Belyea, L.R., Malmer, N., 2004. Carbon sequestration in peatland: patterns and mechanisms of response to climate change. Global Change Biology 10, 1043–1052.

Bennett, A.F., Dao, K.M., Lenski, R.E., 1990. Rapid evolution in response to high-temperature selection. doi:10.1038/346079a0 346, 79–81.

Birgander, J., Rousk, J., Olsson, P.A., 2012. Activity of temperate grassland plants and symbiotic fungi during the winter – implications for community structure and carbon cycling in a changing climate. Nordic Journal of Botany 30, 513–521.

Bliss, C.I., 1958. Periodic regression in biology and climatology. Bulletin 615, 19–21.

Blume, E., Bischoff, M., Reichert, J.M., Moorman, T., Konopka, A., Turco, R.F., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology 20, 171–181.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.-W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20, 30–59.

Bonan, G.B., 2008. Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. Science 320, 1444–1449.

Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological Monographs 75, 139–157.

Borner, A., Kielland, K., Walker, M., 2008. Effects of Simulated Climate Change on Plant Phenology and Nitrogen Mineralization in Alaskan Arctic Tundra. Arctic, Antarctic, and Alpine Research 40, 27–38.

Bosatta, E., Ågren, G.I., 1985. Theoretical analysis of decomposition of heterogeneous substrates. Soil Biology and Biochemistry 17, 601–610.

Bosatta, E., Ågren, G.I., 1999. Soil organic matter quality interpreted thermodynamically. Soil Biology and Biochemistry 31, 1889–1891.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of Soil Microbial Communities: Effects of Agricultural Management, Season, and Soil Type on Phospholipid Fatty Acid Profiles. Microbial Ecology 36, 1–12.

Bradford, M., Fierer, N., Reynolds, J., 2008. Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Functional Ecology 22, 964–974.

Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K., Wallenstein, M.D., 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11, 1316–1327.

Bradford, M.A., Watts, B.W., Davies, C.A., 2010. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. Global Change Biology 16, 1576–1588.

Breland, T.A., 1994. Measured and predicted mineralization of clover green manure at low temperatures at different depths in two soils. Plant and Soil 166, 13–20.

Bremner, J.M., Mulvaney, R.L., 1978. Urease activity in soils, in: Soil Enzymes, Eds. Burns, R.G. Academic Press, London, pp. 149 – 169.

Briones, M.J.I., Garnett, M.H., Ineson, P., 2010. Soil biology and warming play a key role in the release of "old C" from organic soils. Soil Biology and Biochemistry 42, 960–967.

Broadbent, F.E., 1965. Effect of Fertilizer Nitrogen on the Release of Soil Nitrogen. Soil Science Society of America Journal 29, 692–696.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44, 9–20.

Brookes, P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils 19, 269–279.

Brzostek, E.R., Blair, J.M., Dukes, J.S., Frey, S.D., Hobbie, S.E., Melillo, J.M., Mitchell, R.J., Pendall, E., Reich, P.B., Shaver, G.R., Stefanski, A., Tjoelker, M.G., Finzi, A.C., 2012. The effect of experimental warming and precipitation change on proteolytic enzyme activity: positive feedbacks to nitrogen availability are not universal. Global Change Biology 18, 2617–2625.

Burke, I.C., Yonker, C.M., Parton, W.J., Cole, C.V., Schimel, D.S., Flach, K., 1989. Texture, Climate, and Cultivation Effects on Soil Organic Matter Content in U.S. Grassland Soils. Soil Science Society of America Journal 53, 800–805.

Burn, C.R., Kokelj, S.V., 2012. Influence of snow on near-surface ground temperatures in upland and alluvial environments of the outer Mackenzie Delta, Northwest Territories. Canadian Journal of Earth Sciences 49, 895–913.

Burns, R.G., 1982. Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biology and Biochemistry 14, 423–427.

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

Burrows, L.A., Edwards, C.A., 2002. The use of integrated soil microcosms to predict effects of pesticides on soil ecosystems. European Journal of Soil Biology 38, 245–249.

Butler, C.J., García Suárez, A.M., Coughlin, A.D.S., Morrell, C., 2005. Air temperatures at Armagh Observatory, Northern Ireland, from 1796 to 2002. International Journal of Climatology 25, 1055–1079.

Butler, S.M., Melillo, J.M., Johnson, J.E., Mohan, J., Steudler, P.A., Lux, H., Burrows, E., Smith, R.M., Vario, C.L., Scott, L., Hill, T.D., Aponte, N., Bowles, F., 2012. Soil warming alters nitrogen cycling in a New England forest: implications for ecosystem function and structure. Oecologia 168, 819–828.

Callaway, R.M., Thelen, G.C., Rodriguez, A., Holben, W.E., 2004. Soil biota and exotic plant invasion. Nature 427, 731–733.

Campbell, J.L., Mitchell, M.J., Groffman, P.M., Christenson, L.M., Hardy, J.P., 2005. Winter in northeastern North America: a critical period for ecological processes. Frontiers in Ecology and the Environment 3, 314–322.

Chamizo, S., Cantón, Y., Rodríguez-Caballero, E., Domingo, F., Escudero, A., 2012. Runoff at contrasting scales in a semiarid ecosystem: A complex balance between biological soil crust features and rainfall characteristics. Journal of Hydrology 452–453, 130–138.

Chapin, F.S., Sturm, M., Serreze, M.C., McFadden, J.P., Key, J.R., Lloyd, A.H., McGuire, A.D., Rupp, T.S., Lynch, A.H., Schimel, J.P., Beringer, J., Chapman, W.L., Epstein, H.E., Euskirchen, E.S., Hinzman, L.D., Jia, G., Ping, C.-L., Tape, K.D., Thompson, C.D.C., Walker, D.A., Welker, J.M., 2005. Role of Land-Surface Changes in Arctic Summer Warming. Science 310, 657–660.

Chapin, F.S., Van Cleve, K., Chapin, M.C., 1979. Soil Temperature and Nutrient Cycling in the Tussock Growth Form of Eriophorum Vaginatum. The Journal of Ecology 67, 169.

Chin, K.-J., Lukow, T., Conrad, R., 1999. Effect of Temperature on Structure and Function of the Methanogenic Archaeal Community in an Anoxic Rice Field Soil. Applied and Environmental Microbiology 65, 2341–2349.

Chudinova, S.M., Frauenfeld, O.W., Barry, R.G., Zhang, T., Sorokovikov, V.A., 2006. Relationship between air and soil temperature trends and periodicities in the permafrost regions of Russia. Journal of Geophysical Research: Earth Surface 111. DOI: 10.1029/2005JF000342

Chung, H., Zak, D.R., Reich, P.B., Ellsworth, D.S., 2007. Plant species richness, elevated CO2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Global Change Biology 13, 980–989.

Clarholm, M., 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology and Biochemistry 17, 181–187.

Clarke, A., 2004. Is there a Universal Temperature Dependence of metabolism? Functional Ecology 18, 252–256.

Conant, R.T., Drijber, R.A., Haddix, M.L., Parton, W.J., Paul, E.A., Plante, A.F., Six, J., Steinweg, J.M., 2008. Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biology 14, 868–877.

Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., Kirschbaum, M.U.F., Lavallee, J.M., Leifeld, J., Parton, W.J., Megan Steinweg, J., Wallenstein, M.D., Martin Wetterstedt, J.Å., Bradford, M.A., 2011. Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Global Change Biology 17, 3392–3404.

Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008. Experimental Warming Shows That Decomposition Temperature Sensitivity Increases with Soil Organic Matter Recalcitrance. Ecology 89, 2384–2391.

Conrad, R., Klose, M., Noll, M., 2009. Functional and structural response of the methanogenic microbial community in rice field soil to temperature change. Environmental Microbiology 11, 1844–1853.

Cookson, W.., Cornforth, I.., Rowarth, J.., 2002. Winter soil temperature (2–15 °C) effects on nitrogen transformations in clover green manure amended or unamended soils; a laboratory and field study. Soil Biology and Biochemistry 34, 1401–1415.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000a. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408, 184–187.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000b. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408, 184–187.

Cregger, M.A., Schadt, C.W., McDowell, N.G., Pockman, W.T., Classen, A.T., 2012. Soil microbial community response to precipitation change in a semi-arid ecosystem. Applied and Environmental Microbiology. DOI: 10.1128/AEM.02050-12

Crowther, T.W., Bradford, M.A., 2013. Thermal acclimation in widespread heterotrophic soil microbes. Ecology Letters. DOI: 10.1111/ele.12069

Curtis, C.J., Evans, C.D., Helliwell, R.C., Monteith, D.T., 2005. Nitrate leaching as a confounding factor in chemical recovery from acidification in UK upland waters. Environmental Pollution 137, 73–82.

D'Amico, S., Collins, T., Marx, J.-C., Feller, G., Gerday, C., Gerday, C., 2006. Psychrophilic microorganisms: challenges for life. EMBO reports 7, 385–389.

Dale, M.B., McBRATNEY, A.B., Russell, J.S., 1989. On the role of expert systems and numerical taxonomy in soil classification. Journal of Soil Science 40, 223–234.

Dalias, P., Anderson, J., Bottner, P., Coûteaux, M.-M., 2001. Long-term effects of temperature on carbon mineralisation processes. Soil Biology and Biochemistry 33, 1049–1057.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Dawes, M.A., Hagedorn, F., Zumbrunn, T., Handa, I.T., Hättenschwiler, S., Wipf, S., Rixen, C., 2011. Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. New Phytologist 191, 806–818.

Deslippe, J.R., Egger, K.N., Henry, G.H.R., 2005. Impacts of warming and fertilization on nitrogen-fixing microbial communities in the Canadian High Arctic. FEMS microbiology ecology 53, 41–50.

Di, H.J., Cameron, K.C., 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64, 237–256.

Dietzel, R., Wolfe, D., Thies, J.E., 2011. The influence of winter soil cover on spring nitrous oxide emissions from an agricultural soil. Soil Biology and Biochemistry 43, 1989–1991.

Dijkstra, F.A., Blumenthal, D., Morgan, J.A., Pendall, E., Carrillo, Y., Follett, R.F., 2010. Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. New Phytologist 187, 426–437.

Dise, N.B., Wright, R.F., 1995. Nitrogen leaching from European forests in relation to nitrogen deposition. Forest Ecology and Management 71, 153–161.

Dörsch, P., Palojärvi, A., Mommertz, S., 2004. Overwinter greenhouse gas fluxes in two contrasting agricultural habitats. Nutrient Cycling in Agroecosystems 70, 117–133.

Downing, J.A., Mcclain, M., Twilley, R., Melack, J.M., Elser, J., Rabalais, N.N., Jr, W.M.L., Turner, R.E., Corredor, J., Soto, D., Yanez-Arancibia, A., Kopaska, J.A., Howarth, R.W., 1999. The impact of accelerating land-use change on the N-Cycle of tropical aquatic ecosystems: Current conditions and projected changes. Biogeochemistry 46, 109–148.

Duxbury, J.M., Bouldin, D.R., Terry, R.E., Tate, R.L., 1982. Emissions of nitrous oxide from soils. , Published online: 29 July 1982; | doi:10.1038/298462a0 298, 462–464.

Ehrenfeld, J.G., 2003. Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. Ecosystems 6, 503–523.

Elgersma, K.J., Ehrenfeld, J.G., Yu, S., Vor, T., 2011. Legacy effects overwhelm the short-term effects of exotic plant invasion and restoration on soil microbial community structure, enzyme activities, and nitrogen cycling. Oecologia 167, 733–745.

Eliasson, P.E., McMurtrie, R.E., Pepper, D.A., Strömgren, M., Linder, S., Ågren, G.I., 2005. The response of heterotrophic CO_2 flux to soil warming. Global Change Biology 11, 167–181.

Erisman, J.W., Galloway, J., Seitzinger, S., Bleeker, A., Butterbach-Bahl, K., 2011. Reactive nitrogen in the environment and its effect on climate change. Current Opinion in Environmental Sustainability 3, 281–290.

Eskelinen, A., Stark, S., Männistö, M., 2009. Links between plant community composition, soil organic matter quality and microbial communities in contrasting tundra habitats. Oecologia 161, 113–123.

Euskirchen, E.S., McGuire, A.D., Kicklighter, D.W., Zhuang, Q., Clein, J.S., Dargaville, R.J., Dye, D.G., Kimball, J.S., McDonald, K.C., Melillo, J.M., Romanovsky, V.E., Smith, N.V., 2006. Importance of recent shifts in soil thermal dynamics on growing season length, productivity, and carbon sequestration in terrestrial high-latitude ecosystems. Global Change Biology 12, 731–750.

Evans, M.G., Polanyi, M., 1935. Some applications of the transition state method to the calculation of reaction velocities, especially in solution. Transactions of the Faraday Society 31, 875–894.

Evans, R.D., Rimer, R., Sperry, L., Belnap, J., 2001. Exotic plant invasion alters Nitrogen dynamics in an arid grassland. Ecological Applications 11, 1301–1310.

Eyring, H., 1935. The Activated Complex in Chemical Reactions. The Journal of Chemical Physics 3, 107–115.

Fang, C., Smith, P., Moncrieff, J.B., Smith, J.U., 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature 433, 57–59.

Field, C.B., Lobell, D.B., Peters, H.A., Chiariello, N.R., 2007. Feedbacks of Terrestrial Ecosystems to Climate Change*. Annual Review of Environment and Resources 32, 1–29.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176.

Fisher, J.B., Tu, K.P., Baldocchi, D.D., 2008. Global estimates of the land–atmosphere water flux based on monthly AVHRR and ISLSCP-II data, validated at 16 FLUXNET sites. Remote Sensing of Environment 112, 901–919.

Fließbach, A., Martens, R., Reber, H.H., 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biology and Biochemistry 26, 1201–1205.

Frank, D.A., Groffman, P.M., Evans, R.D., Tracy, B.F., 2000. Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. Oecologia 123, 116–121.

Frank, D.A., Inouye, R.S., Huntly, N., Minshall, G.W., Anderson, J.E., 1994. The biogeochemistry of a north-temperate grassland with native ungulates: Nitrogen dynamics in Yellowstone National Park. Biogeochemistry 26, 163–188.

Frankenberger Jr, W.T., Johanson, J.B., 1982. Effect of pH on enzyme stability in soils. Soil Biology and Biochemistry 14, 433–437.

Frankenberger, W.T., Tabatabai, M.A., 1979. Amidase Activity in Soils: I. Method of Assay. Soil Science Society of America Journal 44, 282–287.

Fraser, F.C., Hallett, P.D., Wookey, P.A., Hartley, I.P., Hopkins, D.W., 2012. How do enzymes catalysing soil nitrogen transformations respond to changing temperatures? Biology and Fertility of Soils 49, 99–103.

Freppaz, M., Williams, B.L., Edwards, A.C., Scalenghe, R., Zanini, E., 2007. Labile nitrogen, carbon, and phosphorus pools and nitrogen mineralization and immobilization rates at low temperatures in seasonally snow-covered soils. Biology and Fertility of Soils 43, 519–529.

Fritz, C., Van Dijk, G., Smolders, A.J.P., Pancotto, V.A., Elzenga, T.J.T.M., Roelofs, J.G.M., Grootjans, A.P., 2012. Nutrient additions in pristine Patagonian Sphagnum bog vegetation: can phosphorus addition alleviate (the effects of) increased nitrogen loads. Plant Biology 14, 491–499.

Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723–730.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen Cycles: Past, Present, and Future. Biogeochemistry 70, 153–226.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. Science 320, 889–892.

Garcia-Gil, J.C., Plaza, C., Soler-Rovira, P., Polo, A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology & Biochemistry 32, 1907–1913.

García-Suárez, A.M., Butler, C.J., 2006. Soil temperatures at Armagh Observatory, Northern Ireland, from 1904 to 2002. International Journal of Climatology 26, 1075–1089.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387–1397.

Gianfreda, L., De Cristofaro, A., Rao, M.A., Violante, A., 1995. Kinetic Behavior of Synthetic Organo- and Organo-Mineral-Urease Complexes. Soil Science Society of America Journal 59, 811–815.

Giardina, C.P., Ryan, M.G., 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature 404, 858–861.

Gilichinsky, D.A., Barry, R.G., Bykhovets, S.S., Sorokovikov, V.A., Zhang, T., Zudin, S.L., Fedorov-Davydov, D.G., 1998. A century of temperature observations of soil climate: Methods of analysis and long-term trends, in: Proceedings of the 7th International Conference on Permafrost. pp. 23–27.

Gillabel, J., Cebrian-Lopez, B., Six, J., Merckx, R., 2010. Experimental evidence for the attenuating effect of SOM protection on temperature sensitivity of SOM decomposition. Global Change Biology 16, 2789–2798.

Giovannetti, M., Sbrana, C., Avio, L., Strani, P., 2004. Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. New Phytologist 164, 175–181.

Gould, W.D., Cook, F.D., Webster, G.R., 1973. Factors affecting urea hydrolysis in several Alberta soils. Plant and Soil 38, 393–401.

Goulding, K.W.T., Bailey, N.J., Bradbury, N.J., Hargreaves, P., Howe, M., Murphy, D.V., Poulton, P.R., Willison, T.W., 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. New Phytologist 139, 49–58.

Gregorich, E.G., Monreal, C.M., Carter, M.R., Angers, D.A., Ellert, B.H., 1994. Towards a minimum data set to assess soil organic matter quality in agricultural soils. Canadian Journal of Soil Science 74, 367–385.

Grime, J.P., Pierce, S., 2012. The evolutionary strategies that shape ecosystems. Wiley-Blackwell.

Grogan, P., Michelsen, A., Ambus, P., Jonasson, S., 2004. Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biology and Biochemistry 36, 641–654.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451, 293–296.

Grünwald, N.J., Flier, W.G., 2005. The Biology of *Phytophthora infestans* at Its Center of Origin. Annual Review of Phytopathology 43, 171–190.

Grzymski, J.J., Murray, A.E., Campbell, B.J., Kaplarevic, M., Gao, G.R., Lee, C., Daniel, R., Ghadiri, A., Feldman, R.A., Cary, S.C., 2008. Metagenome analysis of an extreme microbial symbiosis reveals eurythermal adaptation and metabolic flexibility. Proceedings of the National Academy of Sciences 105, 17516–17521.

Gu, L., Post, W.M., King, A.W., 2004. Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: A model analysis. Global Biogeochemical Cycles 18, DOI: 10.1029/2003GB002119.

Guggenberger, G., Christensen, B. t., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. European Journal of Soil Science 45, 449–458.

Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360.

Habekost, M., Eisenhauer, N., Scheu, S., Steinbeiss, S., Weigelt, A., Gleixner, G., 2008. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biology and Biochemistry 40, 2588–2595.

Hamilton, E.W., Frank, D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82, 2397–2402.

Hansen, J., Sato, M., Ruedy, R., 2012. Perception of climate change. Proceedings of the National Academy of Sciences of the United States of America 109, E2415–E2423.

Harder, W., Dijkhuizen, L., 1983. Physiological Responses to Nutrient Limitation. Annual Review of Microbiology 37, 1–23.

Harris, G.P., 2001. Biogeochemistry of nitrogen and phosphorus in Australian catchments, rivers and estuaries: effects of land use and flow regulation and comparisons with global patterns. Marine and Freshwater Research 52, 139–149.

Harrison, K.A., Bardgett, R.D., 2008. Impacts of Grazing and Browsing by Large Herbivores on Soils and Soil Biological Properties, in: Gordon, I.J., Prins, H.H.T. (Eds.), The Ecology of Browsing and Grazing, Ecological Studies. Springer Berlin Heidelberg, pp. 201–216.

Hartley, A.E., Neill, C., Melillo, J.M., Crabtree, R., Bowles, F.P., 1999. Plant Performance and Soil Nitrogen Mineralization in Response to Simulated Climate Change in Subarctic Dwarf Shrub Heath. Oikos 86, 331-343.

Hartley, I.P., Garnett, M.H., Sommerkorn, M., Hopkins, D.W., Fletcher, B.J., Sloan, V.L., Phoenix, G.K., Wookey, P.A., 2012. A potential loss of carbon associated with greater plant growth in the European Arctic. Nature Climate Change 2, 875–879.

Hartley, I.P., Heinemeyer, A., Ineson, P., 2007. Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. Global Change Biology 13, 1761–1770.

Hartley, I.P., Hopkins, D.W., Garnett, M.H., Sommerkorn, M., Wookey, P.A., 2008. Soil microbial respiration in arctic soil does not acclimate to temperature. Ecology Letters 11, 1092–1100.

Hartley, I.P., Hopkins, D.W., Garnett, M.H., Sommerkorn, M., Wookey, P.A., 2009. No evidence for compensatory thermal adaptation of soil microbial respiration in the study of Bradford et al. (2008). Ecology Letters 12, E12–E14.

Hayhoe, K., Wake, C., Anderson, B., Liang, X.Z., Maurer, E., Zhu, J., Bradbury, J., DeGaetano, A., Stoner, A.M., Wuebbles, D., 2008. Regional climate change projections for the Northeast USA. Mitigation and Adaptation Strategies for Global Change 13, 425–436.

Hayhoe, K., Wake, C.P., Huntington, T.G., Luo, L., Schwartz, M.D., Sheffield, J., Wood, E., Anderson, B., Bradbury, J., DeGaetano, A., Troy, T.J., Wolfe, D., 2007. Past and future changes in climate and hydrological indicators in the US Northeast. Climate Dynamics 28, 381–407.

He, Y., Zhang, Y., 2013. Climate change from 1960 to 2000 in the Lancang River Valley, China

Heathwaite, A.L., 2010. Multiple stressors on water availability at global to catchment scales: understanding human impact on nutrient cycles to protect water quality and water availability in the long term. Freshwater Biology 55, 241–257.

Heinemeyer, A., Ineson, P., Ostle, N., Fitter, A.H., 2006. Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. New Phytologist 171, 159–170.

Heinemeyer, A., Wilkinson, M., Vargas, R., Subke, J.-A., Casella, E., Morison, J.I.L., Ineson, P., 2012. Exploring the "overflow tap" theory: linking forest soil CO_2 fluxes and individual mycorrhizosphere components to photosynthesis. Biogeosciences 9, 79–95.

Henry, H.A.L., 2008. Climate change and soil freezing dynamics: historical trends and projected changes. Climatic Change 87, 421–434.

Herrmann, A., Witter, E., 2002. Sources of C and N contributing to the flush in mineralization upon freeze–thaw cycles in soils. Soil Biology and Biochemistry 34, 1495–1505.

Hill, P.W., Farrar, J., Roberts, P., Farrell, M., Grant, H., Newsham, K.K., Hopkins, D.W., Bardgett, R.D., Jones, D.L., 2011. Vascular plant success in a warming Antarctic may be due to efficient nitrogen acquisition. Nature Climate Change 1, 50–53.

Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. Trends in Ecology & Evolution 7, 336–339.

Hobbie, S.E., Schimel, J.P., Trumbore, S.E., Randerson, J.R., 2000. Controls over carbon storage and turnover in high-latitude soils. Global Change Biology 6, 196–210.

Hochachka, P.W., Somero, G.N., 2002. Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, USA.

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485–493.

Hogg, P., Squires, P., Fitter, A.H., 1995. Acidification, nitrogen deposition and rapid vegetational change in a small valley mire in Yorkshire. Biological Conservation 71, 143–153.

Holland, E.A., Parton, W.J., Detling, J.K., Coppock, D.L., 1992. Physiological responses of plant populations to herbivory and their consequences for ecosystem nutrient flow. American Naturalist 14: 685–706.

Holland, J.N., Cheng, W., Crossley, D.A.Jnr., 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia 107, 87–94.

Hooper, D.U., Vitousek, P.M., 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs 68, 121–149.

Hopkins, D.W., Dungait, J.A.J., 2010. Chapter 3 - Soil Microbiology and Nutrient Cycling., in: Soil Microorganisms and Sustainable Crop Production, Eds. Dixon, G. R., Tillson, E. L. Springer, 59 – 80.

Hopkins, D.W., Sparrow, A.D., Elberling, B., Gregorich, E.G., Novis, P.M., Greenfield, L.G., Tilston, E.L., 2006. Carbon, nitrogen and temperature controls on microbial activity in soils from an Antarctic dry valley. Soil Biology and Biochemistry 38, 3130–3140.

Horton, R., Bristow, K.L., Kluitenberg, G.J., Sauer, T.J., 1996. Crop residue effects on surface radiation and energy balance - review. Theoretical and Applied Climatology 54, 27–37.

Houghton, R.A., Davidson, E.A., Woodwell, G.M., 1998. Missing sinks, feedbacks, and understanding the role of terrestrial ecosystems in the global carbon balance. Global Biogeochemical Cycles 12, 25–34.

Hunt, A., Gee, G., 2002. Application of critical path analysis to fractal porous media: comparison with examples from the Hanford site. Advances in Water Resources 25, 129–146.

Ineson, P., Benham, D.G., Poskitt, J., Harrison, A.F., Taylor, K., Woods, C., 1998b. Effects of climate change on nitrogen dynamics in upland soils. 2. A soil warming study. Global Change Biology 4, 153–161.

Ineson, P., Taylor, K., Harrison, A.F., Poskitt, J., Benham, D.G., Tipping, E., Woof, C., 1998a. Effects of climate change on nitrogen dynamics in upland soils. 1. A transplant approach. Global Change Biology 4, 143–152.

Isaksen, M.F., Bak, F., Jørgensen, B.B., 1994. Thermophilic sulfate-reducing bacteria in cold marine sediment. FEMS Microbiology Ecology 14, 1–8.

Jackson, L.E., Calderon, F.J., Steenwerth, K.L., Scow, K.M., Rolston, D.E., 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma 114, 305–317.

Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biology and Biochemistry 41, 2272–2282.

Jarvis, P., Linder, S., 2000. Botany: Constraints to growth of boreal forests. Nature 405, 904–905.

Jarvis, S.C., 1993. Nitrogen cycling and losses from dairy farms. Soil Use and Management 9, 99–104.

Jin, V.L., Evans, R.D., 2007. Elevated CO_2 increases microbial carbon substrate use and nitrogen cycling in Mojave Desert soils. Global Change Biology 13, 452–465.

Johnes, P.J., 1996. Evaluation and management of the impact of land use change on the nitrogen and phosphorus load delivered to surface waters: the export coefficient modelling approach. Journal of Hydrology 183, 323–349.

Johnsson, H., Bergstrom, L., Jansson, P.-E., Paustian, K., 1987. Simulated nitrogen dynamics and losses in a layered agricultural soil. Agriculture, Ecosystems & Environment 18, 333–356.

Jones, C., McConnell, C., Coleman, K., Cox, P., Falloon, P., Jenkinson, D., Powlson, D., 2005. Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. Global Change Biology 11, 154–166.

Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F., Hodge, A., 2005. Dissolved organic nitrogen uptake by plants—an important N uptake pathway? Soil Biology and Biochemistry 37, 413–423.

Jones, D.L., Kielland, K., 2002. Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biology and Biochemistry 34, 209–219.

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytologist 187, 843–858.

Kaiser, E.-A., Kohrs, K., Kücke, M., Schnug, E., Heinemeyer, O., Munch, J.C., 1998. Nitrous oxide release from arable soil: Importance of N-fertilization, crops and temporal variation. Soil Biology and Biochemistry 30, 1553–1563.

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil science 165, 277–304.

Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils 6, 68–72.

Kandeler, F., Kampichler, C., Horak, O., 1996. Influence of heavy metals on the functional diversity of soil microbial communities. Biology and Fertility of Soils 23, 299–306.

Kardol, P., Cregger, M.A., Campany, C.E., Classen, A.T., 2010. Soil ecosystem functioning under climate change: plant species and community effects. Ecology 91, 767–781.

Kardol, P., Martijn Bezemer, T., Van Der Putten, W.H., 2006. Temporal variation in plant–soil feedback controls succession. Ecology Letters 9, 1080–1088.

Keenlyside, N.S., Latif, M., Jungclaus, J., Kornblueh, L., Roeckner, E., 2008. Advancing decadal-scale climate prediction in the North Atlantic sector. Nature 453, 84–88.

Kielland, K., 1994. Amino Acid Absorption by Arctic Plants: Implications for Plant Nutrition and Nitrogen Cycling. Ecology 75, 2373–2383.

Kirschbaum, M.U.F., 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry 27, 753–760.

Kirschbaum, M.U.F., 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? Biogeochemistry 48, 21–51.

Kirschbaum, M.U.F., 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 10, 1870–1877.

Kirschbaum, M.U.F., 2006. The temperature dependence of organic-matter decomposition—still a topic of debate. Soil Biology and Biochemistry 38, 2510–2518.

Kızılkaya, R., Bayraklı, B., 2005. Effects of N-enriched sewage sludge on soil enzyme activities. Applied Soil Ecology 30, 192–202.

Knops, J.M.H., Tilman, D., 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology 81, 88–98.

Knorr, W., Prentice, I.C., House, J.I., Holland, E.A., 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433, 298–301.

Koch, A., 1985. The macroeconomics of bacterial growth. Special Publications of the Society for General Microbiology.

Koponen, H.T., Jaakkola, T., Keinänen-Toivola, M.M., Kaipainen, S., Tuomainen, J., Servomaa, K., Martikainen, P.J., 2006. Microbial communities, biomass, and activities in soils as affected by freeze thaw cycles. Soil Biology and Biochemistry 38, 1861–1871.

Koster, R.D., Dirmeyer, P.A., Guo, Z., Bonan, G., Chan, E., Cox, P., Gordon, C.T., Kanae, S., Kowalczyk, E., Lawrence, D., Liu, P., Lu, C.-H., Malyshev, S., McAvaney, B., Mitchell, K., Mocko, D., Oki, T., Oleson, K., Pitman, A., Sud, Y.C., Taylor, C.M., Verseghy, D., Vasic, R., Xue, Y., Yamada, T., 2004. Regions of Strong Coupling Between Soil Moisture and Precipitation. Science 305, 1138–1140.

Kourtev, P.., Ehrenfeld, J.., Häggblom, M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology and Biochemistry 35, 895–905.

Kuhnert, R., Oberkofler, I., Peintner, U., 2012. Fungal growth and biomass development is boosted by plants in snow-covered soil. Microbial Ecology 64, 79–90.

Ladd, J.N., 1972. Properties of proteolytic enzymes extracted from soil. Soil Biology and Biochemistry 4, 227–237.

Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biology and Biochemistry 4, 19–30.

Lai, C.M., Tabatabai, M.A., 1992. Kinetic parameters of immobilized urease. Soil Biology and Biochemistry 24, 225–228.

Lal, R., 1988. Effect of slope length, slope, gradient, Tillage methods and cropping systems and run-off and soil erosion on a Tropical Alfisol: Preliminary results. International Assoc. Hydrological Sciences Publ 79–88.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Applied and Environmental Microbiology 75, 5111–5120.

Leprince, F., Quiquampoix, H., 1996. Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. European Journal of Soil Science 47, 511–522.

Lesins, G., Duck, T.J., Drummond, J.R., 2012. Surface Energy Balance Framework for Arctic Amplification of Climate Change. Journal of Climate 25, 8277–8288.

Lewis, F.I., McCormick, B.J.J., 2012. Revealing the Complexity of Health Determinants in Resource-poor Settings. American Journal of Epidemiology 176, 1051–1059.

Li, C., Frolking, S., Harriss, R., 1994. Modeling carbon biogeochemistry in agricultural soils. Global Biogeochemical Cycles 8, 237–254.

Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., Li, B., 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177, 706–714.

Lipson, D.A., Schadt, C.W., Schmidt, S.K., 2002. Changes in Soil Microbial Community Structure and Function in an Alpine Dry Meadow Following Spring Snow Melt. Microbial Ecology 43, 307–314.

Lipson, D.A., Schmidt, S.K., 2004. Seasonal Changes in an Alpine Soil Bacterial Community in the Colorado Rocky Mountains. Applied and Environmental Microbiology 70, 2867–2879.

Liski, J., Ilvesniemi, H., Makela, A., Westman, C.J., 1999. CO_2 emissions from soil in response to climatic warming are overestimated - The decomposition of old organic matter is tolerant of temperature. Ambio 28, 171 − 174.

Liu, L., Wu, Y., He, Y., Wu, N., Sun, G., Zhang, L., Xu, J., 2011. Effects of seasonal snow cover on soil nitrogen transformation in alpine ecosystem: a review. The journal of applied ecology 22: 2193-2200.

Liu, X.R., Dong, Y.S., Ren, J.Q., Li, S.G., 2010. Drivers of soil net nitrogen mineralization in the temperate grasslands in Inner Mongolia, China. Nutrient Cycling in Agroecosystems 87, 59–69.

Lõhmus, K., Ivask, M., 1995. Decomposition and nitrogen dynamics of fine roots of Norway spruce *Picea abies* (L.) Karst) at different sites. Plant and Soil 168-169, 89–94.

Lukow, T., Dunfield, P.F., Liesack, W., 2000. Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. FEMS Microbiology Ecology 32, 241–247.

Lundquist, E.., Scow, K.., Jackson, L.., Uesugi, S.., Johnson, C.., 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biology and Biochemistry 31, 1661–1675.

Luo, Y., Su, B.O., Currie, W.S., Dukes, J.S., Finzi, A., Hartwig, U., Hungate, B., Mc Murtie, R.E., Oren, R.A.M., Parton, W.J., 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54, 731–739.

Luo, Y., Wan, S., Hui, D., Wallace, L.L., 2001. Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413, 622–625.

Lützow, M. von, Kögel-Knabner, I., 2009. Temperature sensitivity of soil organic matter decomposition—what do we know? Biology and Fertility of Soils 46, 1–15.

MacDonald, J.A., Dise, N.B., Matzner, E., Armbruster, M., Gundersen, P., Forsius, M., 2002. Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. Global Change Biology 8, 1028–1033.

Mahecha, M.D., Reichstein, M., Carvalhais, N., Lasslop, G., Lange, H., Seneviratne, S.I., Vargas, R., Ammann, C., Arain, M.A., Cescatti, A., Janssens, I.A., Migliavacca, M., Montagnani, L., Richardson, A.D., 2010. Global Convergence in the Temperature Sensitivity of Respiration at Ecosystem Level. Science 329, 838–840.

Maich, L.T., Boltneva, L.I., Sherstyukov, B.G., 2011. Changes of the climate and soil temperature conditions in the second half of the 20th and early 21st century, Lake Baikal. Earth Cryosphere 15, 80 − 90.

Malcolm, G.M., López-Gutiérrez, J.C., Koide, R.T., Eissenstat, D.M., 2008. Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. Global Change Biology 14, 1169–1180.

Mallory, E.B., Griffin, T.S., 2007. Impacts of Soil Amendment History on Nitrogen Availability from Manure and Fertilizer. Soil Science Society of America Journal 71, 964.

Manabe, S., Ploshay, J., Lau, N.-C., 2011. Seasonal Variation of Surface Temperature Change during the Last Several Decades. Journal of Climate 24, 3817–3821.

Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry 35, 453–461.

Marschner, P., Yang, C.-H., Lieberei, R., Crowley, D., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology and Biochemistry 33, 1437–1445.

Martinez, J., Guiraud, G., 1990. A lysimeter study of the effects of a ryegrass catch crop, during a winter wheat/maize rotation, on nitrate leaching and on the following crop. Journal of Soil Science 41, 5–16.

Marx, M.-C., Kandeler, E., Wood, M., Wermbter, N., Jarvis, S.C., 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biology and Biochemistry 37, 35–48.

Matzner, E., Borken, W., 2008. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. European Journal of Soil Science 59, 274–284.

Mawdsley, J.L., Bardgett, R.D., 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. Biology and Fertility of Soils 24, 52–58.

McBean, G., Alekseev, G., Chen, D., Foerland, E., Fyfe, J., Groisman, P.Y., King, R., Melling, H., Vose, R., Whitfield, P.H., 2005. Chapter 2: Arctic Climate: past and present. Arctic Climate Impact Assessment.

McDaniel, M.D., Kaye, J.P., Kaye, M.W., 2013. Increased temperature and precipitation had limited effects on soil extracellular enzyme activities in a post-harvest forest. Soil Biology and Biochemistry 56, 90–98.

McMurtrie, R.E., Medlyn, B.E., Dewar, R.C., 2001. Increased understanding of nutrient immobilization in soil organic matter is critical for predicting the carbon sink strength of forest ecosystems over the next 100 years. Tree Physiology 21, 831–839.

McNamara, N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R., 2003. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Applied Soil Ecology 24, 117–132.

Meals, D.W., Dressing, S.A., Davenport, T.E., 2010. Lag Time in Water Quality Response to Best Management Practices: A Review. Journal of Environment Quality 39, 85-96.

Medina-Roldán, E., Bardgett, R.D., 2011. Plant and soil responses to defoliation: a comparative study of grass species with contrasting life history strategies. Plant and Soil 344, 377–388.

Melillo, J.M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.M., Tang, J., 2011. Soil warming, carbon–nitrogen interactions, and forest carbon budgets. Proceedings of the National Academy of Sciences 108, 9508–9512.

Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil Warming and Carbon-Cycle Feedbacks to the Climate System. Science 298, 2173–2176.

Mellander, P.-E., Bishop, K., Lundmark, T., 2004. The influence of soil temperature on transpiration: a plot scale manipulation in a young Scots pine stand. Forest Ecology and Management 195, 15–28.

Miller, G.R., Baldocchi, D.D., Law, B.E., Meyers, T., 2007. An analysis of soil moisture dynamics using multi-year data from a network of micrometeorological observation sites. Advances in Water Resources 30, 1065–1081.

Miralles, I., Ortega, R., Almendros, G., Gil-Sotres, F., Trasar-Cepeda, C., Leiros, M.C., Soriano, M., 2012. Modifications of organic matter and enzymatic activities in response to change in soil use in semi-arid mountain ecosystems (southern Spain). European Journal of Soil Science 63, 272–283.

Misson, L., Baldocchi, D.D., Black, T.A., Blanken, P.D., Brunet, Y., Curiel Yuste, J., Dorsey, J.R., Falk, M., Granier, A., Irvine, M.R., Jarosz, N., Lamaud, E., Launiainen, S., Law, B.E., Longdoz, B., Loustau, D., McKay, M., Paw U, K.T., Vesala, T., Vickers, D., Wilson, K.B., Goldstein, A.H., 2007. Partitioning forest carbon fluxes with overstory and understory

eddy-covariance measurements: A synthesis based on FLUXNET data. Agricultural and Forest Meteorology 144, 14–31.

Monreal, C.M., Bergstrom, D.W., 2000. Soil enzymatic factors expressing the influence of land use, tillage system and texture on soil biochemical quality. Canadian Journal of Soil Science 80, 419–428.

Moore, T.R., Dalva, M., 2000. Some Controls on the Release of Dissolved Organic Carbon By Plant Tissues and Soils. Soil Science 166, 38–47.

Moore-Kucera, J., Dick, R.P., 2008. PLFA Profiling of Microbial Community Structure and Seasonal Shifts in Soils of a Douglas-fir Chronosequence. Microbial Ecology 55, 500–511.

Moorhead, D.L., Sinsabaugh, R.L., 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. Applied Soil Ecology 14, 71–79.

Morris, J.G., 1974. A Biologist's Physical Chemistry, 2nd Revised edition. Hodder Arnold, London.

Moyo, C.C., Kissel, D.E., Cabrera, M.L., 1989. Temperature effects on soil urease activity. Soil Biology and Biochemistry 21, 935–938.

Mubarak, I., Mailhol, J.C., Angulo-Jaramillo, R., Ruelle, P., Boivin, P., Khaledian, M., 2009. Temporal variability in soil hydraulic properties under drip irrigation. Geoderma 150, 158–165.

Murphy, J.M., Sexton, D.M.H., Jenkins, G.J., Boorman, P.M., Booth, B.B.B., Brown, C.C., Clark, R.T., Collins, M., Harris, G.R., Kendon, E.J., Betts, R.A., Brown, S.J., Howard, T.P., Humphery, K.A., McCarthy, M.P., McDonald, R.E., Stephens, A., Wallace, C., Warren, R., Wilby, R., Wood, R.A., 2009. UK Climate Projections Science Report: Climate change projections. Met Office Hadley Centre, Exeter.

Murty, D., Kirschbaum, M.U.F., Mcmurtrie, R.E., Mcgilvray, H., 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? a review of the literature. Global Change Biology 8, 105–123.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Enzymes in the environment, Eds: Burns, R.G., Dick, R.P. Marcel Dekker, New York 1–33.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M., Högberg, P., 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914–916.

Näsholm, T., Huss-Danell, K., Hogberg, P., 2000. Uptake of Organic Nitrogen in the Field by Four Agriculturally Important Plant Species. Ecology 81, 1155.

Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. New Phytologist 182, 31–48.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917.

Neilsen, C.B., Groffman, P.M., Hamburg, S.P., Driscoll, C.T., Fahey, T.J., Hardy, J.P., 2001. Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. Soil Science Society of America Journal 65, 1723–1730.

Nicolardot, B., Fauvet, G., Cheneby, D., 1994. Carbon and nitrogen cycling through soil microbial biomass at various temperatures. Soil Biology and Biochemistry 26, 253–261.

Norby, R.J., Cotrufo, M.F., Ineson, P., O'Neill, E.G., Canadell, J.G., 2001. Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia 127, 153–165.

Oechel, W.C., Vourlitis, G.L., Hastings, S.J., Zulueta, R.C., Hinzman, L., Kane, D., 2000. Acclimation of ecosystem CO_2 exchange in the Alaskan Arctic in response to decadal climate warming. Nature 406, 978–981.

Olsson, L., Eklundh, L., 1994. Fourier Series for analysis of temporal sequences of satellite sensor imagery. International Journal of Remote Sensing 15, 3735–3741.

Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303–310.

Oztas, T., Fayetorbay, F., 2003. Effect of freezing and thawing processes on soil aggregate stability. CATENA 52, 1–8.

Pagliai, M., Vignozzi, N., Pellegrini, S., 2004. Soil structure and the effect of management practices. Soil and Tillage Research 79, 131–143.

Parfitt, R.L., Yeates, G.W., Ross, D.J., Schon, N.L., Mackay, A.D., Wardle, D.A., 2010. Effect of fertilizer, herbicide and grazing management of pastures on plant and soil communities. Applied Soil Ecology 45, 175–186.

Patil, R.H., Laegdsmand, M., Olesen, J.E., Porter, J.R., 2010. Effect of soil warming and rainfall patterns on soil N cycling in Northern Europe. Agriculture, Ecosystems & Environment 139, 195–205.

Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Guillaumaud, N., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J.I., Roux, X.L., 2006. Effects of management regime and plant species on the enzyme activity and genetic structure of N-fixing, denitrifying and nitrifying bacterial communities in grassland soils. Environmental Microbiology 8, 1005–1016.

Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J.I., Richaume, A., Le Roux, X., 2005. Effects of grazing on microbial functional groups involved in soil N dynamics. Ecological Monographs 75, 65–80.

Paul, E.A., 2007. Soil microbiology, ecology, and biochemistry. Academic Press.

Pelat, C., Boëlle, P.-Y., Cowling, B.J., Carrat, F., Flahault, A., Ansart, S., Valleron, A.-J., 2007. Online detection and quantification of epidemics. BMC Medical Informatics and Decision Making 7, 29.

Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y., Megonigal, J.P., Olsrud, M., Ryan, M.G., Wan, S., 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytologist 162, 311–322.

Perucci, P., 1992. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biology and Fertility of Soils 14, 54–60.

Peterjohn, W.T., Melillo, J.M., Steudler, P.A., Newkirk, K.M., Bowles, F.P., Aber, J.D., 1994. Responses of Trace Gas Fluxes and N Availability to Experimentally Elevated Soil Temperatures. Ecological Applications 4, 617.

Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, J.P., Lee, J.A., 2003. Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. Global Change Biology 9, 1309–1321.

Pienkowski, M.W., Watkinson, A.R., Kerby, G., Akinola, M.O., Thompson, K., Buckland, S.M., 1998. Soil seed bank of an upland calcareous grassland after 6 years of climate and mangement manipulations. Journal of Applied Ecology 35, 544–552.

Piñeiro, G., Oesterheld, M., Batista, W.B., Paruelo, J.M., 2006. Opposite changes of whole-soil vs. pools C : N ratios: a case of Simpson's paradox with implications on nitrogen cycling. Global Change Biology 12, 804–809.

Ping, C.L., Michaelson, G.J., Jorgenson, M.T., Kimble, J.M., Epstein, H., Romanovsky, V.E., Walker, D.A., 2008. High stocks of soil organic carbon in the North American Arctic region. Nature Geoscience 1, 615–619.

Plummer, D.A., Caya, D., Frigon, A., Côté, H., Giguère, M., Paquin, D., Biner, S., Harvey, R., De Elia, R., 2006. Climate and Climate Change over North America as Simulated by the Canadian RCM. Journal of Climate 19, 3112–3132.

Post, W.M., Emanuel, W.R., Zinke, P.J., Stangenberger, A.G., 1982. Soil carbon pools and world life zones. , Published online: 08 July 1982; | doi:10.1038/298156a0 298, 156–159.

Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6, 317–327.

Potthoff, M., Steenwerth, K.L., Jackson, L.E., Drenovsky, R.E., Scow, K.M., Joergensen, R.G., 2006. Soil microbial community composition as affected by restoration practices in California grassland. Soil Biology and Biochemistry 38, 1851–1860.

Power, S.A., Collins, C.M., 2010. Use of *Calluna vulgaris* to detect signals of nitrogen deposition across an urban–rural gradient. Atmospheric Environment 44, 1772–1780.

Qian, B., Gregorich, E.G., Gameda, S., Hopkins, D.W., Wang, X.L., 2011. Observed soil temperature trends associated with climate change in Canada. Journal of Geophysical Research 116, 1984 – 2012.

Quiquampoix, H., Staunton, S., Baron, M.-H., Ratcliffe, R.G., 1993. Interpretation of the pH dependence of protein adsorption on clay mineral surfaces and its relevance to the understanding of extracellular enzyme activity in soil. Colloids and Surfaces A: Physicochemical and Engineering Aspects 75, 85–93.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahn, C.R., Bending, G.D., Lillywhite, R.D., Turner, M.K., 1999. Chemical characterisation of vegetable and arable crop residue materials: a comparison of methods. Journal of the Science of Food and Agriculture 79, 1715–1721.

Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44, 81–99.

Rees, R.M., Farrelly, D., 1990. Biological Electron Transfer, in: The Enzymes: Mechanisms of Catalysis. Academic Press, pp. 38 – 92.

Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M.H., Naeem, S., Trost, J., 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440, 922–925.

Reichstein, M., Beer, C., 2008. Soil respiration across scales: The importance of a model–data integration framework for data interpretation. Journal of Plant Nutrition and Soil Science 171, 344–354.

Reiners, W.A., Bouwman, A.F., Parsons, W.F.J., Keller, M., 1994. Tropical Rain Forest Conversion to Pasture: Changes in Vegetation and Soil Properties. Ecological Applications 4, 363–377.

Rejsek, K., Formanek, P., Pavelka, M., 2008. Estimation of protease activity in soils at low temperatures by casein amendment and with substitution of buffer by demineralized water. Amino Acids 35, 411–417.

Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281–2291.

Ribolzi, O., Patin, J., Bresson, L.M., Latsachack, K.O., Mouche, E., Sengtaheuanghoung, O., Silvera, N., Thiébaux, J.P., Valentin, C., 2011. Impact of slope gradient on soil surface features and infiltration on steep slopes in northern Laos. Geomorphology 127, 53–63.

Rishbeth, J., 1978. Effects of soil temperature and atmosphere on growth of *Armillaria* rhizomorphs. Transactions of the British Mycological Society 70, 213–220.

Rogers, D.J., Hay, S.I., Packer, M.J., 1996. Predicting the distribution of tsetse flies in West Africa using temporal Fourier processed meteorological satellite data. Annals of Tropical Medicine and Parasitology 90, 225–241.

Rollinson, C.R., Kaye, M.W., 2012. Experimental warming alters spring phenology of certain plant functional groups in an early successional forest community. Global Change Biology 18, 1108–1116.

Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., Menzel, A., Root, T.L., Estrella, N., Seguin, B., Tryjanowski, P., Liu, C., Rawlins, S., Imeson, A., 2008. Attributing physical and biological impacts to anthropogenic climate change. Nature 453, 353–357.

Rousk, J., Frey, S.D., Bååth, E., 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. Global Change Biology 18, 3252–3258.

Roux, X.L., Bardy, M., Loiseau, P., Louault, F., 2003. Stimulation of soil nitrification and denitrification by grazing in grasslands: do changes in plant species composition matter? Oecologia 137, 417–425.

Röver, M., Heinemeyer, O., Kaiser, E.-A., 1998. Microbial induced nitrous oxide emissions from an arable soil during winter. Soil Biology and Biochemistry 30, 1859–1865.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., Gcte-News, 2001. A Meta-Analysis of the Response of Soil Respiration, Net Nitrogen Mineralization, and Aboveground Plant Growth to Experimental Ecosystem Warming. Oecologia 126, 543–562.

Rustad, L.E., Huntington, T.G., Boone, R.D., 2000. Controls on soil respiration: Implications for climate change. Biogeochemistry 48, 1–6.

Ryan, M.G., Law, B.E., 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73, 3–27.

Saiya-Cork, K., Sinsabaugh, R., Zak, D., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology and Biochemistry 34, 1309–1315.

Sardans, J., Peñuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology and Biochemistry 37, 455–461.

Sardans, J., Peñuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology 39, 223–235.

Scanlon, B.R., Reedy, R.C., Stonestrom, D.A., Prudic, D.E., Dennehy, K.F., 2005. Impact of land use and land cover change on groundwater recharge and quality in the southwestern US. Global Change Biology 11, 1577–1593.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Schimel, J.P., Bennett, J., 2004. Nitrogen Mineralization: Challenges of a Changing Paradigm. Ecology 85, 591–602.

Schimel, J.P., Bilbrough, C., Welker, J.A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biology and Biochemistry 36, 217 – 227.

Schimel, J.P., Chapin, F.S., 1996. Tundra Plant Uptake of Amino Acid and NH_4^+ Nitrogen in Situ: Plants Complete Well for Amino Acid N. Ecology 77, 2142–2147.

Schimel, J.P., Clein, J.S., 1996. Microbial response to freeze-thaw cycles in tundra and taiga soils. Soil Biology and Biochemistry 28, 1061–1066.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35, 549–563.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20.

Schmidhuber, J., Tubiello, F.N., 2007. Global food security under climate change. Proceedings of the National Academy of Sciences 104, 19703–19708.

Scholefield, D., Lockyer, D.R., Whitehead, D.C., Tyson, K.C., 1991. A model to predict transformations and losses of nitrogen in UK pastures grazed by beef cattle. Plant and Soil 132, 165–177.

Schöll, L.V., Dam, A.M.V., Leffelaar, P.A., 1997. Mineralisation of nitrogen from an incorporated catch crop at low temperatures: experiment and simulation. Plant and Soil 188, 211–219.

Schuur, E.A.G., Bockheim, J., Canadell, J.G., Euskirchen, E., Field, C.B., Goryachkin, S.V., Hagemann, S., Kuhry, P., Lafleur, P.M., Lee, H., Mazhitova, G., Nelson, F.E., Rinke, A., Romanovsky, V.E., Shiklomanov, N., Tarnocai, C., Venevsky, S., Vogel, J.G., Zimov, S.A., 2008. Vulnerability of Permafrost Carbon to Climate Change: Implications for the Global Carbon Cycle. BioScience 58, 701–714.

Schuur, E.A.G., Vogel, J.G., Crummer, K.G., Lee, H., Sickman, J.O., Osterkamp, T.E., 2009. The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. Nature 459, 556–559.

Shackle, V.J., Freeman, C., Reynolds, B., 2000. Carbon supply and the regulation of enzyme activity in constructed wetlands. Soil Biology and Biochemistry 32, 1935–1940.

Sharma, S., Szele, Z., Schilling, R., Munch, J.C., Schloter, M., 2006. Influence of Freeze-Thaw Stress on the Structure and Function of Microbial Communities and Denitrifying Populations in Soil. Applied and Environmental Microbiology 72, 2148–2154.

Shaw, M.R., Harte, J., 2001. Response of nitrogen cycling to simulated climate change: differential responses along a subalpine ecotone. Global Change Biology 7, 193–210.

Sherry, R.A., Zhou, X., Gu, S., Arnone, J.A., Schimel, D.S., Verburg, P.S., Wallace, L.L., Luo, Y., 2007. Divergence of reproductive phenology under climate warming. Proceedings of the National Academy of Sciences 104, 198–202.

Silver, W.L., Neff, J., McGroddy, M., Veldkamp, E., Keller, M., Cosme, R., 2000. Effects of Soil Texture on Belowground Carbon and Nutrient Storage in a Lowland Amazonian Forest Ecosystem. Ecosystems 3, 193–209.

Simmelsgaard, S.E., 1998. The effect of crop, N-level, soil type and drainage on nitrate leaching from Danish soil. Soil Use and Management 14, 30–36.

Singh, B.K., Tate, K., Thomas, N., Ross, D., Singh, J., 2011. Differential effect of afforestation on nitrogen-fixing and denitrifying communities and potential implications for nitrogen cycling. Soil Biology and Biochemistry 43, 1426–1433.

Sinsabaugh, 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular Enzyme Activities and Soil Organic Matter Dynamics for Northern Hardwood Forests receiving Simulated Nitrogen Deposition. Biogeochemistry 75, 201–215.

Sinsabaugh, R.L., Moorhead, D.L., 1994. Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26, 1305–1311.

Sjögersten, S., Wookey, P.A., 2002. Climatic and resource quality controls on soil respiration across a forest–tundra ecotone in Swedish Lapland. Soil Biology and Biochemistry 34, 1633–1646.

Smolders, A.J.P., Lucassen, E.C.H.E.T., Bobbink, R., Roelofs, J.G.M., Lamers, L.P.M., 2010. How nitrate leaching from agricultural lands provokes phosphate eutrophication in groundwater fed wetlands: the sulphur bridge. Biogeochemistry 98, 1–7.

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65–105.

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), 2007. Climate Change 2007: The Physical Science Basis. IPPC Reports Cambridge University Press, Cambridge.

Song, F., Han, X., Zhu, X., Herbert, S.J., 2012. Response to water stress of soil enzymes and root exudates from drought and non-drought tolerant corn hybrids at different growth stages. Canadian Journal of Soil Science 92, 501–507.

Sørensen, L.H., 1972. Stabilization of Newly Formed Amino Acid Metabolites in Soil. Soil Science 114: 5-11

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218–221.

Steinweg, J.M., Dukes, J.S., Wallenstein, M.D., 2012. Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. Soil Biology and Biochemistry 55, 85–92.

Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A., Tanaka, D.L., 2008. Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology and Biochemistry 40, 2722–2728.

Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of Nitrogen Deposition on the Species Richness of Grasslands. Science 303, 1876–1879.

Stiehl-Braun, P.A., Hartmann, A.A., Kandeler, E., Buchmann, N., Niklaus, P.A., 2011. Interactive effects of drought and N fertilization on the spatial distribution of methane assimilation in grassland soils. Global Change Biology 17, 2629–2639.

Stotzky, G., Norman, A.G., 1961. Factors limiting microbial activities in soil. Archives of Microbiology 40, 370–382.

Strömgren, M., Linder, S., 2002. Effects of nutrition and soil warming on stemwood production in a boreal Norway spruce stand. Global Change Biology 8, 1194–1204.

Strum, M., Schimel, J., Michaelson, G., Welker, J.M., Oberbauer, S.F., Liston, G.E., Fahnestock, J., Romanovsky, V.E., 2005. Winter Biological Processes Could Help Convert Arctic Tundra to Shrubland. BioScience 55, 17–26.

Tape, K., Sturm, M., Racine, C., 2006. The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. Global Change Biology 12, 686–702.

Teepe, R., Brumme, R., Beese, F., 2000. Nitrous oxide emissions from frozen soils under agricultural, fallow and forest land. Soil Biology and Biochemistry 32, 1807–1810.

Teepe, R., Brumme, R., Beese, F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biology and Biochemistry 33, 1269–1275.

Thomas, R.Q., Canham, C.D., Weathers, K.C., Goodale, C.L., 2010. Increased tree carbon storage in response to nitrogen deposition in the US. Nature Geoscience 3, 13–17.

Thompson, D.W.J., Wallace, J.M., Jones, P.D., Kennedy, J.J., 2009. Identifying signatures of natural climate variability in time series of global-mean surface temperature: methodology and insights. Journal of Climate 22, 6120–6141.

Thorup-Kristensen, K., Magid, J., Jensen, L.S., 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in Agronomy. 227–302.

Tracy, B.F., Frank, D.A., 1998. Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park. Oecologia 114, 556–562.

Trasar-Cepeda, C., Gil-Sotres, F., Leirós, M.C., 2007. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. Soil Biology and Biochemistry 39, 311–319.

Trenberth, K.E., 2011. Changes in precipitation with climate change. Climate Research 47, 123.

Tscherko, D., Hammesfahr, U., Marx, M.-C., Kandeler, E., 2004. Shifts in rhizosphere microbial communities and enzyme activity of *Poa alpina* across an alpine chronosequence. Soil Biology and Biochemistry 36, 1685–1698.

Tucker, C.L., Bell, J., Pendall, E., Ogle, K., 2013. Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? Global Change Biology 19, 252–263.

Turner, M.M., Henry, H.A.L., 2010. Net nitrogen mineralization and leaching in response to warming and nitrogen deposition in a temperate old field: the importance of winter temperature. Oecologia 162, 227–236.

Ussiri, D.A.N., Lal, R., 2009. Long-term tillage effects on soil carbon storage and carbon dioxide emissions in continuous corn cropping system from an alfisol in Ohio. Soil and Tillage Research 104, 39–47.

Van Dijk, H.F.G., Roelofs, J.G.M., 1988. Effects of excessive ammonium deposition on the nutritional status and condition of pine needles. Physiologia Plantarum 73, 494–501.

Vanhala, P., Karhu, K., Tuomi, M., Sonninen, E., Jungner, H., Fritze, H., Liski, J., 2007. Old soil carbon is more temperature sensitive than the young in an agricultural field. Soil Biology and Biochemistry 39, 2967–2970.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7, 737–750.

Walegur, M.T., Nelson, F.E., 2003. Permafrost distribution in the Appalacian Highlands, northeastern USA. Proceedings of the Eigth International Conference on Permafrost.

Wallenstein, M.D., Weintraub, M.N., 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biology and Biochemistry 40, 2098–2106.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Putten, W.H. van der, Wall, D.H., 2004. Ecological Linkages Between Aboveground and Belowground Biota. Science 304, 1629–1633.

Wedin, D.A., Tilman, D., 1990. Species effects on nitrogen cycling: a test with perennial grasses. Oecologia 84, 433–441.

Weedon, J., Kowalchuk, G., Aerts, R., Van Hal, J., Van Logtestijn, R., Taş, N., F. M. Röling, W., M. van Bodegom, P., 2012. Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. Global Change Biology 18, 138–150.

Weih, M., Karlsson, P.S., 2001. Growth response of Mountain birch to air and soil temperature: is increasing leaf-nitrogen content an acclimation to lower air temperature? New Phytologist 150, 147–155.

Weintraub, M.N., Schimel, J.P., 2003. Interactions between Carbon and Nitrogen Mineralization and Soil Organic Matter Chemistry in Arctic Tundra Soils. Ecosystems 6, 129–143.

Weiss, S.B., 1999. Cows, cars, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. Conservation Biology 13, 1476–1486.

Westover, K.M., Kennedy, A.C., Kelley, S.E., 1997. Patterns of Rhizosphere Microbial Community Structure Associated with Co-Occurring Plant Species. The Journal of Ecology 85, 863.

Wheatley, R., Ritz, K., Griffiths, B., 1990. Microbial biomass and mineral N transformations in soil planted with barley, ryegrass, pea or turnip. Plant and Soil 127, 157–167.

Wipf, S., Stoeckli, V., Bebi, P., 2009. Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. Climatic Change 94, 105–121.

Wolkovich, E.M., Cook, B.I., Allen, J.M., Crimmins, T.M., Betancourt, J.L., Travers, S.E., Pau, S., Regetz, J., Davies, T.J., Kraft, N.J.B., Ault, T.R., Bolmgren, K., Mazer, S.J., McCabe, G.J., McGill, B.J., Parmesan, C., Salamin, N., Schwartz, M.D., Cleland, E.E., 2012. Warming experiments underpredict plant phenological responses to climate change. Nature 485: 494-497.

Wood, R., 2008. Climate change: Natural ups and downs. Nature Reports Climate Change 61–62.

Wu, H., Dannenmann, M., Wolf, B., Han, X.G., Zheng, X., Butterbach-Bahl, K., 2012. Seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia. Ecosphere 3, art34.

Wyland, L.J., Jackson, L.E., Chaney, W.E., Klonsky, K., Koike, S.T., Kimple, B., 1996. Winter cover crops in a vegetable cropping system: Impacts on nitrate leaching, soil water, crop yield, pests and management costs. Agriculture, Ecosystems & Environment 59, 1–17.

Xu, N., Saiers, J.E., 2010. Temperature and Hydrologic Controls on Dissolved Organic Matter Mobilization and Transport within a Forest Topsoil. Environmental Science & Technology 44, 5423–5429.

Young, I.., Ritz, K., 2000. Tillage, habitat space and function of soil microbes. Soil and Tillage Research 53, 201–213.

Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Mooney, H.A., Field, C.B., 2003. Additive effects of simulated climate changes, elevated CO_2, and nitrogen deposition on grassland diversity. Proceedings of the National Academy of Sciences 100, 7650–7654.

Zhang, K., Kimball, J.S., Mu, Q., Jones, L.A., Goetz, S.J., Running, S.W., 2009. Satellite based analysis of northern ET trends and associated changes in the regional water balance from 1983 to 2005. Journal of Hydrology 379, 92–110.

Zhao, D., Li, F., Wang, R., Yang, Q., Ni, H., 2012. Effect of soil sealing on the microbial biomass, N transformation and related enzyme activities at various depths of soils in urban area of Beijing, China. Journal of Soils and Sediments 12, 519–530.

Zhou, X., Chen, C., Wang, Y., Xu, Z., Han, H., Li, L., Wan, S., 2013. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. Science of the Total Environment 444, 552–558.

Zhu, B., Cheng, W., 2011. Constant and diurnally-varying temperature regimes lead to different temperature sensitivities of soil organic carbon decomposition. Soil Biology and Biochemistry 43, 866–869.

Appendix 1: How do enzymes catalysing soil nitrogen transformations respond to temperature change?