DYNAMICS AND APPLICATION OF HOT WATER EXTRACTABLE CARBON AND NITROGEN IN NEW ZEALAND PASTORAL SYSTEMS

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science
in
Agricultural Science

at Massey University, Manawatū,
New Zealand

Nicola Wilson

2024

Abstract

Changes in soil carbon (C) and nitrogen (N) stocks under agricultural land have the potential to reduce greenhouse gas emissions and improve soil health. The health of soils is dependent on the organic matter cycle, biological community and its functions and activity. While current tests used for these parameters are often expensive and time-consuming, hot water extractable C (HWEC) and hot water extractable N (HWEN) measures have been promoted as sensitive and informative tests of labile and microbial C and N. The consensus of studies to date, is that factors such as soil type heavily influence the HWEC and HWEN measures.

However, information on how other characteristics typical of New Zealand farms, such as highly variable topography, different phosphorus (P) fertiliser history, grazing management and stocking densities and crop establishment affect HWEC and HWEN measures is lacking. Furthermore, the responsiveness of these tests to land management changes within a farm system has yet to be fully investigated.

The first study investigated changes in soil HWEC and HWEN pools with varying long term P fertiliser history, slope, aspect and sheep stocking rates. Significant differences in HWEC were not observed between high P fertiliser farmlets and low P fertiliser farmlets, only between the low P fertiliser farmlet and the farmlet which had not received P fertiliser since 1980. Significantly lower HWEC and HWEN concentrations were observed on high, erosion prone slopes when compared to medium and low slopes. The prediction of HWEC using soil physicochemical factors, taking into account P fertiliser history and slope, has advanced our understanding of factors influencing the HWEC fraction. These factors were organic matter, effective CEC, microporosity and sulfate sulfur (S).

The second study investigated the impact of adaptive, high instantaneous (number of animals per unit area at a specific point in time) stocking density grazing on HWEC and HWEN. This study found adaptive grazing did not increase either the HWEC or HWEN pool.

The final study investigated the impact of summer crop establishment using herbicide and power harrowing and herbicide and no-till methods on HWEC and HWEN. Results suggest there was no

detrimental effects on HWEC and HWEN associated with no-till, and power-harrowing yielded a similar result after a period of soil recovery.

Overall, HWEC and HWEN proved to be highly indicative of the total C and N. None of the changes investigated in this study had major effects on the quantity of total C and N pools present as HWEC or HWEN.

Acknowledgements

This research would truly not have been possible without the support of many people. This brief acknowledgement section cannot fully convey the depth of my gratitude for their contributions.

Firstly, I would like to express my deepest gratitude to my supervisors; Dr Lucy Burkitt, for all her work keeping me on track, the time which she took to ensure my work was the best I could achieve and being an incredible inspiration with all her work and contributions in the soil science sector. Dr Alec Mackay, who was always available to discuss ideas, and guided me throughout this research. Dr Maria Minor and Dr Paramsothy Jeyakumar for dedicating their time and generously supporting me. This thesis would not have been possible without such an incredible team by my side throughout the process, and words cannot express my thanks for their unending support and patience.

I would also like to thank Dr Sarah Rosanowski who was always happy to help and advance my limited statistical analysis knowledge. To Emma Noakes and Rachel Cole, Eru Tait-Jamieson and Ben Carter, thank you for helping with all the practical aspects of the sampling done in this thesis, and for providing me with any extra information I needed.

This research was made possible through generous funding from the Fertiliser Association of New Zealand. Thank you for having faith in me to carry out this research. I am also deeply grateful for the support provided by the Brian Aspin Memorial Scholarship and the C V Fife Scholarship. Without these contributions, this work would not have been achievable.

I would also like to thank the farmers whose land has been utilized in the projects included in this thesis. The time and sacrifices required to support the continued improvement of New Zealand's agriculture industry are greatly appreciated.

Finally, to my friends and family for motivating me to complete my Masters degree, thank you for always being a source of motivation and support. To my older sister, Penelope, for being my rock and showing me that with hard work, dedication and a lot of stubbornness I can achieve anything. To my mum, for dedicating so much of her time and effort to allow me to be who I am today. To my dad, who always showed an interest no matter how much I rambled about things he didn't understand.

Your continued support and belief in me means the world.

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Chapter 1

Introduction

This thesis is formatted as three scientific papers, each presented as a chapter. As such, sections of the methodology, specifically chemical analysis, are repeated in each chapter.

The sequestration of carbon (C) and nitrogen (N) in soils and reduction of emissions has large implications in reducing greenhouse gas (GHG) and climate change effects (Lal et al., 2021). Agriculture in New Zealand (NZ) accounts for a large proportion of the countries primary production and land use (Caradus et al., 2023). As such, optimizing agricultural production is important for a multitude of reasons including food security, decreasing environmental impacts, such as GHG emissions as, well as securing exports and the nations' economy on an international scale. With 39% of NZ's land being occupied by pastoral farming there is a large capacity for beneficial changes within agriculture to have major impacts on environmental improvements (Cleverly et al., 2020; Smith et al., 2019; Stats NZ, 2023; Whitehead et al., 2018).

Organic matter (OM) makes up only a small percentage of a soil, but is central to all of the soil properties, functions and services (Dominati et al., 2010). Small changes in soil organic C stocks have been shown to have significant impacts on atmospheric C levels (Smith et al., 2019; Whitehead et al., 2018). Increasing soil C stocks through sequestration presents an opportunity both nationally and internationally (Smith et al., 2019; Whitehead et al., 2018). Initiatives such as the '4 per 1000 initiative: Soils for Food Security and Climate' have been introduced in recent years (4 per 1000, 2022; Whitehead et al., 2018). This initiative aims to increase soil C stocks by 0.4% (4‰) per year. Furthermore, the recent State of Environment report emphasizes the measurement of soil C within NZ agricultural soils as a method for reduced environmental impacts (Ministry for the Environment & Stats NZ, 2022). It has been well recognised that by implementing cost effective improvements to agricultural practices there is great potential for increasing soil C stocks.

Recently, there has been increased interest in the adoption of agricultural practices which are

considered ‘regenerative’ both within NZ and globally. These practices aim to improve soil health, through improved ecosystem function, fertility, soil structure and overall reduction of negative environmental impacts (Grelet et al., 2021; Stanley et al., 2024). These practices view the system as a ‘living system’ and apply adaptable practices which are tailored to the context and need of the farm and surrounding ecosystems (Grelet et al., 2021). Adaptive tillage, which reduces soil disturbance, adaptive grazing practices such as adaptive multi-paddock grazing and diverse pastures are three strategies promoted as part of ‘regenerative’ farming practice. Many studies have shown that alternative tillage and grazing strategies can potentially improve SOM, microbial biomass and C and N sequestration (Abagandura et al., 202; Fonteyne et al., 2022; Mosier et al., 2021; Rutledge et al., 2017; Stanley et al., 2024; Whitehead et al., 2018). However, the capacity of these practices to improve soil C has been questioned due to the current management of NZ pastoral farms being somewhat ‘regenerative’. Many NZ farming systems do not incorporate management strategies which are highly destructive to soil, and a diversity of pasture through the use of legumes and other species within swards is already relatively common (Grelet et al., 2021; Rowarth et al., 2020).

Studies have found that conservational tillage practices with the goal of reducing soil disturbance are beneficial to soil structure, microbial biomass and C and N sequestration within soil. No-till methods such as direct drilling reduces aeration of soil, preventing exposure of soil micro-organisms to additional oxygen, which drives microbial decomposition of OM and carbon dioxide (CO_2) losses (Chahal et al., 2021; Colombi et al., 2018; Mehra et al., 2018). Additionally, detrimental effects of soil disturbance on labile N have been observed, with increased nitrification leading to nitrous oxide (N_2O) losses (Grave et al., 2018; Liu et al., 2018; Yoo et al., 2016; Yuan et al., 2024; Zurovec et al., 2017). Studies have also shown that the physical disturbance of soil resulting from tillage negatively impacts soil structure (Schülter et al., 2018). This exposes the aggregate-protected OM to microbial decomposition, increasing respiration and CO_2 losses (Botenelli et al., 2010; Chahal et al., 2021; Liu et al., 2017; Mehra et al., 2018; Saggar et al., 2004; Zhang et al., 2019).

Evidence suggests that increasing species diversity within a pasture sward has benefits for soil C

and N sequestration, pasture production and animal productivity (Bracken et al., 2020; Cummins et al., 2021; Fornara & Tilman, 2008; Jing et al., 2017; Laubach et al., 2023; McNally et al., 2015). Greater root biomass featuring roots capable of reaching greater depths in the soil profile increases the release of organic and inorganic compounds through rhizodeposition, microbial biomass and overall greater quantities of labile C (Beare et al., 2014; Fornara & Tilman, 2008; McNally et al., 2015; Rutledge et al., 2017; Wall et al., 2021). Studies have also found diverse pastures to be beneficial to N cycling in soils. The ability of deep-rooted species to uptake mineral N from greater depths reduces the potential for N leaching (Bracken et al., 2020; Cummins et al., 2021; Jaramillo et al., 2021; Laubach et al., 2023; Romera et al., 2017).

Additionally, inclusion of legume species capable of biological N fixation reduces the requirements for synthetic N fertiliser (Bracken et al., 2020; Cooledge et al., 2022). However, with the diversity existing in many current NZ pastures, there is an argument that the improvements gained by further diversification may be minimal in many systems (Rowarth et al., 2020).

While mismanagement of grazing livestock can be destructive to soil structure, C and N cycling and microbial communities, optimal grazing has been shown to lead to improvements in soil health (Abagandura et al., 2024; Mosier et al., 2021; Stanley et al., 2024). Adaptive multi-paddock grazing aims to increase soil C and N incorporation through plant litter and subsequently improve microbial biomass and activity, when compared to more conventional grazing practices (Abagandura et al., 2024; Mosier et al., 2021). By utilizing high instantaneous stocking densities, short grazing periods and long recovery periods, preferential grazing is reduced under adaptive grazing due to greater trampling of OM occurs, and urine an~~d dung patche~~s are more evenly distributed (Abagandura et al., 2024; Mosier et al., 2021; Stanley et al., 2024). However, studies have questioned whether these benefits outweigh the potential for greater damage to soil structure, and the resulting detrimental effects on labile C, N and microbial biomass (Abagandura et al., 2024; Khatri-Chhertri et al., 2024).

Phosphorus (P) fertiliser inputs have a large influence on the C and N cycle in grazed pasture in NZ. Applied to pasture, P fertiliser stimulates legume growth and biological N fixation, which

increases grass growth (Parfitt et al., 2005; Saggar et al., 1997). As the amount of P fertiliser applied increases, so does pasture growth and the amount of organic C and N cycling above and below ground within the grazed pasture (Parfitt et al., 2005; Saggar et al., 1997). Bilotto et al. (2022) found the amount of C and N cycling in grazed pasture that had received no fertiliser in the last 40 years was only a fraction of the C and N cycling observed in pasture that had received annual P fertilizer applications over the same period. However, those differences had not translated to a difference in the soils organic C or N stocks. Previous NZ studies (Condron et al., 2012; Schipper et al., 2011) also found little difference in soil organic C stocks in long-established permanent pastures, despite large differences in pasture growth due to P fertiliser inputs. The assumption is made that NZ soils are generally approaching saturation, with regards to organic C storage. Therefore, the additional C cycling through microbial activity is either respired or lost as dissolved organic C. Adding a measure of the labile organic C fraction and size of microbial biomass, alongside the existing measure of total C and N might provide additional insights into the factors governing the cycling of C and N in a grazed system.

Monitoring the status of the labile C and N pools is important for several different reasons. Increased soil C is effective in mitigating CO_2 emissions, while higher C levels sourced from microbial biomass indicates better microbial health of the soil (Voltr et al., 2021). The labile pool of C is rapidly changing and can be highly sensitive to changes in agricultural soil management, and thus reflecting changes in the total OM pool (Lambie et al., 2019; Landgraf et al., 2006).

Therefore, a measurement of the labile C pool within soils could help inform decisions surrounding soil management practices that would trigger the need for a change to prevent soil degradation or realise the potential increases in the soil C pool. This would be more difficult to detect if measures were limited to the total OM.

Soil organic C and N is comprised of a stable slowly decomposed fraction, and a labile rapidly decomposed fraction (Curtin et al., 2006; Ghani et al., 2003; Tirole-Padre et al., 2004; Weigel et al., 2011). The labile C and N pools are highly responsive to changes in land management practices and degradation processes (Ghani et al., 2003; Weigel et al., 2011). These pools are considered to give early indication of changes in the total organic pools, which are relatively slow

changing, making fluctuations difficult to detect in a timely manner (Curtin et al., 2006; Ghani et al., 2003; Weigel et al., 2011).

The current methods for measuring labile soil C and N are regarded as both expensive and time consuming. The promotion of hot water extractable C (HWEC) and hot water extractable N (HWEN) highlights their economic viability and usefulness as indicators of soil organic matter (OM) status, microbial biomass, mineralisable C and N and indicative of general soil health (Curtin et al., 2020; Ghani et al., 2023). Due to the sensitivity of these measures to provide short-term insights in to soil C and N changes by measuring a highly labile pool of C and N, they have the potential to be valuable additions to the current soil health indicator toolbox available for NZ farmers (Ghani et al., 2003). Current research has focussed on the sensitivity of HWEC and HWEN to land use changes (e.g., arable, dairying and dry stock) (Coca-Salazar et al., 2021; Ghani et al., 2003; Lambie et al., 2019; Landgraf et al., 2006). Whereas the sensitivity of HWEC and HWEN to changes in management (e.g., tillage, grazing and fertilisation) within a pastoral land use, as well as the effects of varying topography, has received little attention. Investigating these factors will improve our understanding of what practices influence soil C and N in NZ pastoral systems.

Current knowledge strongly suggests that HWEC and HWEN are useful and informative indicators for monitoring broader land use changes. However, a large gap in research still exists around the use of HWEC and HWEN within a system, to monitor changes caused by management practices. Furthermore, soil factors which influence these tests are not well understood, hindering the application across a broad range of soil types and spatial differences (Curtin et al., 2020). The sensitivity of HWEC and HWEN tests to changes in total C and N pools caused by management practices will give insight into the potential benefits of regenerative practices. Furthermore, this will also provide useful insight of the responsiveness of HWEC and HWEN measures to changes in management.

Chapter 2

Literature Review

2.0 Introduction

This chapter reviews the use of hot water extractable carbon (HWEC) and nitrogen (HWEN) as indicators of soil health and fertility and as early indicators of a change in carbon (C) and nitrogen (N) sequestration in soil. Section 2.1 outlines the contextual importance of soil organic matter (OM) and potentially mineralisable N (PMN) for maintaining soil health and fertility.

Section 2.2 details the use of HWEC and HWEN measures and the origins and forms of C and N extracted in these measures. Section 2.3 investigates the current knowledge of the effects broader land use factors have on these measures, while section 2.4 reviews the current research and knowledge on the effects that management practices within a land use have on HWEC and HWEN measures. Finally, section 2.5 outlines the methods which are currently used for HWEC and HWEN extractions within NZ.

2.1 Soil organic matter

Soil organic matter (OM) is a critical component in soil-plant systems and is crucial in the improvement of soil physical properties, including soil structure and soil water dynamics (Ghani et al., 2003; Voltr et al., 2021). Organic matter plays a crucial role in the soils nutrient supply and retention, buffering capacity, water holding capacity, aggregation, biological and enzymatic activity and minimises topsoil erosion (Ghani et al., 2013; Voltr et al., 2021; Zhao et al., 2013). There are several agricultural anthropogenic activities that accelerate the decline of OM, such as tillage, monocropping, overgrazing and overuse of chemical fertilisers (Ghani et al., 2003; Weigel et al., 2011). Degradation of soil OM leads to losses in agricultural productivity and has negative implications on food security and environmental impacts (Ghani et al., 2003; Voltr et al., 2021). Thus, limiting any loss of OM and or improving the quantity and quality of OM through land management practices will maintain or improve all those soil properties which are integral components of a healthy, productive soil ecosystem.

Soil OM is a small part of the soil and is composed of multiple pools of matter with differing turnover times (Curtin et al., 2020; Ghani et al., 2003). These vary from labile pools with quick turnovers, to stable pools with slow turnovers, to recalcitrant pools which re passive (Curtin et al., 2020; Ghani et al., 2003). The labile C fraction of OM is considered to consist of surface and buried plant residues, microbial biomass, particulate OM (POM) and low molecular weight compounds such as root exudates (Gregorich et al., 2006; Stockmann et al., 2013). The OM complexed to soil mineral particles is largely non-labile, while resistant organic C such as biochar is recalcitrant and highly non-labile (Gregorich et al., 2006). The pools of labile OM exist in the forms of both litter, roots and semi-decomposed OM as a result of microbial respiration (Stockmann et al., 2013). Monitoring this labile pool, which has a rapid rate of turnover, compared to the rate of the total soil OM fraction, has the potential to provide early and timely insights into the changes in the organic components of soil health (Doran et al., 1994).

2.1.0 Soil organic C pools

Agricultural soils can act as a major sink for C, reducing carbon dioxide (CO_2) emissions to the atmosphere and thus contributing to a reduction of greenhouse gas emissions (Johns et al., 2015; Lal, 2013). Carbon in soils consists of two pools; a stable slowly decomposed fraction and a labile, more rapidly decomposed fraction (Tirole-Padre et al., 2004). Monitoring the status of these pools is important for several different reasons. Increased soil C is effective in mitigating CO_2 emissions, while higher C levels sourced from microbial biomass indicates better microbial health of the soil (Voltr et al., 2021). The labile pool of C is rapidly changing and can be highly sensitive to changes in agricultural soil management, and thus reflecting likely changes in the total OM pool (Lambie et al., 2019; Landgraf et al., 2006). Therefore, a measurement of the labile C pool within soils could help inform decisions surrounding soil management practices that would trigger the need for a change to prevent soil degradation or realise the potential increases in the soil C pool. This would be more difficult to detect if measures were limited to the total OM.

2.1.1 Potentially mineralisable N in soil

Potentially mineralisable N (PMN) in soils consists of organic forms of N which can be

converted to inorganic N through mineralisation (Curtin et al., 2017; Wang et al., 2003). This fraction contributes to the plant available, inorganic N mineral pool in the soil (Curtin et al., 2017; Wang et al., 2003). Optimising plant growth relies heavily on meeting the plants inorganic N requirements, which the plant takes up from the soil solution (Curtin et al., 2017). Measurement of the quantity of PMN in soil has implications for soil fertility and pasture management decisions within agricultural systems. Current methods for the quantification of PMN rely largely on an incubation assay, which is laborious and time-consuming (Curtin et al., 2017; Curtin et al., 2006). Development of a reliable and effective method of measuring PMN in soils would offer an advance on the current approach for monitoring N fertility. It would be an important mitigation tool to reduce the negative environmental impacts, by better managing the balance between N mineralisation, immobilisation, N fertiliser use and crop demand (Öborn et al., 2003).

2.2 Hot water extractable C and N measures

Detecting small changes in the OM pool and PMN is difficult, with current laboratory-based measures being laborious, time consuming and in many instances impractical (Ghani et al., 2003; Weigel et al., 2011). Extracting the labile OM fraction with cold or hot water has been investigated as a test to measure short-term changes in the labile C and N pool in the soil (Ghani et al., 2003; Weigel et al., 2011). The water extractable organic matter (WEOM) is defined as the portion of the OM which has particle sizes of less than 0.45µm (Zhao et al., 2013). The release and turnover of this labile OM is indicative of C and N cycling and has been shown to be an accurate indicator of soil microbial biomass (Ghani et al., 2003; Zhao et al., 2013).

Analysis of HWEC and HWEN in the WEOM fraction has the potential to be an easy and applicable measure of labile OM and PMN along with an indication of potential C and N sequestration in soils (Bankó et al., 2021; Ghani et al., 2003). The responsiveness of WEOM to soil management change and correlation with a number of soil health measures, suggests that HWEN and HWEC could be sensitive indicators of a change in total C and N (Ćirić et al., 2016; Ghani et al., 2003; Weigel et al., 2011). Additionally, these measures could be valuable in advancing the monitoring of the biological components of the health of soils to provide early

detection of degradation and or improvements in the microbial community (Ćirić et al., 2016; Ghani et al., 2003; Weigel et al., 2011).

2.2.0 Origins and forms of C compounds in HWEC

A cold-water extraction of C prior to hot water extraction is shown to extract a small amount of highly available C (Landgraf et al., 2006; Zhao et al., 2013). The cold-water extractable C has been found to contain mainly plant debris from within the soil (Ghani et al., 2003; Landgraf et al., 2006; Zhao et al., 2013). A sequential extraction with 80°C water extracts a much larger pool of labile C (Zhao et al., 2013). The higher temperature of extraction results in depolymerization of OM, viscosity of soil humus, alterations of the OM physical state and the death of microbes (Zhao et al., 2013). This HWEC pool has been shown to contain 45-60% of compounds with origins of micro-organisms, enzymes, root exudates and lysates (Lambie et al., 2019). With a high correlation to microbial biomass, HWEC provide an insight into the factors influencing the rate of microbial decomposition, and soil microbial health (Bongiorno et al., 2019; Ghani et al., 2003; Lambie et al., 2019; Landgraf et al., 2006; Weigel et al., 2011). In addition to this relationship, HWEC correlates with the degree of soil micro-aggregation and soil fertility (Weigel et al., 2011; Voltr et al., 2021). This suggests there is potential for HWEC to be used as an easy, applicable and reliable method for the quantification of the active or labile C fraction (Weigel et al., 2011; Voltr et al., 2021).

2.2.1 Origins and forms of N compounds in HWEN

The PMN pool contains organic nitrogenous compounds that have the potential to undergo decomposition and degradation, including ammonium (NH_4^+), nitrate (NO_3^-) and soil microbial biomass N (MBN) (Chen & Sinsabaugh, 2020). Two step water extraction of soil N has shown to be a valuable tool in the quantification of the PMN fraction (Zhao et al., 2013). The initial cold-water extraction step in this method extracts plant derived OM from recently deposited plant debris and decaying microbes (Curtin et al., 2006; Zhao et al., 2013). This OM has potential to contain higher concentrations of less humified and N-enriched compounds (Curtin et al., 2006; Zhao et al., 2013). Cold-water extractable N yielded N compounds from OM which are easily accessible to microbes (Balaria et al., 2009). However, the cold-water extraction extracts a very small fraction of the bioavailable N and therefore has limited utility in quantifying the PMN

within soil (Balaria et al., 2009).

Further extraction using 80°C water to gain the HWEN resulted in a much larger extract of higher quality nitrogenous compounds (Chantigny et al., 2014; Zhao et al., 2013). The HWEN test has been shown to account for up to 40% of changes in total (organic and inorganic) soil N (Lambie et al., 2019). This large extraction of both organic and inorganic N compounds could be explained by the fact that organic N has been shown to contribute to only 80% of the total HWEN extract (Curtin et al., 2006). Hydrolysis of heat-labile organic N compounds results in the release of soluble inorganic N molecules such as peptides, amino acids and amino sugars from OM contributing to the remaining 20% of the HWEN extract (Curtin et al., 2006; Chantigny et al., 2014).

Further analysis of HWEN compared to other measures of soil N such as total N, PMN, POM and anaerobically mineralised N gives insight into the composition of HWEN and its relationship to the total soil N pool (Curtin et al., 2017). The HWEN extract was shown to contain a large quantity of NH_4^+-N (Curtin et al., 2006; Zhao et al., 2013). This NH_4^+-N released during the extraction has high potential to adsorb to cation exchange sites of soil colloids within the sample (Curtin et al., 2006). Further extraction of NH_4^+-N using 2 mol L^{-1} potassium chloride (KCl) in an additional extraction step has proven efficient at extracting these adsorbed N compounds for analysis (Curtin et al., 2006). Qualitative analysis of different extraction methods found the NH_4^+-N extracted in hot water was highly correlated with total soil N (Curtin et al., 2017). The NH_4^+-N extracted using hot water varied between soil types (Curtin et al., 2017). Soil factors influencing this included cation selectivity of soil colloid exchange sites and the concentrations of cations which compete for these exchange sites within the solution (Curtin et al., 2006). These factors will vary between soil types, potentially contributing to the discrepancy found by Curtin et al. (2017). Exclusion of the additional salt (KCl) extraction step has been suggested, with the partitioning constant of NH_4^+-N between exchange and solution phases being used as an estimate of NH_4^+-N using only hot water extracted NH_4^+-N (Curtin et al., 2017). This study showed that, once cation exchange sites with a strong affinity for NH_4^+-N were filled, the partition ratio of NH_4^+-N between exchange sites and solution was 1:1 (Curtin et al., 2017). With the exclusion of

outlying volcanic ash soils, HWEN shows strong correlations with anaerobic and aerobic PMN (Curtin et al., 2006; Curtin et al., 2017).

2.2.2 Correlations between HWEC and HWEN

The C:N ratio is an important indicator of plant N uptake, N mineralisation and immobilisation rates and potential N leaching losses (Zhang et al., 2019). In NZ soils, a C:N ratio of 8-12 is considered optimal for efficient mineralisation nutrient release (Mackay et al., 2013; Schipper & Sparling, 2011). Organic N extracted with cold-water shows little correlation with cold-water extractable C (Balaria et al., 2009; Curtin et al., 2006). This is reflected in a highly variable C:N ratio on extraction with cold-water (Balaria et al., 2009; Curtin et al., 2006). Factors such as recent fertilisation and plant debris in soil are likely contributing factors as these will increase soil N levels while leaving the labile C pool unaffected (Curtin et al., 2006; Zhao et al., 2013).

Comparatively, the correlation between the C:N ratio and the ratio of HWEC and HWEN was considered excellent when the additional step of KCl N extraction was included (Curtin et al., 2006). Exclusion of this KCl extraction step led to a gross overestimation in the C:N ratio of the hot water-soluble OM. The similar relationship between HWEN and mineralisable N suggests that HWEC may be a useful indicator of HWEN and may remove the need for an additional measurement (Curtin et al., 2017).

2.2.3 Relationships between HWEC, HWEN and microbial biomass

Microbial biomass C (MBC) is a quantifying measure of the microbial community within the soil (Vohland et al., 2017). A strong, significant positive correlation has been shown between HWEC and MBC in several NZ (Curtin et al., 2006; Ghani et al., 2003) and international studies (Balaria et al., 2009; Balaria & Johnson, 2013; Zhao et al., 2013). The correlation between HWEC and MBC has however been contradicted by findings of unexpectedly low correlations between MBC measured by chloroform-fumigation extraction and the HWEC measure when compared to the correlation between MBC and a separate labile C measure (Weigel et al., 2011). This was attributed to the likely decrease in microbial activity in some seasons of the year (Weigel et al., 2011). This gives evidence towards HWEC having origins of soil microbial biomass and being indicative of microbial activity and turnover, leading to the potential for HWEC to be used as a quantification tool for the microbial health of the soil (Balaria et al., 2009; Curtin et al., 2006;

Vohland et al., 2017).

The specific origins of the MBC, which can account for up to 40% of HWEC, suggest they have similar molecular composition to that of MBC and HWEC (Balaria et al., 2009). Elemental analysis has been performed on HWEC extracts to determine the composition of these compounds (Balaria et al., 2009). Determining the molar ratios of C/N, H/C and O/C, in conjunction with detection of ^{13}C isotopes through nuclear magnetic resonance spectroscopy ($^{13}CNMR$) found low C/N ratio in extracted molecules (Balaria et al., 2009). This indicates high levels of N functional groups on compounds within the hot water extract (Balaria et al., 2009). These N containing compounds were predominantly amino acids and amides found in microbial OM, rather than heterocyclic compounds found in plant OM (Balaria et al., 2009; Bi et al., 2007; Curtin et al., 2006; Paul, 2016). This elemental analysis further suggested that HWEC contains high levels of carbohydrates and chitin (Balaria et al., 2009). Ghani et al. (2003) found that the HWEC pool was largely comprised of amorphous polysaccharides (carbohydrates) which likely originate from microbial and plant root exudates. Fluorophore analysis of both plant-derived OM and microbial derived OM in hot water extracts showed that plant OM had a lower fluorescence index than microbial OM, indicating higher concentrations of the latter in the extract (Zhao et al., 2013). The ratio of HWEC to total soil organic C (SOC) can be used as an indicator in the success of ecological restoration, as increasing litter and OM leads to an increase in recalcifying bacteria (Yu et al., 2020).

Evidence that the HWEC pool contains a portion of the microbial biomass which is predominantly carbohydrates along with phenols and lignin monomers, promotes HWEC as a measure of a particularly bioavailable pool of C and N (Balaria et al., 2009; Yu et al., 2020). These extracted compounds have higher degradability potentials than other natural biopolymers, and HWE lignin is in the most easily digestible form of monomers rather than polymers which microbes cannot break down and digest (Balaria et al., 2009; Naorem et al., 2021). Therefore, use of HWEC and HWEN as an indicator of the readily available PMN and SOC has potential value as a tool for use in the management of agricultural soils (Naorem et al., 2021).

2.2.4 Alternative methods of labile C quantification

Current analytical quantification of labile C includes methods such as incubation and spectroscopic analysis, physio-chemical methods, and chemical extraction methods. However, many of these methods have limitations due to time required to process and associated costs and limited ability to identify the diverse range of compounds that can potentially make up the labile C pool (Johns et al., 2015; Weigel et al., 2011).

Chemical extraction involves oxidising C compounds using potassium permanganate ($KMnO_4$) (Huang et al., 2021). The $KMnO_4$-oxidisable C pool is supposed to represent the biologically oxidised pool of C naturally occurring in soil (Huang et al., 2021). However, the actual nature of the C extracted is not always defined as the labile C pool (Tirole-Padre et al., 2004). While studies have shown correlations of this C pool with the microbial biomass, research suggests that the $KMnO_4$-oxidisable C extract represents a more stable pool of C (Culman et al., 2012; Huang et al., 2021). It is suggested that the chemically oxidised C does not represent the biologically degraded C pool in soil (Huang et al., 2021; Tirole-Padre et al., 2004). Comparatively, the HWEC measure has been shown to measure the labile pool, which is a more readily bioavailable pool of C and indicative of the readily biologically degradable C in soil (Ghani et al., 2003). Furthermore, effects of clay and silt ratios suggest that soil texture influences the oxidisable C fraction due to the physical protection of oxidisable C groups (Tirole-Padre et al., 2004). Reports of $KMnO_4$-oxidisable C having positive correlations with particulate C and a more stable fraction of C suggests that this method of analysis is better suited to applications where monitoring long-term effects of management changes on C sequestration is the objective (Culman et al., 2012; Huang et al., 2021). Therefore, the use of a measure of more labile C pools such as HWEC could be better suited to measuring the short-term available C pool.

2.2.5 Alternative methods of mineralisable N quantification

Current methods for assessing mineralisable soil N have been shown to have large limitations in the quantification of N, and the degree of representation of the PMN present in soil (Curtin et al., 2017). Biological methods of PMN analysis analyse the inorganic N produced under either anaerobic or aerobic conditions during a long incubation method (Ros et al., 2010). These methods measure the amount of soil N produced by the soil microbial biomass under controlled conditions (Drinkwater et al., 1996). The time taken for the incubation test can range from 7 days

to 30 weeks (Curtin et al., 2017; Drinkwater et al., 1996; Ros et al., 2010). These measures of the PMN pool have also shown to be poorly correlated with the measured mineral N contents, due to the limited ability of the test conditions to replicate the environmental conditions found in the field (Curtin et al., 2017; Drinkwater et al., 1996; Ros et al., 2010). These factors have contributed to the limited use of biological assays of PMN, in the absence of agronomic field calibration (Curtin et al., 2017).

Chemical methods are used to extract inorganic and organic N from soils (Ros et al., 2010). Use of salt extractants have demonstrated variable outcomes, depending on a number of factors including salt type, pH, temperature and molarity of the salt (Curtin et al., 2006; Ros et al., 2010). KCl is a commonly used salt in the extraction of NH_4^+-N and NO_3^--N, used as a stand- alone measure and in conjunction with HWEN (Curtin et al., 2006). With quantities of N extracted depending on a large number of factors including salt type, molarity, duration of extraction and temperature of extraction, interpretation and use of the test is limited (Ros et al., 2010). Therefore, application of this measure to agriculture practices is limited beyond a general insight of soil N fertility.

2.3 Factors influencing HWEC and HWEN measures

2.3.0 Seasonality

Temporal changes in both HWEC and HWEN are commonly observed (Ghani et al., 2003; Uchida et al., 2012; Zhao et al., 2013). With climate factors having the potential to be a regulator of soil microbial activity, seasonal reduction in the mineralisation of C and N, along with overall microbial biomass, leading to a reduction in the amount of C and N that microbes contribute to HWEC and HWEN (Bardgett et al., 2005). Seasonal changes in HWEC have been reported as being less than 10% (Ghani et al., 2003). These variations are shown to be more affected by soil moisture content than temperature, with large reductions in HWEC and HWEN when soil moisture drops below optimum conditions for plant growth and microbial activity in the summer months (Ghani et al., 2003; Zhao et al., 2013). Increases in soil moisture content in winter have been shown to increase HWEC and HWEN fractions (Li et al., 2017).

2.3.1 Soil type

While the implications of varying soil types influencing the amount of HWEC and HWEN are well documented (Curtin et al., 2022; Filimonova et al., 2016; Harrison-Kirk et al., 2014; Lambie et al., 2019; Schipper et al., 2014; Voltr et al., 2021), there is still some uncertainty around the effect which individual soil parameters have on the amount of C and N extracted by hot water (Voltr et al., 2021). Response of HWEC to changes in total C is more pronounced in a silt loam soil than clay loam soil (Harrison-Kirk et al., 2014; Voltr et al., 2021). Saturation of the C adsorption capacity of the soil was shown to increase HWEC, potentially influencing the cation and anion storage capacities of soils (Voltr et al., 2021). This difference is due to higher stability of OM in the clay fraction than the silt fraction of soil. Higher physical protection of OM leading to increased stability occurs in these clay soils due to adsorption to the fine mineral fraction, reducing the bioavailable OM (Curtin et al., 2022). These differences between soils may also be due to differences in the underlying fertility of fine-textured soils and associated differences in OM input from plant growth (Harrison-Kirk et al., 2014; Ortner et al., 2021). Effects on HWEN in volcanic ash soils was high due to elevated concentrations of iron (Fe) and aluminum (Al) oxide (ferrihydrite compound) surfaces available for NH_4-N to re-adsorb, before the separation of the hot water extract resulting in lower recovery in the hot water extract (Curtin et al., 2006). Similarly, smaller proportions of total soil N have been extracted as mineral N from allophanic soils than from sedimentary soils of similar fertility (Curtin et al., 2017). Stabilisation of OM in allophanic soils due to their nanostructure providing stabilising effects and minerals such as ferrihydrite (Fe oxides) (Curtin et al., 2017; Filimonova et al., 2016; Schipper et al., 2014). Conflicting evidence shows there are no significant alterations in the HWEC across allophanic, brown and gley soils (Lambie et al., 2019). The influence which soil physico-chemical factors have on hot water extractable C and N fractions is not well understood and further investigation into this would be beneficial in the application of HWEC as a comparative measure across these soil types (Curtin et al., 2022).

2.3.2 Topography

Topography including slope and aspect, influences parent material, soil development and depth and climate, all of which influence pasture growth and the behaviour of animals (López et al., 2003). Lambert et al. (1983) and Lambert & Roberts (1976) showed large differences in pasture

growth with slope and aspect. Large amounts of nutrient (N, P and S) and C can be transferred from high slopes (>25 °) to low slopes (1-12°) due to greater deposits of animal excreta on low slope areas (Bilotto et al., 2022; Letica et al., 2006; Saggar et al., 2004). Most of the N deposited is in the form of inorganic N, and differing grazing intensities have not been shown to impact the dissolved organic N across slope classes (Hoogendorn et al., 2016; Letica et al., 2006; Mackay et al., 2018; Saggar et al., 2004). Due to the presence of inorganic N in the HWEN extract, the spatial distribution of excreta across different topographical units may influence this fraction (Curtin et al., 2006; Chantigny et al., 2014).

Redistribution of labile C and N rich topsoil through erosion in high slope areas has been well established as a major source of labile C and N losses from these areas (Berhe et al., 2012; Fissore et al., 2017; Gao et al., 2024; Liu et al., 2023). Additionally, dissolved organic N (DON) and dissolved organic D (DOC) leaching has been found to occur at higher rates from high slope than low slope positions (Hoogendorn et al., 2016). The leachate includes both DON and inorganic N (Hoogendorn et al., 2016). The deposition of this soil to down slope results in the accumulation of topsoil and of labile C and N (Berhe et al., 2012; Fissore et al., 2017; Gao et al., 2024). This labile C and N is potentially buried under the eroded soil which protects it from decomposition, likely through the burial process rather than by mineralisation or stabilisation on mineral surfaces leading to lower rates of OM turnover (Berhe et al; 2012; Fissore et al., 2017). Conversely, in some studies this accumulated SOC has been found to result in increased C mineralisation by providing increased substrate for microbial communities (Gao et al., 2024; Liu et al., 2023; Wang et al., 2014).

Lower soil moisture is characteristic of high slope areas, which has also been shown to lead to the fragmentation of soil aggregates, leading to further exposure of OM for decomposition (Gao et al., 2024). There is rivalling evidence on whether labile C present on high slope, erosion prone areas is stabilised through incorporation onto freshly exposed mineral surfaces, or whether a lack of physical protection due to topsoil erosion leads to greater respiration rates and C mineralisation (Doetterl et al., 2012; Fissore et al., 2017). A critical gap in this research is the specific influence which topography may have on the HWEC and HWEN fractions.

Investigating HWEC and HWEN in this context will provide further insights into the dynamics of these pools across the range of topographical units found in hill country.

2.3.3 Land use

Different land uses have differing effects on the physico-bio-chemical soil characteristics, which in turn influences the size, structure and occurrence of soil microbial community and associated processes (Coca-Salazar et al., 2021). Therefore, with a direct relationship between microbial biomass and HWEC and HWEN measures, these two measures are potentially effective tools in monitoring the impacts which different land uses have on soil functions, particularly C and N cycling (Coca-Salazar et al. 2021; Lambie et al., 2019; Landgraf et al., 2006).

Agricultural systems in NZ consist of a range of pastoral systems, including drystock (sheep/beef) and dairying, as well as cropping systems (Ghani et al., 2003). The specific land use has greater effects on the soils labile C pool and the HWEC fraction than the SOC pool (Curtin et al., 2017; Ghani et al., 2003; Jinbo et al., 2006). Between land uses, HWEC concentrations have been shown to be highest on average under sheep/beef pastures (3400 μgOC/g soil), followed by dairy pastures (3000 μgOC/g soil) and lowest under annual cropping systems (1000 μgOC/g soil) (Curtin et al., 2017; Ghani et al., 2003;).

Studies suggest that long-term pastoral systems accumulate OM, effectively increasing the soils labile C pool and HWEC concentration when compared to long-term arable cropping systems which decline over time (Coca-Salazar et al., 2021; Curtin et al., 2022; Ghani et al., 2003; Loke et al., 2019). Soils with a history of arable and vegetable cropping were also shown to have lower organic N concentrations, and rates of N mineralisation than soils under long-term pasture use (Curtin et al., 2017). Greater organic N in pasture soils has been attributed to a greater amount of OM returned under grazed perennial pasture (Coca-Salazer et al., 2021; Curtin et al., 2022; Loke et al., 2019). Decreases in C inputs from root exudates and fractions of carbohydrates, along with the lower total OC content, are considered two major factors contributing to the decrease of HWEC under arable cropping, as these C sources contribute to the HWEC pool (Curtin et al., 2022; Ghani et al., 2003; Li et al., 2007). Further declines in HWEC were seen under arable

systems utilising extensive cultivation and fallowing, where fallowing refers to incorporation of remaining aboveground biomass into the soil through tillage, followed by leaving the area unmanaged (Coca-Salazar et al., 2021; Loke et al., 2019). The HWEC measured labile C pool contains substrates that are more bioavailable to micro-organisms (Landgraf et al., 2006). Therefore, aggregate breakdown through fallowing will likely increase the decomposition of these compounds (Coca-Salazar et al., 2021; Li et al., 2007).

A negative correlation has been found between soil labile C and water holding capacity under land use which disturbs soil, and this correlation has been reflected in the HWEC measure (Li et al., 2007). Overall, there is strong evidence that HWEC reflects the loss in labile C which has been measured under arable cropping and also that this decline in HWEC gives insight into other soil health parameters such as water holding capacity and soil aggregate structure (Curtin et al., 2022; Ghani et al., 2003; Landgraf et al., 2006; Li et al., 2007).

2.4 Sensitivity of HWEC and HWEN to management practices in pastoral systems

The ability of HWEC and HWEN measures to indicate changes within a land use, and across different management practices, is crucial in the integration of these measures as tools in improving soil health in agricultural systems. To be useful as an indicator for monitoring land resources and informing decisions effecting C and nutrient management, the HWEC and HWEN tests need to have the ability to detect changes in C and N cycles, pools and their stocks in the soil as a consequence of changes in management practices in a pastoral system. Using these measures to make management decisions and monitor soil management changes would provide invaluable information from both a farm productivity and environmental reporting standpoints.

2.4.0 P fertiliser

Soil fertility (N and P) both influence the above and below ground C and N cycle, including the size and turnover of microbial mass in a pastoral system, and therefore are likely to influence the size of the HWEC fraction (Ghani et al., 2003). Net primary production (NPP) is the quantity of vegetation C gained by photosynthesis, minus the C lost via respiration (Collati et al., 2019).

Applications of P fertiliser in the absence of N fertilisers increases NPP and subsequently increases the amount of C fixed and returned to the soil as plant leaf litter, roots and root exudates (Condron et al., 2012; Ortas & Bykova, 2020; Poeplau et al., 2016; Schipper et al., 2013; Wakelin et al., 2017). However, despite increases in returned biological material to the soil, multiple studies have shown that when N is limited, SOC does not follow the observed NPP increase (Ghani et al., 2003; Ortas & Bykova, 2020; Peixoto et al., 2022; Poeplau et al., 2016; Voltr et al., 2021; Wakelin et al., 2017). This suggests that in a system with P inputs and where N is limited, labile C becomes output driven (Ghani et al., 2003; Ortas & Bykova, 2020; Peixoto et al., 2022; Poeplau et al., 2016; Voltr et al., 2021; Wakelin et al., 2017).

Mechanisms that have been suggested to explain why SOC does not follow the observed NPP increases are as follows:

1. Stimulation of microbial heterotrophic respiration through the addition of readily available P as a nutrient for the microbial community results in decomposition of OM, and an efflux of CO_2 from the soil (Ortas & Bykova, 2020; Poeplau et al., 2016; Wakelin et al., 2017). The average C:P demand of microbial biomass is 60:1 (Poeplau et al., 2016; Wakelin et al., 2017). Global average C:P ratios are around 186:1. Thus, the addition of P to soils overcomes the stoichiometric constraint of P for microbial growth and activity (Poeplau et al., 2016). Long-term increases in mineralisation of C and CO_2 losses have been observed throughout multiple P fertilisation trials when N is limited to N fixation from legumes, reducing the labile C present (Mackay et al., 2021; Poeplau et al., 2016; Wakelin et al., 2017). The resulting effect of this increased microbial respiration causes faster turnover of C (Poeplau et al., 2016; Wakelin et al., 2017).
2. Arbuscular mycorrhizal fungi (AMF) are root-obligate biotrophs which play an important role in allowing plants to access nutrients such as P throughout the soil profile (Chen et al., 2018; Berruti et al., 2016). A reduction in the colonization of roots by AMF (proportion of roots occupied by AMF) has been observed when readily available P is applied (Ortas & Bykova 2020; Poeplau et al., 2016). Hyphae contribute a significant quantity of MBC to the soil, along with translocating C away from areas with high microbial activity (Cheng et al., 2012; Poeplau et al., 2016; Wilson et al., 2009). This

promotes C sequestration and contributes to the protection of SOC from decomposition by promoting soil aggregation (Cheng et al., 2012; Poeplau et al., 2016; Wilson et al., 2009). Reducing abundance of AMF thus decreases a source of labile C and the protection of other sources of labile C (Poeplau et al., 2016).

3. The root:shoot ratio of grasses and legumes as well as the spatial distribution of roots has been shown to be influenced by P fertiliser inputs (Haling et al., 2016; Lopez et al., 2022; Poeplau et al., 2016). Deficiency of P has been shown to cause an increased allocation of biomass to roots while suppressing shoot growth, while a decreased root:shoot ratio is seen under high P fertiliser inputs (Haling et al., 2016; Kim & Li, 2016; Lopez et al., 2022; Poeplau et al., 2016). Preferential root development within topsoil where higher concentrations of P are located is often seen in P deficient soils (Haling et al., 2016; Lopez et al., 2022).

   In addition to greater root biomass, P deficiency has been shown to result in the release of carboxylate by roots, allowing for the mobilisation of P through the capture of Fe oxides and Al oxides/hydroxides due to carboxylates ligand properties (Lambers et al., 2015; Lopez et al., 2022; Tshewang et al., 2017). This larger presence of root biomass and carboxylate within the topsoil will likely increase the labile C pool sourced from root exudates and measured by HWEC (Curtin et al., 2022; Dodla et al., 2012; Ghani et al., 2003; Guo et al., 2014; Lambie et al., 2019; Li et al., 2007; Yu et al., 2016).

4. Nutrient mining by microbial communities occurs when specific nutrients are limited (Majumdar et al., 2016; Meyer et al., 2017; Meyer et al., 2018; Poeplau et al., 2016; Zeng et al., 2023). In the presence of long-term P fertilisation with no N additions, it has been found that N mining can often occur to provide microbes with a source of N (Meyer et al., 2017; Poeplau et al., 2016). Microbes decompose more recalcitrant, energy poor OM in search of the required N (Majumdar et al., 2016; Meyer et al., 2017; Meyer et al., 2018; Poeplau et al., 2016). To gain energy for this process, labile C is used as an energy source rendering it less efficient for biosynthesis use (Meyer et al., 2017; Meyer et al., 2018; Poeplau et al., 2016; Zeng et al., 2023). Furthermore, with the increased inputs as a result of changes in NPP under P fertilisation, there is a higher microbial demand for N to mineralise labile C within the decomposed OM (Meyer et al.,

2017; Poeplau et al., 2016; Zeng et al., 2023). Therefore, due to the co-limitation of microbial activity and decomposition by both P and N, increased use of labile C and C mineralisation may occur leading to lower levels of HWEC in soils which have experienced long-term P fertilisation (Meyer et al., 2017; Meyer et al., 2018; Poeplau et al., 2016; Zeng et al., 2023).

Increased microbial growth has resulted in increased HWEC measured in soils under some studies (Wakelin et al., 2017). The ability of soils to support larger microbial communities and faster microbial turnover with higher P inputs increases the levels of MBC (Peixoto et al., 2022; Wakelin et al., 2017) Therefore, despite a decrease in SOC contributing to the labile C pool, the high contribution of MBC to HWEC may counteract the net SOC losses resulting from stimulated microbial activity overcoming increased NPP under P fertiliser input (Ghani et al., 2003; Poeplau et al., 2016; Wakelin et al., 2017). Increases in soil P content (i.e., soil Olsen P) correlated strongly with an increase in the aromaticity of HWEC (Wakelin et al., 2017). The prevalence of cyclic functional groups will lower the degradability of this C, suggesting that at higher Olsen P concentrations, the HWEC pool will have higher prevalence of condensed C and will be less bioavailable. Studies surrounding long term P fertilisation and labile C are limited due to the scarcity of long-term trials. Gaining further insight into the effect of P fertilisation, where N inputs are limited to N from the legume component of the sward on labile C and N pools is crucial. Strong coupling between C and P cycles, and a close relationship with microbial activity allows HWEC to monitor changes in OM initiated by P fertiliser (Wakelin et al., 2017). However, most research focuses on the effects of nutrient management on HWEC. Further investigation into the impacts on HWEN should be undertaken to gain further understanding of this measure.

2.4.1 N fertiliser

Applications of mineral N fertilisers have OM building effects, with higher growth rates of plants leading to higher plant litter returns to the soil (Voltr et al., 2021). However, N fertiliser inputs can also promote decomposition processes, leading to the depletion of SOC (Ghani et al., 2003; Moran et al., 2005; Voltr et al., 2021). Excessive N fertiliser has been shown to cause a decline in the HWEC of a soil (Ghani et al., 2003; Voltr et al., 2021). This knowledge could be used to

ensure the N fertiliser programme maintains HWEC at a level which ensures the sustainability of soil production and environmental protection.

2.4.2 Glyphosate herbicide

Reliance on glyphosate herbicide as a tool in weed management has increased with the rise of adaptive tillage strategies such as direct drilling (Nguyen et al., 2016). Glyphosate works by inhibiting the production of the key enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) within the Shikimate pathway of plants (Chávez-Ortiz et al., 2022; Nguyen et al., 2016; Vázquez et al., 2021). This prevents the synthesis of aromatic amino acids essential for plant growth and survival (Chávez-Ortiz et al., 2022; Nguyen et al., 2016; Vázquez et al., 2021).

Research has shown conflicting results surrounding the effects of glyphosate on the microbial community composition, biomass and respiration. Issues with certain micro-organisms such as bacteria, fungi and algae have been identified due to their reliance on the Shikimate synthesis pathway as a vital energy pathway (Kepler et al., 2020; Vázquez et al., 2021). It is believed that factors such as application rate, soil type, environmental conditions, soil pH and soil organic C content play a large role in the positive or negative response of microbial communities to glyphosate application.

Glyphosate is an amphoteric molecule (acting as both base and acid), with 3 polar functional groups; amino, carboxylic and phosphonic (Chávez-Ortiz et al., 2022; Galicia-Andrés et al., 2020; Zhan et al., 2018). Certain micro-organisms, particularly bacteria and certain species of fungi are able to utilise the glyphosate molecule as a C, N and P source (Chávez-Ortiz et al., 2022; Imparato et al., 2016; Lane et al., 2012; Nguyen et al., 2016; Sun et al., 2019; Zhan et al., 2018). Consistent applications of glyphosate have been shown to lead to the selection of micro-organisms capable of metabolising glyphosate as an energy source, and micro-organisms which are not negatively affected by glyphosate (Busse et al., 2001; Chávez-Ortiz et al., 2022; Imparato et al., 2016). This leads to a decrease in microbial diversity within soils which are subject to regular glyphosate applications (Busse et al., 2001; Chávez-Ortiz et al., 2022). Decreased soil microbial diversity has been shown to alter C and N cycling in soil and potentially drive greater

decomposition of the most degradable C and N sources due to lowered metabolic capabilities to decompose OM (Busse et al., 2001; Li et al., 2015; Maron et al., 2018; Qiu et al., 2023; Zhou et al., 2012). Some studies have shown no major impacts of glyphosate on microbial community structure (Kepler et al., 2020; Lane et al., 2012). It has commonly been found that unless soils are subject to intensive and regular glyphosate applications, changes to microbial diversity are generally short-lived (Imparato et al., 2016; Lane et al., 2012; Weaver et al., 2007). Limited research has been conducted on the impacts a shift in microbial diversity has on labile C and N and the decomposition of OM (Chávez-Ortiz et al., 2022; Imparato et al., 2016; Nguyen et al., 2016).

Glyphosate is a potential energy source for microbial communities and glyphosate application has been shown to have the potential to increase microbial respiration (Busse et al., 2001; Chávez-Ortiz et al., 2022; Nguyen et al., 2016). With a low C:N ratio of 3:1, glyphosate is a favourable substrate for micro-organisms (Lane et al., 2012; Rampoldi et al., 2011). This ncreased respiration causes mineralisation of labile soil C and an efflux of CO_2 (Lane et al., 2012; Nguyen et al., 2016). However, the use of glyphosate as a substrate has been shown to not support microbial biomass growth, due to the co-metabolism of this substance (Busse et al., 2001; Lane et al., 2012). Inconsistency has been found across studies around the stimulating effect glyphosate may have on microbial respiration. Application rates below 200 mg kg^{-1} have been shown to be detrimental to microbial respiration rates (Chávez-Ortiz et al., 2022; Imparato et al., 2016; Nguyen et al., 2016). These changes in microbial respiration however, have not been shown to have effects on HWEC and HWEN measures (Imparato et al., 2016). This is likely due to the speed at which utilisation of glyphosate as a C and N source occurs, and the lack of response seen in the microbial biomass (Imparato et al., 2016). There is high variability of the impacts which glyphosate has on labile C and N due to soil type, application rates and other environmental factors (Chávez-Ortiz et al., 2022; Imparato et al., 2016; Nguyen et al., 2016).

Utilising HWEC and HWEN measures to monitor the best management practices could minimize any negative impacts caused. These measures may prove beneficial in monitoring whether glyphosate is having positive or negative impacts on microbial biomass and respiration for each

individual situation.

2.4.3 Tillage methods

Tillage practices within agricultural systems have the potential to be a large short-term source of C and N effluxes (Rutledge et al., 2017). The use of conservational, minimally disruptive pasture renewal practices (e.g., no-till) have been shown to drive maintenance and improvements of general soil health, fertility, soil structure and reduce the losses of soil C and N (Rutledge et al., 2017). Direct drilling with no prior tillage is a common method used to limit the disturbance to the soil (Fonteyne et al., 2022; Rutledge et al., 2017; Whitehead et al., 2018). Comparatively, conventional, non-inversion tillage methods involve some disturbance and disruption of the topsoil through methods such as shallow power-harrowing (Kobierski et al., 2020).

Disruptive conventional tillage practices cause the rupture of soil aggregates, which are crucial in providing physical protection of OM (Botenelli et al., 2010; Chahal et al., 2021; Colombi et al., 2018; Mehra et al., 2018; Zhang et al., 2019). This exposes organic C and N sources from within aggregates as well as that located in inter aggregate zones to attack from micro-organisms, driving the decomposition of OM (Botenelli et al., 2010; Chahal et al., 2021; Colombi et al., 2018; Liu et al., 2017; Mehra et al., 2018; Zhang et al., 2019). Decomposition of OM leads to losses of the labile C and N measured in HWEC and HWEN (Bankò et al., 2021; Bongiorno et al., 2019; Shen et al., 2021; Sun et al., 2017). Degradation of SOC has further negative impacts on aggregation stability due to the loss of the cementing and binding properties of OM in the promotion of soil aggregation (Bongiorno et al., 2019; Bottenelli et al., 2010; Chahal et al., 2021; Mehra et al., 2018; Zhang et al., 2019).

Aeration of soil occurring during disruptive tillage stimulates microbial activity and respiration (Chahal et al., 2021; Colombi et al., 2018; Mehra et al., 2018). Increased rates of microbial respiration through greater access to OM as a substrate and introduction of O_2 to the soil facilitates the oxidation of C to CO_2 through mineralisation (Colombi et al., 2018; Mehra et al., 2018). The sources of labile C subject to decomposition as a result of tillage have been found to be compounds commonly measured in HWEC (Colombi et al., 2018; Mehra et al., 2018).

Furthermore, exposure of labile N has the potential to increase nitrous oxide (N_2O) emissions because of microbial activity (Cummins et al., 2021; Laubach et al., 2023). The use of HWEC and HWEN following either conservational or conventional tillage is currently constrained because of the limited research that has been conducted calibrating these two measures with tillage practices. More knowledge on the influence differing tillage practices have on these fractions, could aid in further determining the optimal practice to reduce detrimental impacts which tillage has on C and N pools in the soil.

2.4.4 Diverse pastures

Diversifying pasture types has been advantageous in some agricultural systems, with increased yields, lower drought vulnerability and improved animal performance (Bracken et al., 2020; Cummins et al., 2021; Jing et al., 2017), with some studies finding greater C and N sequestration (Alemu et al., 2019; Fornara & Tilman, 2008; Laubach et al., 2023; McNally et al., 2015). An increased number of pasture species within a sward allows for a more diverse root systems within the soils compared to monoculture pastures (Fornara & Tilman, 2008; McNally et al., 2015; Rutledge et al., 2017). Deeper rooted plants have the potential to deposit C compounds to greater depths within the soil profile, which are typically less C saturated than topsoil and have higher C sequestration potential (Beare et al., 2014; Fornara & Tilman, 2008; McNally et al., 2015; Rutledge et al., 2017; Wall et al., 2021). Incorporating plant species with finer root systems into the pasture also increases the protection of intra-aggregate SOC, through the stabilisation of aggregates by these roots (Beare et al., 2014; McNally et al., 2015). Diverse pastures have been shown to have greater root biomass, which is a source of C with a longer residence time within soil than aboveground plant litter (Fornara & Tilman, 2008; McNally et al., 2015; Wall et al., 2021). While the increased C inputs as a result of root turnover have been well researched, this does not account for the potential increase in C caused by rhizodeposition (Beare et al., 2014; McNally et al., 2015). Root exudates and cell sloughing release forms of labile C into the soil which would be measured in HWEC (Beare et al., 2014; Curtin et al., 2022; Dodla et al., 2012; Ghani et al., 2003; Guo et al., 2014; Lambie et al., 2019; Li et al., 2007; McNally et al., 2015; Yu et al., 2016). While impacts may not be picked up in a total C measure, HWEC might offer a useful method to measure the effects of diverse pasture on labile C within soils.

A number of mechanisms in diverse pasture swards have been shown to improve soil N dynamics and sequestration (Bracken et al., 2020; Cummins et al., 2021; Jaramillo et al., 2021; Jing et al., 2017; Laubach et al., 2023). Diverse pastures have been found to have a lower demand for synthetic N inputs than monocultures due to the presence of legumes and the ability of diverse root systems to take up N from greater depths in the soil profile, while maintaining equivalent N and DM yields in plant biomass, reducing costs for farmers (Bracken et al., 2020; Cummins et al., 2021; Jaramillo et al., 2021; Laubach et al., 2023; Romera et al., 2017). Sowing a diverse range of pasture species with a greater range of rooting systems can have a direct, positive effects on plant water uptake and gas diffusivity, reducing N_2O emissions as these factors directly influence nitrification and denitrification pathways of microbes (Cummins et al., 2021; Laubach et al., 2023). This increased rooting depth also allows plants to take up plant available N from greater depths within the soil. Not only does this increase nutrient uptake efficiency but also reduces the potential for N losses via leaching through the soil (Bracken et al., 2020; Jing et al., 2017). Diverse pasture swards often include plantain (*Plantago*) species (Bracken et al., 2020; Cooledge et al., 2022; Cummins et al., 2021). Plantain reduces N_2O emissions due the production of biological nitrification inhibitor (BNI) compounds in root exudates (Bracken et al., 2022; Cooledge et al., 2022; Cummins et al., 2021). By preventing the transformation of NH_4^+ to NO_3^-, there is increased N available for plant uptake and greater promotion of N stabilisation (Cooledge et al., 2022; Cummins et al., 2021). Legume species have the ability to biologically fix atmospheric N in the soil (Bracken et al., 2020; Cooledge et al., 2022). By providing biologically sourced plant available N to other species within the pasture sward, the demand for synthetic N can be reduced when compared to a monoculture with no legume and therefore fertiliser inputs should be altered to account for this increase in plant available N to reduce N leaching (Bracken et al., 2020; Cooledge et al., 2022). While individual species have specific diverse functionality in reducing soil N losses, studies have shown that N cycling in monocrops of grasses, legumes and herbs is not positively impacted to the same extent as diverse pastures composed of these three plant groups (Cummins et al., 2021; Laubach et al., 2023). This has been hypothesised to be due to a synergistic effect between the multiple pasture types (Cummins et al., 2021; Laubach et al., 2023).

2.4.5 Grazing practices

Pastoral soils have proven to be more efficient in maintaining HWEC and HWEN pools than soils under arable use (Ghani et al., 2003). The impact that different livestock and grazing practices within pastoral systems have on HWEC is less well understood. Current research indicating that soils under pastures grazed by dairy cows contain less HWEC, than soils under pastures grazed by sheep (Ghani et al., 2003). Differences in HWEN in soils under dairy and other livestock grazing systems were not significant (Curtin et al., 2017). To date, little research has been undertaken examining how grazing practices, including rotational, continuous or more intensive activities within a livestock system influence HWEC and HWEN.

Grazing management of permanent pastures under NZ conditions varies from continuous (i.e. set stocked) grazing systems to those that involve a rotation that varies in length throughout the year. Many sheep and beef farmers use a mix of the two approaches, set stock over lambing and then into a rotation after weaning. While dairy systems generally use a rotation system year-round, with target pre- and post-grazing pasture masses determining the rotation length. There is surprisingly little data on the influence these grazing practices have on OM or the labile C and N fractions.

Interest in alternative grazing management strategies as opposed to the more conventional rotational systems has gained momentum in recent years for a number of reasons (Grelet et al., 2021). These practices have been shown to alter C and N cycling and sequestration within soils (Abagandura et al., 2024; Kim et al., 2023; Khatri-Chhertri et al., 2024; Ma et al., 2021; Mosier et al., 2021). One of these alternative grazing practices aims to achieve higher pre- and post-grazing covers, utilise higher instantaneous stocking densities (e.g., up to 10 times higher than conventional systems) and implement short grazing events (e.g., hours rather than days) and long recovery periods in order to promote soil health and increase C and N stocks (Abagandura et al., 2024; Kim et al., 2023; Ma et al., 2021; Mosier et al., 2021).

Under conventional grazing, cattle tend to selectively graze higher quality components of the pasture, depleting the cover in these areas, which reduces overall pasture regrowth and pasture composition of regrowth and alters C and N cycling (Abagandura et al., 2024; Mosier et al.,

2021). Higher grazing pressure implemented by alternative grazing practices, reduces the opportunity for selective grazing, resulting in more even distribution of residual pasture cover across the soil (Abagandura et al., 2024; Mosier et al., 2021). Additionally, higher stocking density results in greater trampling of plant litter, increasing organic C and N inputs into the soil (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). This incorporation of plant litter into the soil has the potential to increase microbial decomposition resulting in lowered C and N sequestration (Abagandura et al., 2024.

Physical damage caused by higher stocking densities may negatively impact soil macro-aggregates, exposing aggregate protected OM to microbial decomposition (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). High stocking densities across a paddock for a short period of time has also been shown to result in more even distribution of animal excreta and subsequently C and N deposits across the paddock (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). The effects of alternative grazing practices on soil C and N are poorly understood and believed to be largely influenced by soil type and climatic factors rather than grazing management factors (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). Currently no research has investigated the effects of alternative grazing strategies on soil HWEC and HWEN pools.

2.5 Analysis methods for HWEN and HWEC

The extraction method for both N and C was developed by Ghani et al. (2003). This includes an initial extraction in cold-water to remove any readily soluble N or C (Curtin et al., 2006). Sources of C can include recently applied lime, animal excreta and soluble plant residues (Ghani et al., 2003). The soils then undergo hot water extraction and are analysed for both C and N compounds. Some research has shown that the amount of HWEC recovered in the extract varies with extraction time. After four hours of extraction at 80 °C, the ratio of plant carbohydrates to microbial carbohydrates increased (Chantigny et al., 2014). Their study also found that the ratio of carbohydrate to phenolic compounds and glucose declines after 1 hour of extraction at 20 °C (Chantigny et al., 2014).

2.5.0 Cold-water extraction method

Oven-dried soil samples are sieved to a particle size of <2mm. Three grams of <2mm soil samples are weighed and added into 50mL polypropylene centrifuge tubes. 30mL of distilled water is then added to the centrifuge tubes, and the soil samples are extracted for 30 minutes in an end-for-end shaker at 30 rpm. These samples are then centrifuged at a rate of 3500 rpm for 20 minutes at 20 °C to separate the extractant and soil supernatant. Using a 0.45 µm cellulose NO_3^- membrane filter, the extractant is filtered for cold-water extractable C and N analysis. The supernatant of this extraction is kept for further (hot water extractable) analysis.

2.5.1 Hot-water extraction method

A volume of 30mL of distilled water is added to the polypropylene centrifuge containing the cold-water extracted soil supernatants. These supernatants are then resuspended in the solution using either a vortex shaker for 10s or inverting by hand. Hot water extraction is then performed by submerging the soil samples in a water bath at 80 °C for 16hr. Suspension of the hot water C is ensured by inverting the tubes. These are then centrifuged at 3500rpm for 20 minutes. Using a 0.45 µm cellulose NO_3^- membrane filter, the extractant is filtered. The supernatant is collected for further N extraction. Soil supernatants from the hot water extraction can be further extracted to remove any adsorbed NO_3^--N using 30 mL 2M KCl (Curtin et al., 2006).

2.5.2 Analysis of cold and hot water extracted C and N

Both cold-water and hot water filtrates are analysed for organic and inorganic C and N using a total organic C (TOC) analyser. Each sample filtrate is measured three times to reduce error and ensure reproducibility.

The final extract is injected into the detection chamber of the TOC analyser. Following a 9-minute analysis, the total C and total N in the samples is measured using non-dispersive infrared (NDIR). The difference between the total C and total inorganic C, and total N and inorganic N in the extracts is calculated to give the total organic C and N extracted.

2.6 Research gaps

The soil HWEC and HWEN fractions have been proven to contain highly labile forms of C and N and are representative of microbial biomass (Curtin et al., 2006; Chen & Sinsabaugh, 2020; Balaria et al., 2009; Balaria & Johnson, 2013; Ghani et al., 2003; Zhao et al., 2013). However,

current knowledge of how these indicators change in soils is largely limited to comparisons between land uses (e.g., arable, dairying, sheep and beef) (Coca-Salazar et al., 2021; Curtin et al., 2022; Ghani et al., 2003; Loke et al., 2019). While it is clear that HWEC and HWEN vary between land uses, the use of these tests for monitoring the effects of agricultural management on total C and N within a specific land use has not been investigated thoroughly. This is important, as the utility of HWEC and HWEN as indicators of sequestered C and N largely depends on their responses to management practices within a land use. Current research shows that factors such as soil type and season play a crucial role in determining the amount extracted in hot water extracts. However, the short- and long-term effects of land management practices and spatial factors such as topography on these extracts have yet to be investigated. Research into the HWEC and HWEN measures and their response to management factors such as fertiliser use, cropping and grazing practices within a land use, along with how they might be influenced by topography would further our knowledge and understanding of the application of these tests. The potential for HWEC and HWEN to indicate changes in the larger C and N pools is promising, as these pools are important indicators of soil health and greenhouse gas mitigation and having cost effective methods of monitoring changes in response to management, will be critical.

2.7 Conclusions

This review of current research supports the potential use of HWEC and HWEN as simple, informative and economical diagnostic methods for measuring labile C and N fractions in soil that would add to the current soil health indicator toolbox. It is well established that factors that influence the soil microbial biomass and total C and N pools are rapidly and directly reflected in the HWEC and HWEN extracts. Factors such as land use (e.g., arable, dairying and sheep and beef) have been researched to determine the impact these activities have on the HWEC and HWEN pools. There is however a large gap in research surrounding the influence soil management changes within a land use have on the HWEC and HWEN fractions. Until the influence which management impact factors such as grazing practices, pasture type, P and N fertiliser inputs, tillage and topography have on the HWEC and HWEN fraction, the usefulness of these two measures as indicators of change the OM cycle and biology of the soil within agricultural systems will continue to be limited.

Chapter 3

Effects of long-term phosphorus fertiliser use and topography on Hot Water Extractable Carbon and Nitrogen

3.0 Introduction

Hot water extractable carbon (HWEC) and nitrogen (HWEN) have been added to the current suite of soil tests available commercially in NZ to provide farmers with a measure of the labile carbon (C) and nitrogen (N) pool within soils. The HWEC test is also promoted as a proxy for soil microbial biomass C (MBC) (Curtin et al., 2006; Balaria et al., 2009; Balaria & Johnson, 2013; Ghani et al., 2003; Zhao et al., 2013). Although there has been some study of the HWEC and HWEN fractions under different land uses (arable, dairying, sheep and beef, forestry etc.) the degree to which management practices (fertiliser inputs, stocking rates etc.) within a land use effect the HWEC and HWEN fractions has received little attention. More research is also required to better understand what changes in HWEC and HWEN means in terms of biological processes, C and N cycling, nutrient supply and primary production. Furthermore, little is known about the influence topographical factors, such as slope and aspect, might have on the HWEC and HWEN fractions in soils from these locations. At the present time, soil samples collected from transects established on the average slope of a land unit for monitoring soil fertility, are assumed to also provide a representative sample for monitoring HWEC and HWEN concentrations from that land unit (Morton et al., 2000; Schon et al., 2022).

New Zealand hill country pastoral systems rely heavily on legume-based pastures as a source of biologically fixed N to sustain grass growth within the sward. Since legumes are less competitive than grasses for phosphorus (P), P based fertilisers are applied annually to maintain the legume component of the sward. Increased Olsen P values up to 20-25 mg/L as a result of P fertiliser inputs leads to increased pasture productivity (net primary production (NPP)), higher animal

stocking rates and therefore more excreta and plant litter returns to the soil (Mackay et al., 2021).

The increase in the quantity of plant litter, excreta return, and Olsen P concentrations increases the biological activity of the soil, specifically earthworm abundance and biomass (Schon et al., 2019). This has also been shown to increase C and N cycling within the system and therefore could be expected to increase the quantity and rate of turnover of the labile C and N pool in the soil (Bilotto et al., 2022; Wakelin et al., 2017).

Despite this, a number of long term P fertiliser input and grazing livestock studies have shown that despite large increases in NPP, increased biological activity and amounts of C and N cycling in the soil, no increases have been reported in SOC stocks (Bilotto et al., 2022; Condron et al., 2012; Mackay et al., 2021; Ortas & Bykova, 2020; Poeplau et al., 2016; Schipper et al., 2013; Wakelin et al., 2017). Determining whether HWEC and HWEN measures can quantify the increases in C and N cycling as P fertiliser inputs, pasture production and Olsen P increases, would provide insights into the rate of change in soil fertility. It would also provide an indication of any differences in the size and activity of the microbial community. Additionally, it may also highlight the associated increase in the risk of losses of C and N from the very labile fraction as its size and rate of turnover increases.

Differences in topographical features (slope and aspect) across a landscape result in associated differences in soil characteristics and depths. These can also have a range of microclimates, which in turn influences soil temperature and photosynthetically active radiation (PAR), both of which influence pasture growth and the hydrological cycle (Lambert & Roberts, 1976). Slope also influences the behaviour of grazing animals. While animals may graze across all slope classes, a disproportionate amount of excreta is returned to low slope areas which animals prefer to occupy while ruminating and resting (Saggar et al., 1990). This transfer of nutrient fertility through excreta reinforces the influence of slope and aspect on pasture growth. Pasture production in NZ has been shown to be greater on low slopes and north facing aspects, due to increased soil moisture, higher PAR and greater OM returns enhancing soil fertility (Lambert et al., 1983; Mackay et al., 2021).

Erosion can transfer topsoil rich in OM and the associated HWEC and HWEN from high slope (>25°) areas to depositional, low slope (1-12°) areas (Berhe et al., 2012; Fissore et al., 2017; Gao et al., 2024; Liu et al., 2023; Mackay et al., 2018). Studies have shown that this accumulation of OM-rich soil on low slopes facilitates the mineralisation of C and N due to increased availability of microbial substrates, increasing the MBC on low slopes (Gao et al., 2024; Liu et al., 2023; Wang et al., 2014). Lower soil moisture on high slopes and low soil temperatures on south facing aspects has been shown to limit pasture growth and promote the fragmentation of soil aggregates, exposing OM to microbial decomposition (Gao et al., 2024). A focussed study examining how HWEC and HWEN are influenced by slope and aspect and how that changes with P fertility and sheep grazing will help to understand the contribution which these tests might offer in developing a more complete picture of C and N cycling in hill country. This study will also help to understand where best to sample to monitor labile C and N pools.

The wider long-term study at the AgResearch Ballantrae Hill Country Research Station has been monitoring the effects of varying P fertiliser inputs and sheep grazing has had on (i) soil Olsen P and N fertility, (ii) soil organic C and N, (iii) macro- and micro-biology, (iv) net primary production and (v) animal production since its establishment in 1975 (Bilotto et al., 2022; Garbuz et al., 2021; Mackay et al., 2021). However, the impacts that varying P fertiliser inputs and associated sheep grazing, and slope and aspect has had on HWEC and HWEN measures is yet to be established, as is the influence of slope and aspect.

In this study, the influence of P fertiliser and sheep grazing history, slope and aspect on HWEC and HWEN was investigated across the four farmlets that make up the long-term P fertiliser and sheep grazing experiment at AgResearch Ballantrae. To obtain further insights into the significance of any differences in HWEC or HWEN found with P fertiliser history or topography, the relationship between HWEC and HWEN, and a wide range of other soil and plant measures (e.g., soil Olsen P, exchangeable cations, pH, total C, total N and pasture growth, etc.) were also examined as part of the study. Establishing where best to soil sample across this hill country agricultural landscape to provide a representative measure of HWEC and HWEN was also a

focus of the present study.

3.0.0 Objectives

Using soil samples collected from the AgResearch Ballantrae long term hill country P fertiliser and sheep grazing study in 2021, the objectives of the current study were to:

- To determine the impact of long-term P fertiliser application, sheep grazing and topographical factors (aspect and slope) on HWEC and HWEN concentrations.
- To explore the correlations between HWEC, HWEN, and total C and N pools.
- To investigate the relationships between HWEC, soil physicochemical factors, soil fertility and NPP under varying P fertiliser application and sheep stocking rates.

Hypothesis 1: *Null:* The mean soil HWEC measured from all slope classes are equal to those measured from medium slope classes.

Alternative: The mean soil HWEC and HWEN measured from all slope classes are not equal to those measured from medium slope classes.

3.1 Methods

3.1.0 Study site

The long-term P fertiliser and sheep grazing trial at the AgResearch Ballantrae Hill Country Research Station is located in Southern Hawkes Bay, NZ (40.8180°S, 175.8500°E) at 300m above sea level. The long-term average annual temperature is 12.8°C and average annual rainfall is 1270mm (Mackay et al., 2021). Soils throughout the farmlets are classified as Brown and Pallic soils. Both these soils have silt-loam textures and the Pallic soils are imperfectly drained (Lambert et al., 2000).

Four 5-10 ha farmlets were established on the Research Station in 1975. Historic P applications and current P fertiliser treatments are described in Table 3.1. Two farmlets received superphosphate (SSP) at 156 kg/ha/yr (14 kg P/ha/yr) from 1975 to1980. Since then, one farmlet has continued to receive 125 kg SSP/ha/yr (11.25 kg P/ha/yr) (LFLF) while the other has received none (LFNF). Two other farmlets received 500 kg SSP/ha/yr (45 kg P/ha/yr) from 1975 to 1980; after that one farmlet has received 375 kg SSP/ha/yr (33.75 kg P/ha/yr) (HFHF) and the other has received no further P fertiliser (HFNF). Farmlets are grazed by breeding ewes, stocked at 6.9 stock units/ha on LFNF and HFNF farmlets, 10.6 stock units/ha on LFLF farmlets and 14 stock units/ha on the HFHF farmlet (Table 3.1) with different stocking rates reflecting differences in pasture production as a result of the different P fertilizer inputs. Within each of the four farmlets soil fertility is measured across three slope classes: low slope (LS; 1-12°), medium slope (MS; 13-25°), high slope (HS; >25°), and three aspects: east (E; 35-155°), southwest (SW; 275-35°), northwest (NW; 155-275°). There are duplicate sites for each slope/aspect combination, making a total of 18 sites per farmlet. A total of 72 samples were taken. For this study, the soil samples to a depth of 7.5 cm were taken and analysed in September, 2021.

Table 3.1: SSP fertiliser applications (kg SSP/ha/yr), P application rates (hg P/ha/yr) and stocking rates (units/ha) for Ballantrae experimental farmlets since 1975, Farmlets: LFLF (125 kg SSP/ha/yr since 1980), LFNF (No fertiliser since 1980), HFHF (375 kg SSP/ha/yr since 1980), HFNF (No fertilizer since 1980). AgResearch Ballantrae Hill Country Research Station.

Farmlet	SSP rate (kg SSP/ha/yr)	P rate (kg P/ha/yr)	Stocking rate (units/ha)
LFLF	125	11.25[A]	10.6
LFNF	0	0[A]	6.9
HFHF	375	33.75[B]	14
HFNF	0	0[B]	6.9

[A]Between 1975 and 1980 these farmlets received 14kg P/ha/year.

[B]Between 1975 and 1980 these farmlets received 45kg P/ha/year.

3.1.1 Chemical analysis

3.1.1.1 HWEC and HWEN

Hot water extractable C and N were analysed using the method of Ghani et al. (2003). Three grams of soil was added into 50mL polypropylene centrifuge tubes. Thirty mL of distilled water was then added to the centrifuge tubes, and the soil samples were extracted for 30 minutes on an end-for-end shaker at 30rpm. These samples were then centrifuged at a rate of 3500rpm for 5 minutes at 20 °C to separate the extractant and soil supernatant. Using a 0.45 µm cellulose nitrate (NO_3^-) membrane filter, the extractant was filtered for cold-water extractable C analysis. The supernatant was kept for further (hot water extractable) analysis.

Thirty mL of distilled water was added to the polypropylene centrifuge tube containing the cold-water extracted soil supernatants. These supernatants were then resuspended in the solution by inverting by hand. Hot water extraction was then performed by submerging the soil samples in a water bath at 80 °C for 16 hours, followed by centrifugation at 3500 rpm for 5 minutes. The extractant was then filtered using a 0.45 µm cellulose NO_3^- membrane filter.

Hot water filtrates are analysed for organic C using a Shimadzu TOC-V_{CSH}/TNM-1 analyser. Forty µl of the extract was injected into the detection chamber of the analyser. Following a 9- minute

analysis, the total C and inorganic C in the samples were measured using non-dispersive infrared (NDIR). The difference between the total C and total inorganic C in the extracts is calculated to determine the organic water-soluble C concentration. Using a SKALAR analyser, ammonium-N (NH_4^+-N) and NO_3^--N was measured in the samples. The difference between these measures determines the quantity of water-soluble organic N in the extracts.

3.1.1.2 Analysis of total C % and total N %

Total C % and Total N % was measured using the Dumas method of combustion (Nelson & Sommers, 1996), and a VarioMAX Elementar Analyser. Five hundred g of soil was combusted at 900 °C in a pure oxygen atmosphere, completely decomposing all N containing compounds and converting them to nitrous oxides (NO and NO_2) and all C compounds, converting them to methane (CH_4) and carbon monoxide (CO). These nitrous oxides were then reduced to elemental N (N_2) and C gasses were oxidised to carbon dioxide (CO_2) using the catalysts copper oxide and platinum. The percentage of elemental N and CO_2 in the sample was measured using thermal conductivity to determine total N % and total C %.

3.1.1.3 Analysis of physicochemical factors

The physicochemical variables organic matter (OM) %, effective cation exchange capacity (CEC), total recoverable potassium (K), pH, calcium (Ca) base saturation, magnesium (Mg) base saturation, sodium (Na) base saturation, K base saturation, exchangeable Ca, exchangeable Mg, total fluoride, Na MAF quick test and sulfate sulfur (S) were determined using the methods of Eurofins (2022). Olsen P was determined by the method of Lambert et al. (2014). Dry bulk density and soil C and N stocks were determined by the method detailed by Mackay et al. (2021). The C stocks were calculated using the following equation: C stocks = Bulk density (g/cm^3) × total C (%) × (Soil depth (mm) / 100). The N stocks were calculated using the following equation: N stocks = Bulk density (g/cm^3) × total N (%) × (Soil depth (mm) / 100).

3.1.2 Statistical analysis

R version 4.2.3 (2023-03-15) was used for all statistical analysis. Descriptive statistics (minimum, maximum, mean and standard deviation) were calculated for all outcome variables (HWEC, HWEN, total C, total N and HWEC:HWEN ratio) under each farmlet, slope and aspect.

Effects of fertiliser, aspect and slope on HWEC, HWEN and HWEC:HWEN ratio were analysed using 3-way ANOVA. The differences between fertiliser treatments (HFHF, HFNF, LFLF and LFNF), aspect (NW, SW and E) and slope (Low, Med and High) were evaluated at 95% confidence interval using Tukey's honestly significant difference (HSD) test if the overall F-test was significant. A two-sided, two-sample t-test assuming unequal variance was performed to determine whether means of HWEC were statistically different between medium slopes compared to all slope classes (Hypothesis 1).

Correlations between HWEC, HWEN, total C, total N, HWEC:HWEN ratios, C:N ratios, C stocks and N stocks were determined using parametric correlation (Pearson) and non-parametric correlation (Spearman), depending on the outcome of the Shapiro-Wilk normality test for each factor. The significance of each correlation was tested, with $p < 0.05$ considered significant. The amount of HWEC was calculated as a percentage of the total C pool.

3.1.2.1 Multi-level regression model

A nested multi-level regression model was used to predict the HWEC using chemical and physical soil characteristics (Table 3.2). This model was used to account for the hierarchical interactions between topographical, physical and chemical effects influencing HWEC (Gili et al., 2013). Within this model, physicochemical variables were nested within topographical factors, which were further nested within P fertiliser treatments (farmlets).

The mixed effect model was defined as:

(3.1)

$$\pi_{ijk} = \left[\gamma_{000} + \gamma_{001}\chi_{ijk} + \gamma_{010}\chi_{ik} + \gamma_{100}\chi_{k} + e_{ijk} + \mu_{1jk}\chi_{ijk} + \mu_{jk} + \mu_{k}\right]$$

Where π_{ijk} is the HWEC value for physicochemical variables I at slope j in fertiliser farmlet k.

γ_{000} is the fixed intercept of level 1 (physicochemical) variables, γ_{001} is the random effect associated with physicochemical variables, γ_{010} is the random effect associated with slope and γ_{100} is the random effect associated with fertiliser farmlet.

χ_{ijk} is the X value for physicochemical variables *i* in slope *j* and fertiliser farmlet *k,* χ_{ik} is the X value for physicochemical variables *i* in fertiliser farmlet *k* and χ_k is the X value of physicochemical variables in farmlet *k*.

e_{ijk} is the error term unique to physicochemical variables *i* under slope *j* for fertiliser farmlet *k,* μ_{1jk} is the slope error term, μ_{jk} is the combined error term for slope *j* and fertiliser farmlet *k* and μ_k is the fertiliser farmlet *k* error term (Liljequist 2019; Vitali, 2010).

Table 3.2: Physicochemical variables and used to develop a mixed effect model to predict HWEC.

Level	Variable
Outcome Variable (π_{ijk})	HWEC
Level 1 Factors (χ_k)	P Fertiliser treatments: HFHF, HFNF, LFLF, LFNF Slope Low, Medium, High
Level 2 Factors (χ_{jk})	Aspect E, NW, SW Slope Low, Medium, High
Level 3 Factors (Physiochemical variables) (χ_{ijk})	OM (%) Effective CEC (cmol+/kg) Total Recoverable K (mg/kg) Dry bulk density (g/cm^3) pH Ca Base Saturation (%) Olsen P (mg/kg) K Base Saturation (%) Exchangeable Mg (cmol+/kg) Exchangeable Ca (cmol+/kg) Microporosity (%) Na Base Saturation (%) Mg Base Saturation (%) Total Fluoride (mg/kg) Na MAF QT Macroporosity (%) Sulfate S (mg/kg)

Initial analysis of data was performed to determine the normality, distribution, correlation and relationship of each chemical and physical predictor variable with HWEC to ensure the assumptions of the multi-level model were met. Quantile-Quantile (Q-Q) plots were produced to examine the distribution of the outcome, and each predictor variable. Shapiro-Wilk tests were performed to determine whether each variable met the normality assumptions.

Linear regression models were conducted to determine the relationship between the soil physicochemical variables and HWEC. Variables with a strong linear relationship ($p < 0.05$) were selected and used to further develop a model of the best predicting variables of HWEC under varying fertiliser treatment and topographical factors (P fertiliser, slope and aspect).

3.1.2.1.1 Quantile-Quantile regression of predictor variables

To determine whether the relationship between HWEC and each predictor variable met the linearity of the multi-level regression model, quantile regression was used. Quantile regression of each quartile was determined, and this was subject to Wald's ANOVA test. Significant ANOVA results ($p <0.05$) showed that there was a significant difference between the effect of HWEC on the predictor variable across quantiles and therefore a non-linear relationship was observed.

Variables which did not meet the linearity assumptions were transformed from numerical to categorical variables to account for the non-linear relationship which incurred between the predictor variable and HWEC.

Through quantile-quantile regression of the 4 quartiles of each predictor variable, it was determined that the relationship between Olsen P and HWEC and K MAF QT and HWEC were not linear. Significant ($p < 0.05$) variances in the relationship coefficients between each quartile showed that these relationships did not follow a linear trend. All other variables showed a linear relationship with HWEC, with no significant variations between quantiles ($p > 0.05$).

Figure 3.1 shows the 95% confidence interval (dotted red line) for linear regression least squares estimate and the 95% confidence interval for each of the quantile-quantile regression estimates (grey shaded area). These show that the quantile-quantile regression estimates of K MAF QT and

Olsen P are not within the bounds of the linear regression estimates. Hence there is a significant difference between the overall linear relationships and those as estimated by each quartile. To account for this non-linear relationship within the model, Olsen P and K MAF QT were converted from numerical to categorical variables by quartiles.

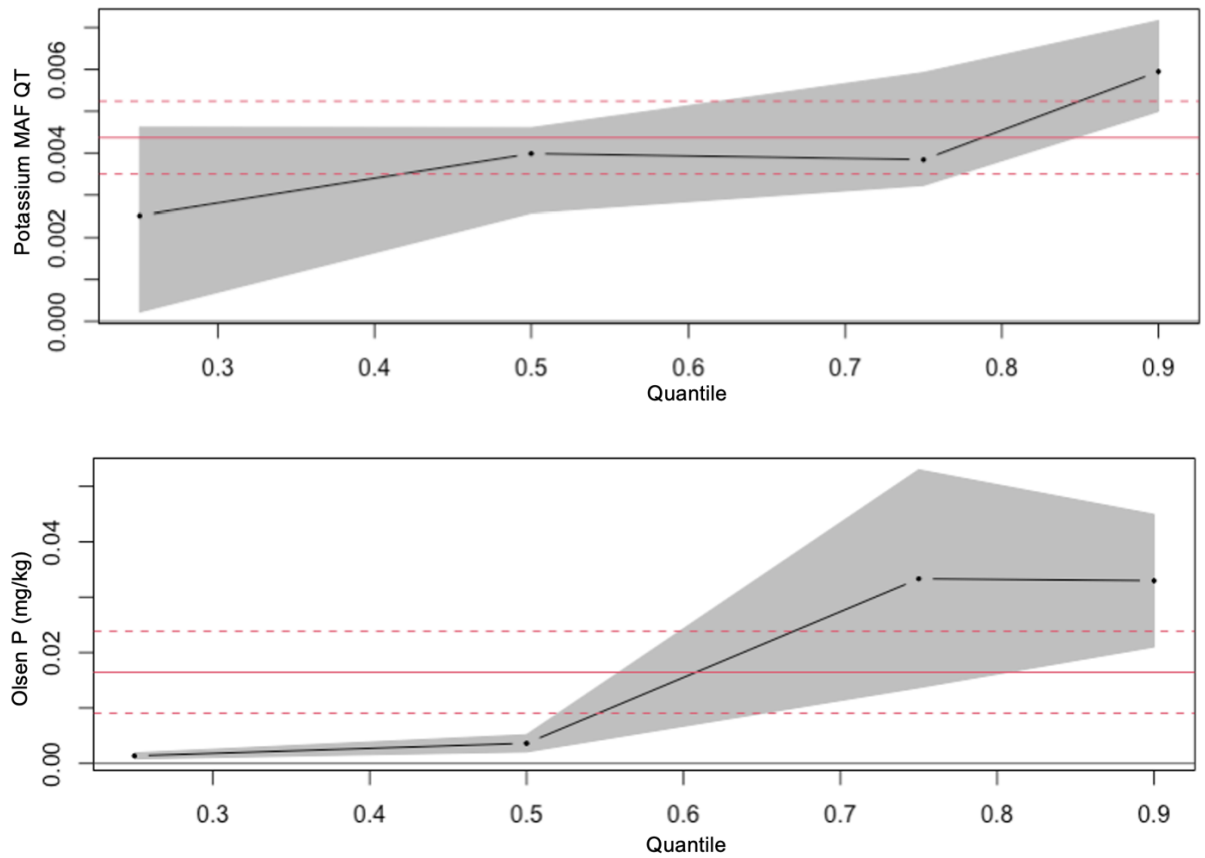

Figure 3.1: Quantile-quantile regression plots of K MAF QT and Olsen P against HWEC. Dotted red line shows 95% confidence interval of quantile-quantile regression estimates shown by the grey shaded area.

3.1.2.1.2 Multicollinearity

Variables selected as significantly correlated to HWEC from both Spearman correlation for non- parametric predictor variables and Pearsons correlation for parametric predictor variables, and quantile-quantile regression were then analysed for correlation. For variables with correlation coefficients greater than 0.7, one of the two were excluded from the model based on best fit to control for multicollinearity. Variables were removed from the model in preference to reducing overfitting due to multicollinearity as the purpose of this predictive model was to reduce the number of tests and costs induced for the agricultural sector.

Explanatory variables removed to prevent multicollinearity were as follows:

- Total Recoverable Cadmium
- Total Recoverable S
- Exchangeable Ca
- Exchangeable K
- Exchangeable Mg
- Exchangeable Na
- Ca MAF QT
- Total C %
- Total N %

3.1.2.1.3 Random intercept model

Initially, a random intercept (null) model was performed with only fertiliser to gain the intraclass correlation coefficient (ICC) ρ. To provide a base model, a null (intercept-only) model was run with no explanatory variables, establishing the baseline of variance on HWEC caused by the level 1 variable farmlet (P fertiliser treatment). The ICC was used to determine the percentage of variation in HWEC which could be attributed to the differences between level 1 variable (P fertiliser farmlet) groups.

This can be defined by:

(3.2)

$$\rho = \left(\sigma_{\mu 0}^2\right)/\left(\sigma_{\mu 0}^2 + \sigma_e^2\right)$$

Where ρ is the ICC, $\sigma_{\mu 0}2$ is the variance of the random intercept of the model and σ_e^2 is the variance of a standard logistic distribution.

3.1.2.1.4 Adding the random slope (level 2) variables to the multilevel model

Random effect variables (level 2 variables - slope and aspect) were included in the model to determine the significance of association which these variables had with HWEC, the dependent variable. These were tested to see if they improved the model established in section 3.2.2.1.3 as random slopes. A Likelihood Ratio (LR) test, using the *lrtest* function from the *lmtest* package

(Kleiber & Zeileis, 2002) in R was used to determine if these variables improved the models' goodness of fit. Variables were retained in the model if the p value of the LR was less than 0.001.

3.1.2.1.5 Adding the fixed effect (level 3) variables to the multilevel model

Using the base model established in section 3.2.2.1.4, level 3 (soil physicochemical) explanatory variables were added stepwise as fixed effects. The fixed effect variables were analysed using a bottom down method. Beginning with the simplest model and adding variables in order of decreasing correlation with HWEC, the level 3 variables were analysed to determine between-group variations of HWEC. Inferential tests for fixed effects were conducted using LR tests using the *lrtest* function from the *lmtest* package (Kleiber & Zeileis, 2002) in R was used to determine if these variables improved the models' goodness of fit. Variables were retained in the final model if the p value of the LR was less than 0.001.

3.1.2.1.6 Model diagnostics

To ensure the validity of the final model, model diagnostic checks were performed. To determine whether residuals of the model fit normality assumptions, a Q-Q plot of model residuals was produced. A Shapiro-Wilk test was also used to determine whether the residuals were normally distributed. To test the final model for homoscedasticity, a Brausch-Pagan test and White's test was performed. These tested the null hypothesis that the variance of the residuals was homogenous. Residuals were also plotted against predicted HWEC values to visualise the variation. To ensure multicollinearity in the final model was reduced, variation inflation factors (VIF) were calculated for each variable. Variables with a VIF of greater than 10 were considered to be linear combinations of other variables in the final model and a cause of multicollinearity.

3.2 Results

3.2.0 Effect of P fertiliser and sheep grazing history on HWEC and HWEN

The amount of C and N extracted by hot water varied from 2602 to 3134 µg OC/g and 181 to 239 µg N/g, respectively, across the four farmlets. The HWEC:HWEN ratio ranged narrowly from a minimum of 14:1 in the HFHF farmlet to a maximum of 15:1 in the LFLF farmlet. The P fertiliser and sheep grazing history (farmlet) had a significant effect on HWEC ($p < 0.05$), HWEN ($p < 0.05$) and the ratio between the two extracts ($p < 0.001$) (Table 3.3, Fig. 3.2).

Few differences in HWEC and HWEN concentrations were found across all four farmlets, despite the long-term differences in the P fertiliser application and sheep grazing histories of the farmlets (Table 3.3, Fig. 3.2). For example, there was no significant difference in either HWEC and HWEN concentrations between the high-P (HFHF and HFNF) farmlets, despite the different P fertiliser histories. Also, there was no significant differences in HWEC and HWEN concentrations between the low-P (LFLF and LFNF) farmlets. The HWEN concentrations were significantly higher under HFHF than either the LFLF or LFNF farmlets. HWEC concentrations were not significantly different under HFHF, LFLF and LFNF farmlets. The amount of HWEC was significantly higher under the HFNF farmlet than the LFLF farmlet. The amount of HWEN was not significantly higher under the HFNF farmlet compared to the LFLF and LFNF.

The only significant difference in the HWEC:HWEN ratio was between the HFHF and the two (HFNF and LFNF) farmlets that had not received fertiliser since 1980.

Table 3.3: Mean soil HWEC and HWEN concentrations and the HWEC:HWEN ratio measured across *four farmlets* at *Ballantrae Research Station in 2021. Farmlets: LFLF (125 kg SSP/ha/yr since 1980), LFNF (No fertiliser since 1980), HFHF (375 kg SSP/ha/yr since 1980), HFNF (No fertilizer since 1980). Values are presented with standard deviations in parentheses. Different letters within columns indicate significant differences according to Tukey's test ($p < 0.05$).*

	HWEC (µg OC/g) Mean (± SD)	**HWEN (µg N/g) Mean (± SD)**	**HWEC:HWEN Ratio Mean (± SD)**
Farmlet			
LFLF	2602 (± 655) b	184 (± 59) b	15.0 (± 1.7) ab
LFNF	2654 (± 703) ab	181 (± 53) b	14.5 (± 1.8) a
HFHF	2995 (± 1001) ab	239 (± 112) a	14.1 (± 2.0) b
HFNF	3134 (± 938) a	215 (± 101) ab	14.4 (± 2.2) a

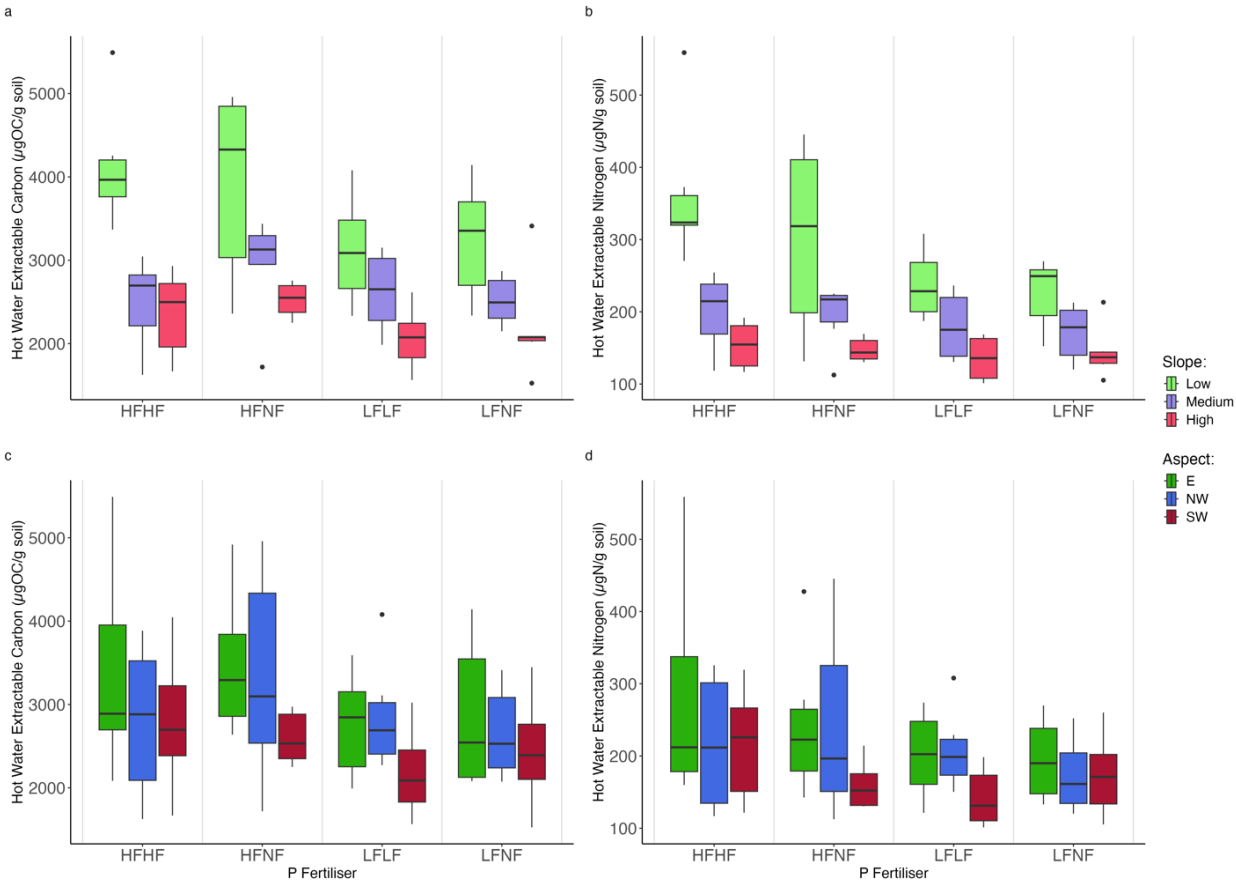

Figure 3.2: Effect of (a) slope on HWEC, (b) slope on HWEN, (c) aspect on HWEC, (d) aspect on HWEN across all four farmlets (LFLF (125 kg SSP/ha/yr since 1980), LFNF (No fertiliser since 1980), HFHF (375 kg SSP/ha/yr since 1980), HFNF (No fertiliser since 1980)) of Ballantrae Research Station, from soils sampled in September, 2021.

3.2.1 Effect of slope and aspect on HWEC and HWEN

Slope had a significant effect on the HWEC ($p < 0.001$), HWEN ($p < 0.001$) and HWEC:HWEN ratio ($p < 0.001$) (Fig. 3.2a, b). On average, HWEC was significantly higher on low slopes than medium and high slopes (Table 3.4). The amount of HWEC on low slopes was over 57% higher than from the high slopes. The amount of HWEN was higher on low slopes than medium slopes (51%), with a further decrease on high slopes (94%). This difference was also reflected in the ratio between HWEC and HWEN fractions, which increased from 13.3 to 14.4 to 15.8, from low slope to medium and to high slope, respectively (Table 3.4). Aspect also had a significant effect on HWEC ($p = 0.001$) and HWEN ($p = 0.004$) (Fig. 3.2c, d). The amount of HWEC was significantly lower on SW aspects than on E and NW aspects (Table 3.4). Significantly lower HWEN concentrations were observed on SW aspects compared to E aspects. Aspect had no significant effect on the HWEC:HWEN ratio ($p > 0.1$).

Table 3.4: Effect of slope and aspect on the mean values of HWEC, HWEN and the HWEC:HWEN ratio in soils collected across the four farmlets of Ballantrae, September 2021. Values are presented with standard deviations in parentheses. Different letters within columns indicate significant differences according to Tukey's test ($p < 0.05$).

	HWEC (µgOC/g) Mean (± SD)	**HWEN (µgN/g) Mean (± SD)**	**HWEC:HWEN ratio Mean (± SD)**
Slope			
Low	3607 (± 891) a	282 (± 101) a	13.3 (± 2.0) a
Medium	2644 (± 500) b	187 (± 45) b	14.4 (± 2.0) b
High	2288 (± 464) b	145 (± 29) c	15.8 (± 1.4) c
Aspect			
E	3122 (± 934) a	230 (± 102) a	14.2 (± 2.0) a
NW	2909 (± 877) a	210 (± 87) ab	14.4 (± 2.2) a
SW	2508 (± 619) b	173 (± 59) b	15.0 (± 2.0) a

3.2.2 HWEC measured from medium slopes compared with all slopes

The mean soil HWEC concentration measured from the medium slope positions was not significantly different compared to all three slope classes combined ($p > 0.05$) (Fig. 3.3).

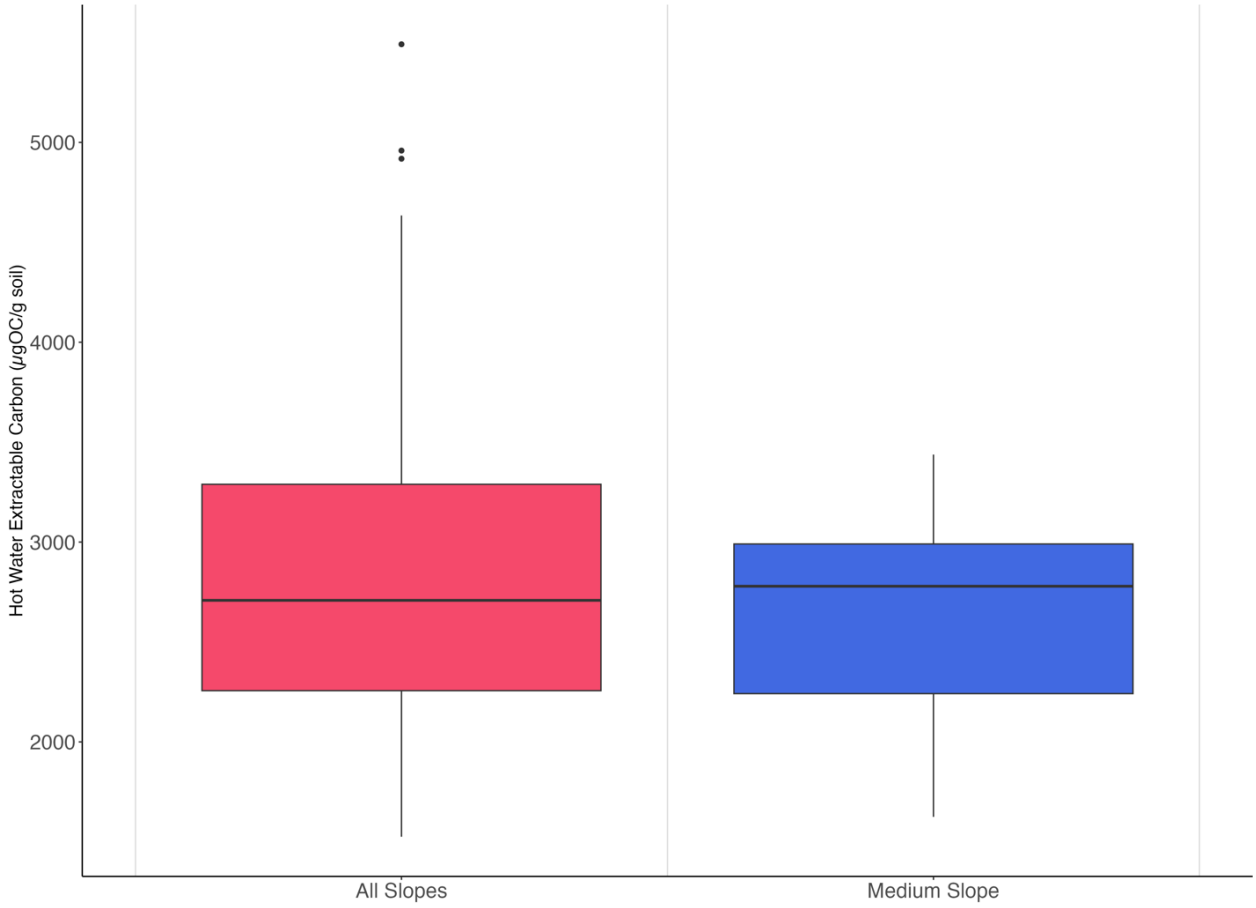

Figure 3.3: Comparison of soil HWEC measured from soils collected from medium slopes compared to all slopes (low, medium and high), across all four farmlets of Ballantrae Research Station, from soils sampled in September, 2021.

3.2.3 Relationships between HWEC and HWEN and total C % and total N %

The HWEC and HWEN concentrations measured in the top 7.5 cm soil layer were strongly positively correlated, with a Spearman correlation coefficient of 0.94 ($p < 0.001$) (Fig. 3.4a). The ratio between the HWEC and HWEN showed significant and strong positive correlation with the total C:N ratio (r = 0.64, $p < 0.001$). Despite this significant correlation, however, the HWEC:HWEN ratios were significantly wider ($p < 0.001$) than C:N ratios. This is shown by the majority of the datapoints falling below the x = y line (Fig. 3.4b).

The HWEC concentrations showed a significant strong positive correlation with total C and a significant positive correlation with C stocks (r = 0.78, $p < 0.001$ and r = 0.65, $p < 0.001$, respectively) (Fig. 3.4c, d). The linear relationship showed that for every unit increase in HWEC, the total C % increased by the factor of 1.65, with a negligible constant of -0.00122.

The total C present in the form of HWEC measured across slopes and aspects was 5.5%, 6.0%, 5.5% and 5.2% for the HFHF, HFNF, LFLF and LFNF farmlets, respectively. With slope averaged across all aspects and farmlets, the percentages were 6.0%, 5.2% and 5.4% for low, medium and high slope, respectively. With aspect averaged across all slopes and farmlets, percentages were 6.1%, 5.8% and 4.8% for NW, E and SW aspects, respectively.

The HWEN concentrations showed a significant and strong positive correlation with total N and N stocks (r = 0.81, $p < 0.001$ and r = 0.76, $p < 0.001$, respectively) (Fig. 3.4e, f). The linear relationship showed that for every unit increase in HWEN, the total N % increased by the factor of 1.06 e^{-3}, with a constant of -0.175.

The amount of total N present as HWEN across slopes and aspects was 6.0%, 5.4%, 5.1% and 4.7% for the HFHF, HFNF, LFLF and LFNF farmlets, respectively. With slope averaged across all aspects and farmlets, the percentages were 5.8%, 4.8% and 4.8% for low, medium and high slope, respectively. With aspect averaged across all slopes and farmlets, percentages were 5.8%, 5.4% and 4.4% for NW, E and SW aspects, respectively.

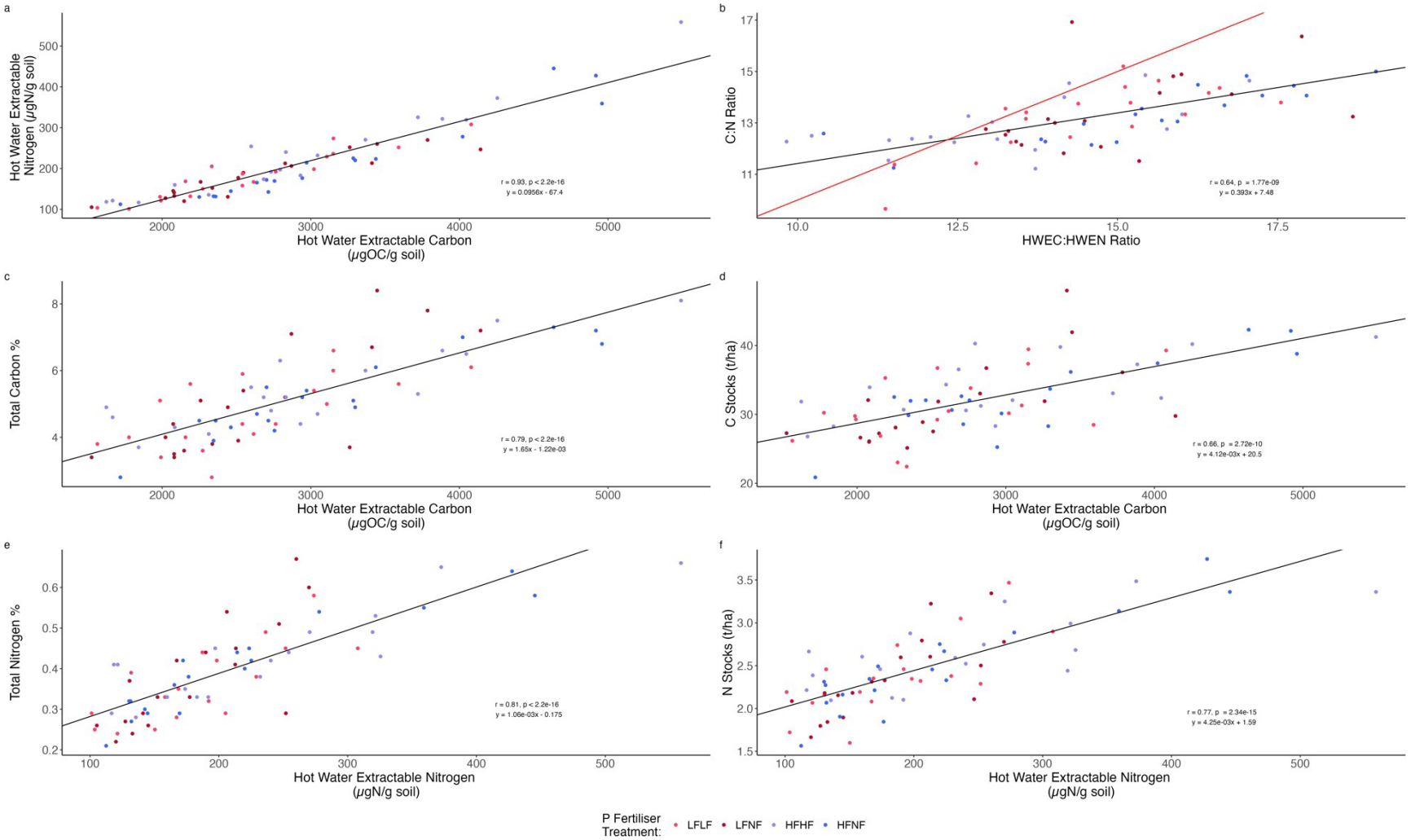

Figure 3.4: Relationship between (a) HWEC and HWEN ($p < 0.001$), (b) HWEC:HWEN and C:N ratio, ($p < 0.001$). The red line indicates the 1:1 ratio of C:N and HWEC:HWEN, (c) HWEC and total C ($p < 0.001$), (d) HWEC and C stocks ($p < 0.001$), (e) HWEN and total N ($p < 0.001$), (f) HWEN and N stocks ($p < 0.001$) across the four farmlets of Ballantrae Research Station, from soils sampled in September, 2021.

3.2.4 Multi-level model

3.2.4.1 Random intercept model

The random intercept model factored only P fertiliser farmlet as a random effect to predict HWEC. Between group variance ($\tau^2 = 144487$) of this model indicates substantial difference between P fertiliser farmlets. The within group variance ($\sigma^2 = 682353$) indicates variance within P fertiliser farmlets and accounts for total unexplained variance of 826,840.

An ICC (ρ) of 0.17, calculated using unexplained variance tells us that 17% of variance in HWEC can be attributed to difference between farmlets (P fertiliser history) (Eq. 3.2). This leaves 83% of variance in HWEC within farmlet groups explained by factors other than the farmlet and remaining unexplained by this model.

3.2.4.2 Adding the random effect (level 2) variables to the multilevel model

The results showed that the model based on slope as a random slope effect within P fertiliser farmlet groups had the best fit ($p < 0.001$) compared to the model based on slope and aspect ($p < 0.05$) and the random intercept only model (Section 3.2.5.1). The model which factored only aspect as a random slope effect within the model was not analysed due to the lack of convergence.

3.2.4.3 Adding the fixed effect (level 3) variables to the multilevel model

This final model accounted for the random intercept effect of farmlet (P fertiliser history), random slope effect of slope and fixed effects of the explanatory variables: OM, effective CEC, microporosity and sulfate S (Table 3.4). The LR test result showed it was the best fit for the Ballantrae data ($p < 0.001$).

Multilevel equation to predict HWEC:

(3.3)

lmer(HWEC (µgOC/g soil) ~ OM (%) + Effective CEC (cmol+/kg) + Microporosity (%) + Sulfate S (mg/kg) + (1 + Slope | Farmlet))

The positive and significant effects ($p < 0.001$) for OM and effective CEC suggest that increase in these variables is strongly associated with higher HWEC concentrations (Table 3.5). The outcome of this model showed that (when all other variables were held constant) for each 1% increase in OM, HWEC increased by 200.82 µgOC/g soil and for each cmol+/kg increase in effective CEC, HWEC increased by 114.83 µgOC/g soil. The negative and significant effects ($p< 0.05$ and $p < 0.001$, respectively) indicated that increased microporosity and sulfate S are associated with lower HWEC concentrations (Table 3.5). For each 1% increase in microporosity, HWEC decreased by 16.32 µgOC/g soil and for each mg/kg increase in sulfate S, HWEC decreased by 18.98 µgOC/g soil.

Table 3.5: Random intercept linear regression model, for the prediction of soil hot water extractable carbon (HWEC) from Ballantrae Research Station.

	HWEC (µg OC/g)		
Predictors	***Estimates***	***Confidence interval***	***P value***
(Intercept)	763.42	267.38 – 1259.46	0.003
OM (%)	200.82	158.43 – 243.22	<0.001
Effective CEC (cmol+/kg)	114.83	88.87 – 140.79	<0.001
Microporosity (%)	-16.32	-26.71 – -5.93	0.003
Sulfate S (mg/kg)	-18.98	-27.37 – -10.59	<0.001

Output of linear model for prediction of HWEC - lmer(HWEC (µgOC/g soil) ~ OM (%) + Effective CEC (cmol+/kg) + Microporosity (%) + Sulfate S (mg/kg) + (1 + Slope | Farmlet)). Estimates represent the predicted effect of each predictor on the response variable (HWEC). The confidence interval provides a within which the true effect of the predictor is likely to fall with 95% confidence. P value < 0.05 indicates significant effect of predictor variable on response variable (HWEC).

3.2.4.4 Model diagnostics of the final model

The distribution of observed HWEC concentrations compared to the HWEC concentrations predicted by the model suggests that the model performs well (Fig. 3.5a). Clustering of observed HWEC concentrations along the line of predicted HWEC concentrations suggests there is a high correlation between the two outcomes. The model appears to account for a high level of variance in HWEC measured across different P fertiliser farmlets and slopes. Residuals of the final model met the assumptions of normality ($p > 0.05$) (Appendix A). The residuals showed constant variance around the predicted HWEC values (Fig. 3.5b), meeting the assumption of homoscedasticity within the model ($p > 0.05$). The VIFs for OM (VIF = 1.3), effective CEC (VIF = 1.3), microporosity (VIF = 1.2) and sulfate S (VIF = 1.2) confirmed multicollinearity within the final model was not significant.

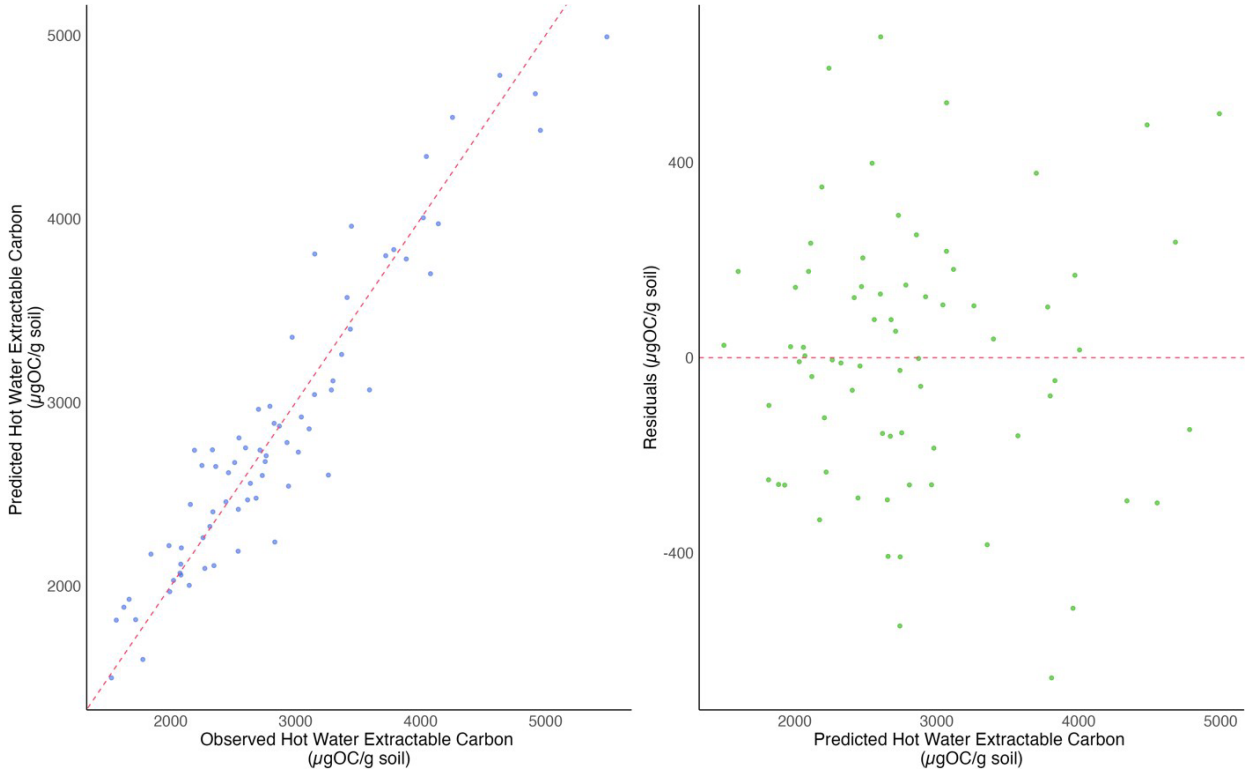

Figure 3.5: HWEC model outcomes for Ballantrae Research Station for soils sampled in 2021. (a) Relationship between the observed HWEC (blue points) and the outcome of the HWEC predictive model (red dashed line). (b) Residuals of the predictive HWEC model for each predicted outcome.

3.3 Discussion

3.3.0 Effect of P fertiliser and sheep grazing history on HWEC and HWEN

The large differences in P fertiliser inputs between the four farmlets dating back to 1975 has resulted in large differences in NPP (6-14 tonnes/ha), sheep stocking rate (6 to 14 su/ha) and P fertility based on Olsen P (7-50 mg/L) across the farmlets of Ballantrae Research Station (Fig. 3.6) Mackay et al., 2021). Despite the large differences in primary production and nutrient (P, C and N) cycling between farmlets, changes in soil HWEC concentrations did not reflect changes in fertility and primary production across the farmlets. Across the four farmlets, HWEC concentrations ranged from only a minimum of 2602 to a maximum of 3134 µg OC/g.

The effects of long-term P fertiliser application rates have been shown to have varying effects on soil C pools across different studies (Anderson et al., 2021; Wakelin et al., 2017). Data from Ballantrae Hill Country Research Station for soils sampled in 2021, 41 years after the original P fertiliser rates were established has shown that high and low P fertiliser applications have had no significant effects on soil HWEC concentrations. This is supported by samples taken from the NZ Winchmore long-term fertiliser trial taken in 2021, which found no increase in HWEC between no, low (188 kgSSP/ha/yr) and high (376 kgSSP/ha/yr) P fertiliser applications with concentrations of 1609, 1591 and 1656 µgOC/g, respectively (Anderson et al., 2021). In an earlier study from Winchmore, Wakelin et al. (2017) reported much lower HWEC concentrations for the no P fertiliser farmlet (1609 µgOC/g) compared to the low and high fertiliser farmlets (2000 and 2050 µgOC/g respectively) (Wakelin et al., 2017). These HWEC concentrations were lower than those measured at Ballantrae in the present study, with HWEC ranging from 2602 to 3134 µgOC/g across the four farmlets.

Under HF (HFHF) treatments on the Ballantrae Hill Country Research Station, studies have found that NPP and animal production has increased significantly as a result of increased Olsen P concentrations when compared to LF (LFLF) treatments (Mackay et al., 2021) (Fig. 3.6). This effect was not reflected in the HWEC and HWEN measured in the current study, with no

significant difference between the LFLF and HFHF farmlets. The amount extracted from the two HF farmlets (2995 and 3134 µg OC/g for the HFHF and HFNF, respectively) and two LF farmlets (2602 and 2654 µg OC/g for the LFLF and LFNF, respectively) appear to fall into two groups and reflect their respective P fertiliser history prior to 1980. This has occurred despite the fact that the HFNF and LFNF have not received any P fertiliser since 1980, with an associated decline in primary production and the amount of C cycled. This is consistent with the findings of Wakelin et al. (2017) at Winchmore, where increased NPP did not correspond with an increase in HWEC, as measured using the method of Ghani et al. (2003).

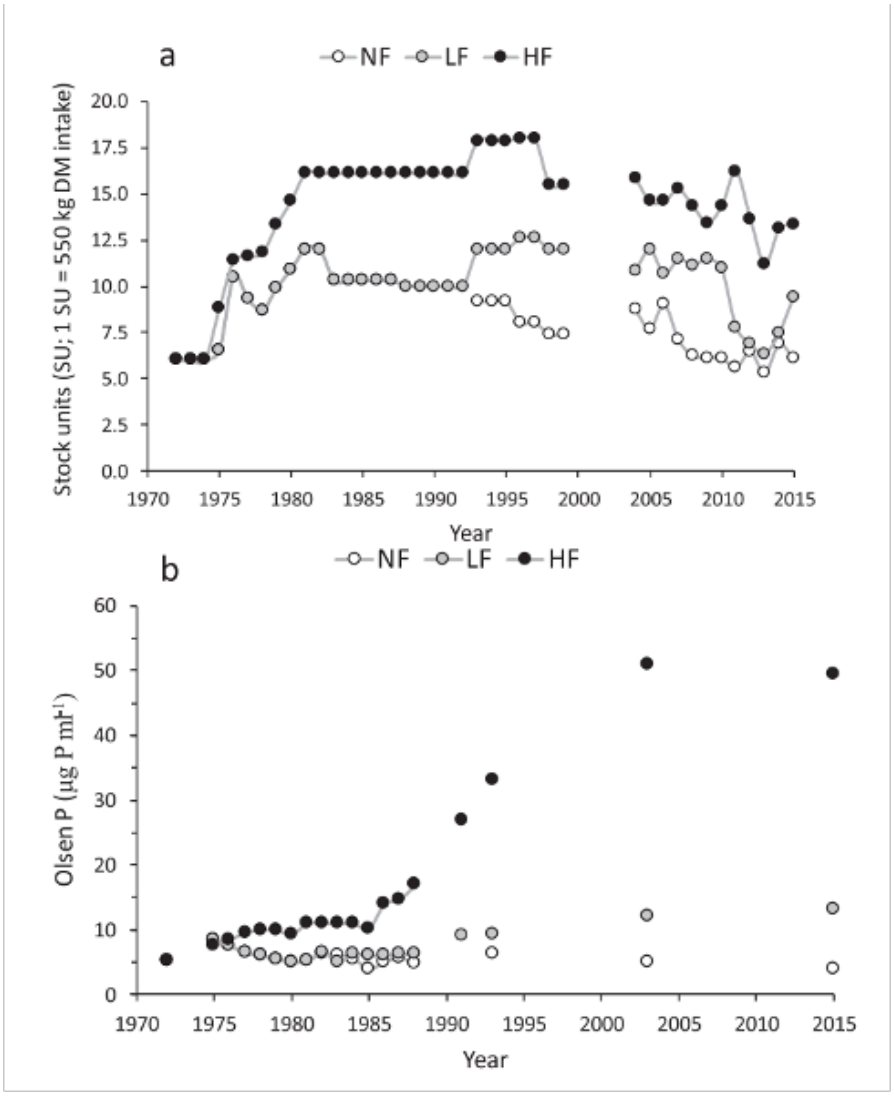

Figure 3.6: Historic stock unit and Olsen P data from the Ballantrae Hill Country Research Station. a) Sheep stocking rates (su/ha) across fertiliser farmlets LFNF and HFNF (NF), LFLF (LF) and HFHF (HF) from 1975 to 2015, (b) Olsen P concentrations (µg/ml) across fertiliser farmlets LFNF and HFNF (NF), LFLF (LF) and HFHF (HF) from 1975 to 2015 (Mackay et al., 2021).

A separate study by Garbuz et al. (2021) at Ballantrae Hill Country Research Station found the amount of root biomass on the low slopes of the LFNF farmlet was higher than on the low slopes of the HFHF farmlet. Despite higher root biomass in LF farmlets, the highest amount of C allocated from root biomass and rhizodeposition was observed from the HF farmlet. This could be attributed to the greater NPP and above ground plant matter under HF farmlets, and therefore greater C and N fixation by plants being returned to the soil. Root biomass and rhizodeposition was the main source of soil C stocks at this long-term experiment, contributing to 62-68% of C stocks (Bilotto et al., 2022). This was followed by litter decomposition (20-29%) and C from dung (9-13%) (Bilotto et al., 2022). The greater root biomass in the LFNF treatment corresponded with an increase in cellulase activity (Bilotto et al., 2022). As a result of the greater cellulase activity it can be expected to see greater quantities of this enhanced root biomass converted to glucose, a compound which is measured in HWEC, and thus increasing HWEC in LFNF farmlets (Bilotto et al., 2022). Garbuz et al. (2021) found the HFHF treatment to have greater phosphatase, NO_3^- reductase, dehydrogenase and peridoxase activity than the LFNF treatment potentially leading to greater rates of C and N cycling. There were some small differences in HWEN between farmlets with 239 µg N/g from the HFHF, declining to 215, 184, and 181 µg N/g for the HFNF, LFLF and LFNF, respectively.

Although not significant, HWEN was higher on the HFNF than the LFLF, despite the fact the HFNF farmlet has not received any P fertiliser for 41 years, while the LFLF has received inputs every year since 1980. This supports the findings of Bilotto et al. (2022) and Wakelin et al. (2017) that C and N cycling is greater with higher P inputs. This gives evidence to suggest that despite greater C and N cycling rates, the microbial biomass and the HWEC and HWEN pools may remain constant but have higher rates of activity under higher P inputs. The greater quantities of dehydrogenase in higher P input soils are indicative of higher microbial activity, and HWEC extracts have been shown to be rich in microbial and plant exudates (Ghani et al., 2003). With P fertiliser inputs comes increased NPP, C and N inputs to the soil and also increased C and N decomposition rates due to a combination of an increase in the amount and quality of the litter returns. These changes may adapt microbial communities to cycle C and N and at a more rapid rate, increasing their activity and more effectively removing C and N from the labile pools through mineralisation in the HFHF farmlets measure by HWEC and HWEN (Wakelin et al.,

2017).

3.3.1 Effects of slope and aspect on HWEC and HWEN

Variations in topography such as slope and aspect create microclimates that affect temperature, moisture, plant growth, grazing and the cycling of C and N, which includes the microbial biomass and labile pools of C and N (Bilotto et al., 2022; Letica et al., 2006; López et al., 2003; Saggar et al., 2004). The effects of slope on HWEC and HWEN measured at Ballantrae were prevalent in the present study, with both measures higher under low slopes than medium or high slopes across all farmlets. Livestock tend to spend more time on low slopes, increasing C and N returns in dung and urine on these areas (Letica et al., 2006; Saggar et al., 2004). This consequentially increases soil fertility which supports greater pasture production (Bilotto et al., 2022). Higher potential erosion rates generally occur on high slope areas (Behre et al., 2012; Fissore et al., 2017; Gao et al., 2024). This can result in the depletion of topsoil which is rich in organic C and N from these areas. Eroded soil commonly deposits on the downslope, lower slope areas, further increasing the difference in HWEC and HWEN measured.

The E and NW aspect was found to have higher HWEC and HWEN concentrations than the SW aspect. Studies have found increased PAR on N facing aspects to increase pasture production (Mackay et al., 2021). This increased pasture production and the resulting OM deposition was reflected by increases in HWEC and HWEN pools. Microclimate effects on labile C and N measured by HWEC and HWEN caused by changes in both slope and aspect were found to be significant.

3.3.2 Relationships between microbial biomass and HWEC

The HWEC measure has been proposed as an estimate of MBC in soils by NZ soils, using the equation MBC = 0.13 x HWEC + 36, calculated from MBC extracted through chloroform fumigation of Ghani et al. (2003). Based on that equation and the data from the current study, the MBC on low slopes of HFHF and LFNF would be estimated to be 562 and 448 µg OC/g, respectively. Comparatively, the MBC values derived by Garbuz et al. (2021) on the low slope of HFHF and LFNF farmlets at ballantrae were 1950 and 3100 µg OC/g for the HFHF and LFNF controls, respectively This suggests that factors such as soil types, sampling time and climate

conditions, which influenced the samples used by Ghani et al, (2003) to develop this equation may be too influential to implement a single predictive equation for MBC using HWEC concentrations.

The measures reported by Garbuz et al. (2021) cannot be directly compared with those reported by Ghani et al. (2003), due to a difference in extraction methods. The MBC reported by Garbuz et al. (2021) is derived from the sum of fungal and bacterial biomass C using substrate induced respiration. Although these measures cannot be directly compared due to the difference in extraction methods used by Garbuz et al. (2021) and Ghani et al. (2003), the HWEC estimated MBC is 1.2- fold larger under the HFHF farmlet than the LFNF farmlet, while the MBC calculated by Garbuz et al. (2021) was 1.6-fold larger under the LFNF farmlet than the HFHF farmlet. Interestingly, the absolute HWEC values for the low slope of the HFHF and LFNF of 4128 and 3247 µg OC/g, respectively, were higher than the values of 1950 and 3100 µg OC/g, respectively reported by Garbuz et al. (2021). This indicates that over a short period of time, the HWEC concentrations in the HFHF farmlet have overtaken the LFNF farmlet. The large disparity between the MBC measured and that estimated from the HWEC concentrations suggests that P fertiliser as a management strategy on a hill country sheep farm may impact the pool of MBC extracted by HWEC. Similarly, Ortner et al. (2022) found HWEC to be more strongly correlated with SOC (R^2 = 0.75), and the correlation to MBC was relatively weak (R^2 = 0.55). Further research should be conducted to determine the actual MBC at these sites, and how they correlate with the HWEC measured to see if this reflects the findings of Ghani et al. (2003).

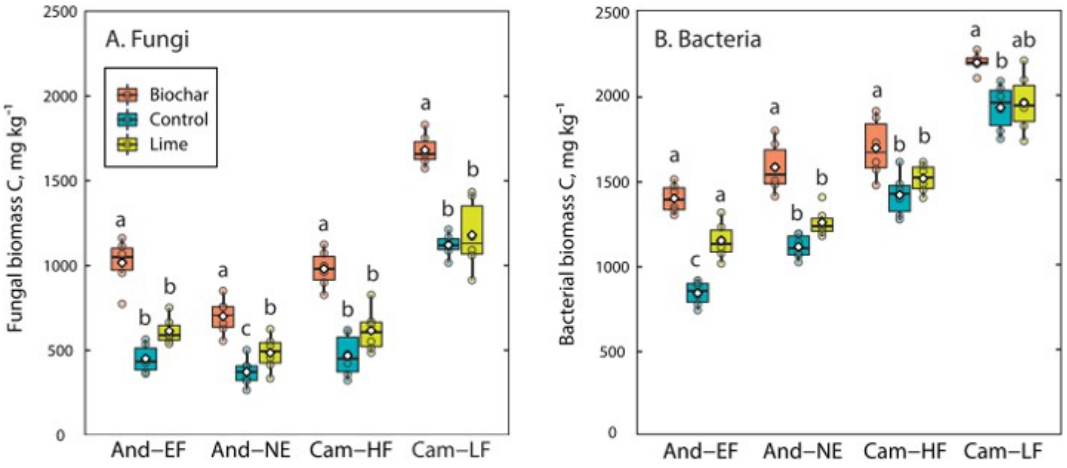

Figure 3.7: Biomass carbon associated with a) fungi and b) bacteria measured from the Ballantrae Hill Country Research Station from soils sampled in 2018 for the Ballantrae HFHF farmlet (Cam-HF) and Ballantrae LFNF farmlet (Cam-LF). Red: willow biochar treatment, blue: no amendments (control), yellow: liming equivalent of biochar treatment. (Garbuz et al., 2021).

3.3.3 The amount of HWEC measured on medium slope compared with all slopes

Soil fertility monitoring on NZ hill country farms is usually conducted along transects on medium slope classes (Schon et al., 2022). The usefulness of HWEC as part of a suite of soil fertility tests will depend largely on how samples taken from these transects represent the HWEC across all slope classes. The HWEC results generated in the current study show that despite variations in HWEC across each slope class, medium slopes are representative of low, medium and high slope classes and as such, analysis of soil samples from the medium slope would be appropriate.

3.3.4 Prediction of HWEC using soil physico-chemical characteristics

Research by Ghani et al. (2003) suggests the HWEC measure as an integrated indicator of soil health and fertility, however, the factors which influence HWEC are not fully understood (Curtin et al., 2020). While the use of the HWEC measure and the factors influencing this pool have been investigated at a broader land use scale, investigations within a land use under different topographical and land management factors are very limited. The findings of the multi-level model showed that P fertiliser and the topographical factor slope had effects on the HWEC outcome, with the latter accounting for more variation. Aspect did not have a significant effect. When these two factors were considered, increased OM and effective CEC had positive effects

on HWEC concentrations, while increased microporosity and sulfate S had negative effects on HWEC concentrations. The reliability of this model to predict HWEC was well supported (Section 3.2.5.4).

The OM content of soil has been well established by multiple studies to contribute to the HWEC pool, however Wakelin et al. (2017) showed that OM was not correlated with HWEC under the Winchmore, NZ P fertiliser study (Ghani et al., 2003). In contrast, the result of the current study supports the use of HWEC to predict this pool under long-term P fertiliser use and under varying topographies, and suggests that P fertiliser applications will not negatively impact the correlation between HWEC and OM. While HWEC is shown to depend on both quantity and quality of OM, there has been suggestion that the CEC and the occupation of these sites by exchangeable cations in the soil may influence the water solubility of OM in soil (Curtin et al., 2020). Findings of Curtin et al. (2020) also showed that OM which has been desorbed from CEC surfaces could be largely contributing to the HWEC extract, with OM compounds extracted in HWEC being predominantly negatively charged.

Significant correlations between HWEC and other labile organic nutrients such as N, P and S have led to the suggestion that the HWEC extract may also provide an indication of the levels of these nutrients in the soil (Ghani et al., 2003). High correlations between HWEC and the HWEN and the total N pool supports the use of HWEC as an indicator of labile and total N. However, the weaker correlation of HWEC with Olsen P in the current study, even when accounting for P fertiliser application, does not support the suggestion made by Ghani et al. (2003).

The multi-level predictive HWEC equation does support HWEC as an indicator of inorganic S when slope and P fertiliser are accounted for. However, the nature of the relationship between HWEC and sulfate S in this model was negative. Potential causes for this are not certain. Increasing sulfate S causes acidification, decreasing soil pH. Decreases in pH have been shown to reduce the water solubility of labile C, reducing HWEC concentrations measured in soil (Curtin et al., 2016; Curtin et al., 2020). However, the lack of correlation between pH and sulfate S suggests that this is unlikely to be a mechanism in this relationship. Studies under long-term P

(superphosphate) fertiliser use found that C bonded (organic) S was the dominant form of this compound in these soils (Curtin et al., 2007). Due to the likely origins of sulfate S in the soils at Ballantrae being mineralized OM, increases in sulfate S could suggest increases in decomposition of OM by microbes. Increases in sulfate S may also overcome stoichiometric C:S constraints of microbes (Curtin et al., 2007). This would therefore lead to increase C sequestration and removal of C from the labile pool (Giweta et al., 2014). This would reduce the OM pool and therefore the HWEC concentrations in soil. However, sulfate S and OM showed a positive correlation in this study, which negates this theory. No research under long term P fertiliser use has been conducted on the relationship between labile C, and inorganic S. Findings by Curtin et al. (2007) have, however, found that under long term P fertilisation there was no significant relationship between inorganic S and total C.

Compounds extracted in HWEC are critical in the formations of aggregates, due to their cementing and binding properties (Botenelli et al., 2010; Chahal et al., 2021; Mehra et al., 2018; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001; Zhang et al., 2019). Due to this mechanism, it could be expected that microporosity and HWEC would show a positive relationship, with increasing HWEC concentrations enhancing aggregation and subsequently microporosity. However, the results of the model developed in the current study contrast this, with increasing HWEC concentrations corresponding with a decrease in microporosity and vice versa. Increased presence of micropores may indicate greater aggregation of soil (Regelink et al., 2015). The OM protected within water-stable aggregates may therefore not be detected in the HWEC test (Regelink et al., 2015).

3.3.5 HWEC, HWEN, total C and total N correlations

The HWEC and HWEN measures were highly correlated with each other and also with their respective total C and N pools. This supports other research that HWEC and HWEN are accurate predictors of the larger and slower fluctuating C and N pools and C and N sequestration potential (Bankó et al., 2021; Ghani et al., 2003). While there were some changes in the amounts of HWEC, HWEN and total C and N between fertiliser treatments, this was not significant and did not appear to have any effect on the ratio of the HWEC and HWEN pools to the total C and N pools.

The fact that few differences were found in HWEC between farmlets is consistent with the fact that there are few differences in soil organic C stocks to 300mm between the farmlets (Mackay et al., 2021). Mackay et al. (2021) suggested in that study that there was slightly more organic C in the 0-75mm soil layer in the HFHF (5.42% C) than either the LFLF (5.22% C) or LFNF (5.17% C). Other studies have also found few differences in soil organic C stocks under different P fertiliser rates in long-term pastures (Condron et al., 2012; Schipper et al., 2013). It would appear that, like total organic C, the labile organic C fraction extracted by hot water is close to a fixed percentage of total C across HFHF (5.5%), HFNF (6.0%), LFLF (5.5%) and LFNF (5.2%) farmlets. The source of organic C and the mechanisms governing the size of the labile organic C fraction appear to vary with increasing P fertiliser inputs and the associated increase in NPP, C and nutrient cycling, but these are not reflected in the fraction extracted by hot water.

Surprisingly, the HWEC:HWEN ratio was wider than the total C:N ratio in the samples where the ratio was greater than 12.5:1 (Fig. 3.4). The majority of these samples above the 1:1 line which had a greater HWEC:HWEN ratio than the C:N ratio were from the HFHF farmlet. The fact that the HWEC:HWEN ratio in > 70% of the samples was greater than the C:N ratio indicates that HWEC has extracted more organic C compounds than HWEN has organic N compounds. This finding aligns with that of Curtin et al. (2006) who found approximately a large proportion (approximately 20%) of HWEN to consist of heat-labile organic compounds. Due to the hydrolysis of these compounds to NH_4^+-N during the extraction, the newly formed inorganic N would have a strong affinity to cation exchange sites to which it may adsorb and be removed from the HWEN extract (Curtin et al., 2006). This mechanism resulting in lowered recovery of N in HWEN is believed to lead to a gross underestimation of HWEN, increasing the HWEC:HWEN ratio (Curtin et al., 2006).

3.4 Conclusions

The results from the current study suggest that the higher levels of NPP and C and N cycling in the HFHF farmlet than the other three farmlets has resulted in greater OM return to soil but has not necessarily contributed to the labile C and N pools. This has implications for the application

of HWEC and HWEN as an indicator of changes in the labile C and N pools that would be expected with a change in NPP. Furthermore, the relationship between HWEC and MBC deviates largely from the equation used to estimate MBC using HWEC which has been suggested by Ghani et al. (2003). Although the samples which this equation is based off were taken years prior, this equation is being promoted by NZ laboratories as a method of MBC estimation. Topographical effects on both HWEC and HWEN were as expected, with low slopes and NW and E aspects measuring higher HWEC and HWEN concentrations. Furthermore, samples taken from medium slopes were shown to be representative of HWEC across all slope classes. This evidence is significant when identifying representative sites in hill country for monitoring soil health.

The multi-level model indicated that when P fertiliser and slope were accounted for, OM, effective CEC, sulfate S and microporosity were the best predictors for HWEC. While this supports the HWEC fraction being largely derived from OM, the negative impacts of sulfate S and microporosity were unexpected.

The HWEC and HWEN measures were highly correlated with each other and were representative of their respective total C and total N pools under varying P fertiliser inputs and sheep grazing history, slope and aspects. This shows that HWEC and HWEN can provide an accurate indication of total C and total N in the soil across a diversity of landscape units and under different P fertiliser inputs and grazing strategies.

Chapter 4

Changes in Hot Water Extractable Carbon and Nitrogen following regenerative and contemporary tillage and summer fodder cropping

4.0 Introduction

Conservational tillage practices such as direct drilling with no prior cultivation have been shown to protect soil carbon (C) and nitrogen (N) dynamics within soils when compared to contemporary tillage methods such as power-harrowing (Bottinelli et al., 2017; Mehra et al., 2018; Bongiorno et al., 2019; Rutledge et al., 2017; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001; Silva-Olaya et al., 2012). Whereas soil disturbance through cultivation can decrease soil structure (aggregate stability), stimulate the oxidation of soil organic matter (OM) and increase greenhouse gas emissions (CH_4, CO_2 and N_2O) (Bottinelli et al., 2017; Mehra et al., 2018; Bongiorno et al., 2019; Rutledge et al., 2017).

Prior to direct drilling, the application of herbicide to control weeds and remove current pasture is a common practice (Espig et al., 2023). Glyphosate, an organophosphate, is the most commonly used herbicide in New Zealand (NZ) (Chávez-Ortiz et al., 2022; Harrington & Ghanizadeh, 2023; Lane et al., 2012). Glyphosate has the potential to degrade microbial communities (Chávez-Ortiz et al., 2022; Lane et al., 2012). The extent of this degradation largely relies on the application rates of glyphosate (Chávez-Ortiz et al., 2022).

Selection pressures on microbial communities can result in the selection for microbial organisms which are able to use glyphosate as an energy source. This results in changes in the microbial community (Chávez-Ortiz et al., 2022; Lane et al., 2012). Both negative and positive implications on microbial respiration have been reported, however, these are mostly observed under long-term applications of glyphosate (Chávez-Ortiz et al., 2022; Haney et al., 2017; Lane et al., 2012).

These impacts on microbial respiration result in either decreased or increased C mineralisation and CO_2 emissions, respectively (Chávez-Ortiz et al., 2022; Haney et al., 2017; Lane et al., 2012). No impacts on N mineralisation following glyphosate application have been reported (Haney et al., 2017).

Soil C and N dynamics are greatly impacted by the form and intensity of tillage used within a system (Bongiorno et al., 2019; Chahal et al., 2021; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001). The soil disturbance resulting from tillage practices influences soil C largely due to aeration of the soil (Chahal et al., 2021). Exposure of soil micro-organisms to O_2 stimulates microbial respiration, leading to the oxidation of organic C, the release of CO_2, and the mineralisation and sequestration of C (Colombi et al., 2018; Mehra et al., 2018). Labile C compounds broken down during this process are typically those measured in hot water extractable carbon (HWEC), such as glucose (Colombi et al., 2018; Mehra et al., 2018). Conflicting results have been found regarding the emission of nitrous oxide (N_2O) following conservational and no-till practices. Increased aeration of the soil has the potential to alter the aerobic condition of the soil and therefore to increase the N_2O fluxes in the soil as a by- product of nitrification (Grave et al., 2018; Liu et al., 2018; Yoo et al., 2016; Yuan et al., 2024; Zurovec et al., 2017).

In addition to this, direct physical impacts caused by tillage, accelerates decomposition of soil organic carbon (SOC) (Mehra et al., 2018; Saggar et al., 2004). Physical protection plays a large role in the protection and turnover of SOC and total N, and the breaking up of soil macro-aggregates through tillage, exposes this C and N to attack by decomposing micro-organisms (Botenelli et al., 2010; Chahal et al., 2021; Liu et al., 2017; Mehra et al., 2018; Zhang et al., 2019). SOC enhances soil physical properties including soil aggregation through cementing and binding properties (Botenelli et al., 2010; Chahal et al., 2021; Mehra et al., 2018; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001; Zhang et al., 2019). Thus, the loss of this C can lead to detrimental effects on macro- and micro-aggregation, hydraulic properties of soil, O_2 diffusion and habitat provision for biodiversity, all of which are important in maintaining general soil health (Bongiorno et al., 2019; Botenelli et al., 2010; Chahal et al., 2021; Mehra et al., 2018;

Zhang et al., 2019).

Dairy pastures in NZ are generally ryegrass (*Lolium perenne*) and clover based (*Trifolium* spp). However, the use of hyper-diverse multispecies pastures has been proposed as one way of increasing root biomass and C and N sequestration (Rowarth et al., 2020). Multi-species pastures can increase rooting diversity leading to the potential for C and N sequestration at greater depths within the soil profile (Beare et al., 2014; Fornara & Tilman, 2008; McNally et al., 2015; Rutledge et al., 2017; Wall et al., 2021). With the high levels of C sequestered in NZ topsoils, this mechanism could optimise the capability of soils to sequester greater quantities of C (Beare et al., 2014; Fornara & Tilman, 2008; McNally et al., 2015; Rowarth et al., 2020; Rutledge et al., 2017; Wall et al., 2021).

Increased diversity of roots can also increase the root niche occupation within the soil, leading to greater OM input by root biomass and increased protection of intra-aggregate OM (Beare et al., 2014; McNally et al., 2015). Furthermore, greater utilization of plant available N from greater depths throughout the soil profile, reduces the potential for N losses via leaching. Diverse rooting systems have also been shown to mitigate conditions leading to denitrification and N_2O emissions due to their positive effects on water uptake and gas diffusivity within the soil (Cummins et al., 2021; Laubach et al., 2023).

The hot water extractable C (HWEC) and N (HWEN) method extracts highly labile C and N pools, which are effective in capturing the immediate effects of soil management practices, compared to the time required to observe changes in total soil C stocks (Benbi et al., 2012; Toosi et al., 2012). These measures have also been suggested to be early indicators of an increase in the sequestration of total C and N pools (Bongiorno et al., 2019; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001). As previously determined, increased tillage activity and intensity alters the turnover and labile C and N within the soil. Tillage has also been shown to affect microbial activity and microbial communities. Therefore, HWEC and HWEN measurements have been identified as an early indicator of change in the labile soil C and N as well as microbial biomass C and N, with which it is highly correlated, following tillage (Bongiorno et al., 2019; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001).

Common laboratory-based measures used to monitor labile C in soil, in response to cultivation include dissolved organic C (DOC), hydrophilic DOC (Hy-DOC), permanganate oxidisable carbon (POXC), HWEC and particulate OM C (POMC) (Bongiorno et al., 2019; Plaza-Bonilla et al., 2014; Saggar et al., 2004; Shen et al., 2021; Shepherd et al., 2001). Studies have determined that POXC and HWEC extract similar fractions of total C and are the most sensitive to tillage of the previously mentioned measures (Bongiorno et al., 2019; Shen et al., 2021). HWEC is a more cost-effective measure, requiring less labour for preparation, fewer reagents and less equipment. This makes it an economical and effective measure of providing farmers with valuable insight into how cultivation practices are impacting soil C.

Measurements of labile N following cultivation include microbial biomass nitrogen (MBN), potentially mineralisable nitrogen (PMN) and HWEN (Liu et al., 2018; Zuber et al., 2018). HWEN is the most cost-effective, as it has a less intensive requirement for labour and equipment than the other labile N measures. It has also been shown to be highly sensitive to changes in the total N pool within the soil (Bankò et al., 2021; Sun et al., 2017).

The quantities of N fertiliser used in NZ dairying systems is greater than in sheep and beef systems (Thatcher, 2019). Increases in net primary production (NPP) resulting from N fertiliser use, leads to increased return of plant residues to the soil and the potential for increased SOC (Voltr et al., 2021). This increased return of plant residues to the soil promotes microbial decomposition and has the potential to have either a positive or detrimental effect on the SOC pool and on mineralisable N, measured by HWEC and HWEN (Ghani et al., 2003; Moran et al., 2005; Voltr et al., 2021). Tracking changes in HWEC and HWEN pools may offer an option to ensure N fertiliser use delivers increased pasture growth but does not impact negatively on the soils OM pool.

4.0.0 Objectives

While a large number of studies have demonstrated the advantages of reduced tillage on labile and total C and N in soils, the use of HWEC and HWEN as early sensitive indicators of changes in this fraction and in the total C and total N pools is limited. The introduction of summer crops using a range of tillage and crop management practices to an established grazing pasture provided an opportunity to explore the following objectives:

- To assess the impact of summer fodder cropping, including herbicide application and tillage methods on the HWEC, HWEN, total C %, and total N % contents of soil sown with a diverse pasture in relation to contemporary practice.
- To investigate the relationship between HWEC and total C % and HWEN and total N % throughout the cultivation, establishment and growth of the fodder crop to determine if the ratio between these measures change over the course of the cropping cycle.

4.1 Methods

4.1.0 Study site

The Whenua Haumanu dairy farmlet is located within Massey University's Dairy One research farm, located in Palmerston North, NZ (40.376°S, 175.613°E). The farmlet study consists of a 12-ha treatment area and is situated at 35m above sea level. Landscape is flat to rolling with Recent alluvial soils which are weakly structured and have textures varying from sandy loam with stones to silt loam. The average annual rainfall on the farm is 980mm and average 10cm soil temperatures are 7°C in July and 18.5°C in January, based on long-term (30yr) climate data (NIWA, 2024). Lactating dairy cattle are grazed across the farm, with once-a-day milking and spring calving.

This long term (7 yr) study established in 2022 has three farmlet treatments which aim to explore the effects of diverse pasture and regenerative management on a range of soil, pasture, environmental and animal parameters. The background farmlet treatments include (a) standard ryegrass-legume based pasture under contemporary management (StdCon), (b) diverse species pasture under regenerative management (DivRgn) and (c) diverse species pasture under contemporary management (DivCon), each stocked at 2.5 cows/ha in the 2023-2024 season.

Contemporary management is described as having lower post-grazing residuals and the use of mineral fertiliser and chemicals as required. Regenerative management is described as having longer grazing intervals, higher post-grazing residuals and low to no mineral fertiliser and chemical use.

Standard pastures established as part of the farmlet study were sown in April 2022 and consisted of diploid and tetraploid ryegrass, red clover (*Trifolium pratense*), large leaved white clover (*Trifolium repens*) and medium large leaved white clover (*Trifolium repens*). The diverse species pastures were established at the same time and consisted of diploid and tetraploid perennial ryegrass, tall fescue (*Festuca arundinacea*) meadow fescue (*Festuca pratensis*), cocksfoot (*Dactylis glomerata*), timothy (*Pleum pratense*), Pharalis (*Pharalis aquatica*), prairie grass

(*Bromus catharticus*), red clover, large leaved and medium large leaved white clover, chicory (*Chicorium intybus*), plantain (*Plantago lanceolata*), crimson clover (*Trifolium incartum*), Balansa clover (*Trifolium michelianum*), Persian clover (*Trifolium resupinatum*), vetch (*Vicia sativa*) and lucerne (*Medicago sativa*).

Three paddocks were selected from the broader farmlet study to establish a summer crop in November 2023 (51A (StdCon cropped, 1.03ha), 51B (DivRgn cropped, 1.0ha) and 51C (DivCon cropped, 1.0ha). Another 3 paddocks of the same soil type that remained in pasture over the study period were used as a paired comparison (16A (StdCon not cropped, 1.08ha), 16B (DivRgn not cropped, 1.08ha), 16C (DivCon not cropped, 1.09ha).

In this study, conducted within the pre-established farmlet system, only three paddocks were cropped. Therefore, not cropped plots monitored for background HWEC, HWEN, total C % and total N % changes under the existing pasture could not be situated within the same paddocks as cropped plots. Therefore, direct comparisons could not be made between cropped and not cropped treatment plots, due to their spatial separation and possible differences in historical management. As a consequence, each of the cropped and not cropped areas have been treated as a separate case on which to collect baseline data on the likely sensitivity of the labile and total C and N under existing pasture management, during the climate and soil temperatures associated with the study period.

4.1.1 Climate

Climate data was obtained from the Palmerston North Ews NIWA weather station 21963 (40.381°S, 175.609°E). This station is situated 650m away from the study site, at 21m above sea level. Data obtained from this station included daily rainfall (mm), average daily temperature (°C) and soil temperature (°C) measured to a depth of 10cm under permanent pasture. Data was collected from 30 days prior to pre-spraying soil sampling (19/9/23) until the end of the study (16/2/23), covering the entire research period.

4.1.2 Treatments

Due to the pre-existing farmlet design and the fact that only one paddock for each treatment was cropped as part of the farm system (as described in section 4.1.1), the decision was made to select six paddocks with the same soil type, with three of these paddocks being sown to a summer fodder crop and three paddocks remained in their original pasture treatment.

The six study paddocks contained Manawatū Fine Sandy Loam (Selw_122a.1) soils (Manaaki Whenua Landcare Research, 2024). This soil type only made up approximately half of each of paddocks 16A-C, so sampling areas were restricted to within these soil types (Fig. 4.3).

Manawatū Fine Sandy Loam is a Typic Alluvial Recent Soil present to 15cm over 70cm of sand, over gravel (Manaaki Whenua Landcare Research, 2024). This soil is well-drained, with moderate to high water holding capacity and moderate N leaching vulnerability (Manaaki Whenua Landcare Research, 2024). Manawatū Fine Sandy Loam has high structural vulnerability at the surface 30cm depth (Manaaki Whenua Landcare Research, 2024). The three treatment paddocks under the control pasture treatment (not cropped) and the three treatment paddocks transitioning into a summer crop (cropped) (Fig. 4.1) were subject to the management described in Table 4.1.

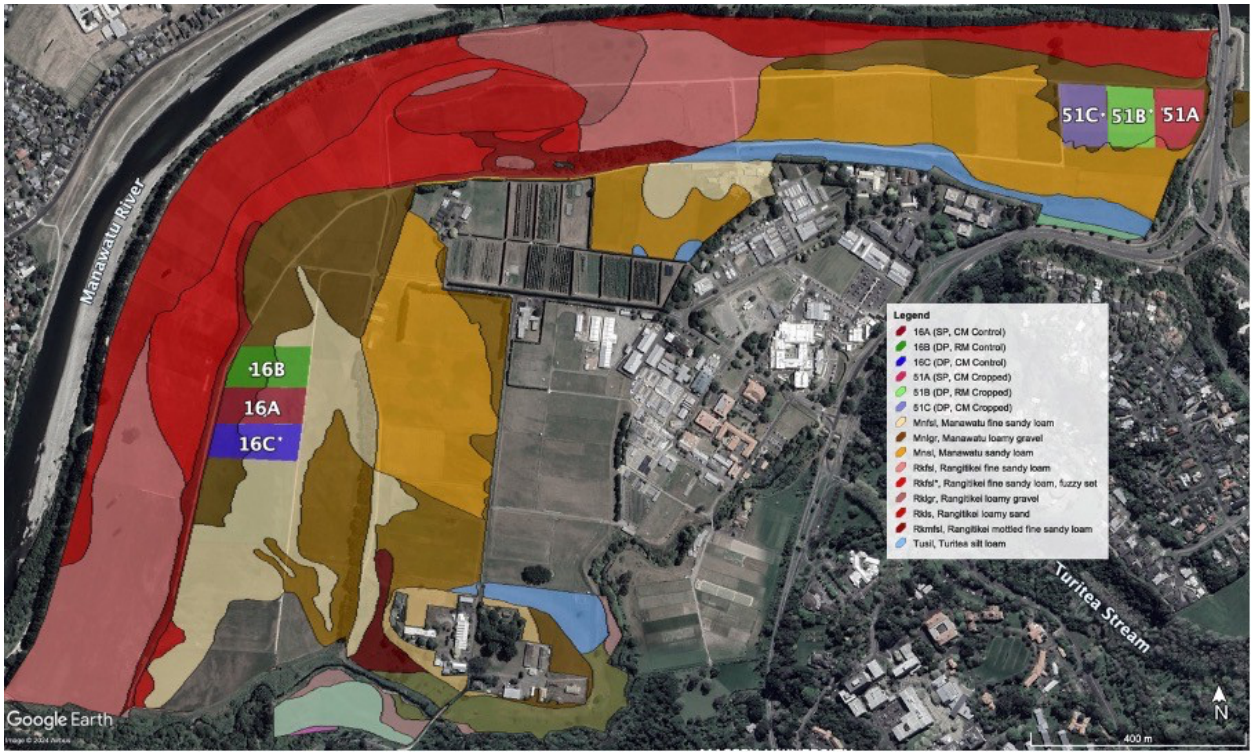

Figure 4.1: Treatment paddock locations within Dairy One farm (Whenua Haumanu project) in Palmerston North, NZ. Paddocks 51A, 51B and 51C were summer cropped and paddocks 16A, 16B and 16C remained in their existing pasture treatments.

Table 4.1: Grazing, fertiliser, herbicide and cropping activities for the period 19/9/23 to 16/02/23 for cropped and not cropped StdCon, DivCon and DivRgn treatments within the Whenua Haumanu farmlet study.

Date	**Not cropped**			**Cropped**		
	StdCon	***DivCon***	***DivRgn***	***StdCon*** *Turnips*	***DivCon*** *Diverse crop*	***DivRgn*** *Diverse crop*
19/9/23 - 23/9/23					Grazing	
10/9/23 - 1/10/23		Grazing				
26/9/23 - 28/9/23				Grazing		
1/10/23 - 2/10/23	Grazing					
4/10/23 (Week 0)	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling
9/10/23			45 kg/ha Ammonium Sulphate [a] (9kgN/ha) 40kg/ha Sulphur 90 [b]			
14/10/23				**Herbicide spraying [c]**	**Herbicide spraying [c]**	**Herbicide spraying [c]**
17/10/23				**Power-Harrowing**		
20/10/23 (Week 2)	Soil sampling			Soil sampling		Grazing
21/10/23 - 23/10/23					Grazing	
24/10/23				**Direct drilling [d]**	**Direct drilling [e]**	**Direct drilling [e]**
27/10/23 (Week 3)	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling
27/10/23 - 29/10/23	Grazing	Grazing				
31/10/23 - 2/11/23			Grazing			
1/11/23	1000 kg/ha AgLime [f]	2000 kg/ha AgLime [f]		1000 kg/ha AgLime [f]		
23/11/23 - 25/11/23	Grazing					
24/11/23 - 26/11/23		Grazing				
8/12/23 (Week 9)	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling
10/12/23 - 12/12/23			Grazing			
20/12/23	180 kg/ha Ammonium Sulphate [g] (26kgN/ha)	180 kg/ha Ammonium Sulphate [g] (26kgN/ha)		180 kg/ha Ammonium Sulphate [g] (26kgN/ha)	180 kg/ha Ammonium Sulphate [g] (26kgN/ha)	
24/12/23 - 26/12/23	Grazing					
31/12/23 - 4/1/24		Grazing	Grazing			
27/1/24 - 30/1/24	Grazing					
31/1/24 - 3/2/24		Grazing	Grazing			
23/1/24 - 16/2/23				Grazing [h]	Grazing [h]	Grazing [h]
16/02/23 (Week 19)	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling	Soil sampling

[a]: N:P:K:S – 9:0:0:10, [b]:N:P:K:S – 0:0:0:36, [c:] Glyphosate 360 at 3L/ha, [d]: Sown in Dynamo turnips (*Brassica rapa*) (3kg/ha), [e]: Sown in spitfire brassica (*Brassica napus*), balansa clover (*Trifolium michelianum*), crimson clover (*Trifolium incarnatum*), red clover (*Trifolium pratense*), forage maize (*Zea mays*), chicory (*Cichorium intybus*), buckwheat (*Fagopyrum esculentum*), Japanese millet (*Echinochloea esculenta*), sorghum/sudan hybrid grass (*Sorghum bicolor* x *Sorghum sudanese*), sunflowers (*Helianthus anuus*) and diakon radish (*Raphanus sativus*) (34 kg/ha), [f]: (Ca 39%), [g]: (N:P:K:S - 26:0:0:30), [h]: Paddock break-fenced, 250 m^2 grazed daily.

4.1.3 Soil sampling

Within each treatment paddock, four plots (8.66 x 15m) were established from within the bounds of the Manawatū Fine Sandy Loam soil type, as replicates to assess the variation in soil measurements associated with summer cropping and undisturbed pasture (Fig. 4.3, Fig. 4.4). The limitations of this approach are acknowledged, but unavoidable due to the restrictions described in section 4.1.1 and 4.1.3. Real time kinematic (RTK) GPS was used to mark the plots to ensure these areas were accurately sampled throughout the study. Within each plot, three transect lines arranged in a Z configuration were sampled, with 10 soil cores sampled to a depth of 7.5cm along each transect (Fig. 4.2). The total of 30 cores from each plot were bulked for analysis.

Baseline soil samples were taken at week 0, prior to spraying of herbicide in cropped treatment plots (4/10/23). StdCon treatment plots were sampled at week 2, post-power-harrowing of the cropped treatment plots (20/10/23). All treatment plots were sampled at week 3, three days post drilling of cropped treatment plots (27/10/23), at week 9, six weeks post-drilling of cropped treatment plots (27/10/23) and at week 19, 16 weeks post-drilling of cropped treatment plots (16/2/24) (Table 4.1). Soil samples were oven-dried at 40 °C immediately post-sampling and ground to pass through a 2mm sieve in preparation for chemical analysis.

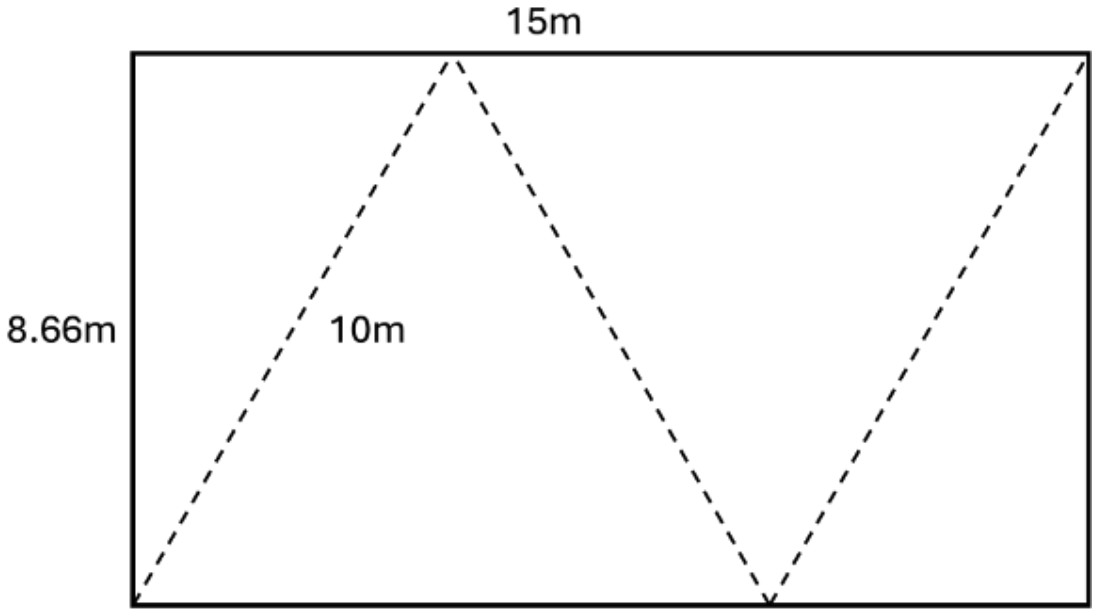

Figure 4.2: Dimensions of sampling plots and sampling transect lines within plots measured in each Whenua Haumanu farmlet.

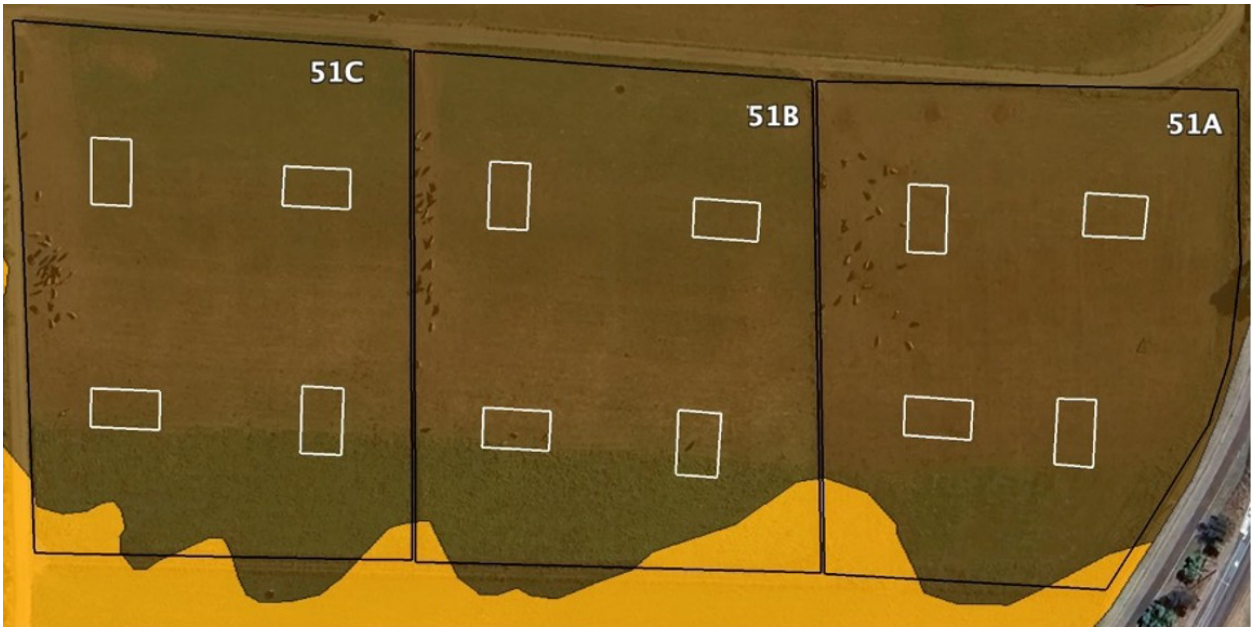

Figure 4.3: Sampling plot layout within the Whenua Haumanu treatment paddocks. Treatments were (51A) StdCon – power-harrowing, direct drilling, turnips; (51C) DivCon cropped – direct drilling, diverse summer fodder crop and (51B) DivRgn cropped – direct-drilling, diverse summer fodder crop. Pasture and management strategies prior to cropping are as follows: Std: ryegrass and white clover pastures, Div: diverse pastures, Con: contemporary management and Rgn: regenerative management.

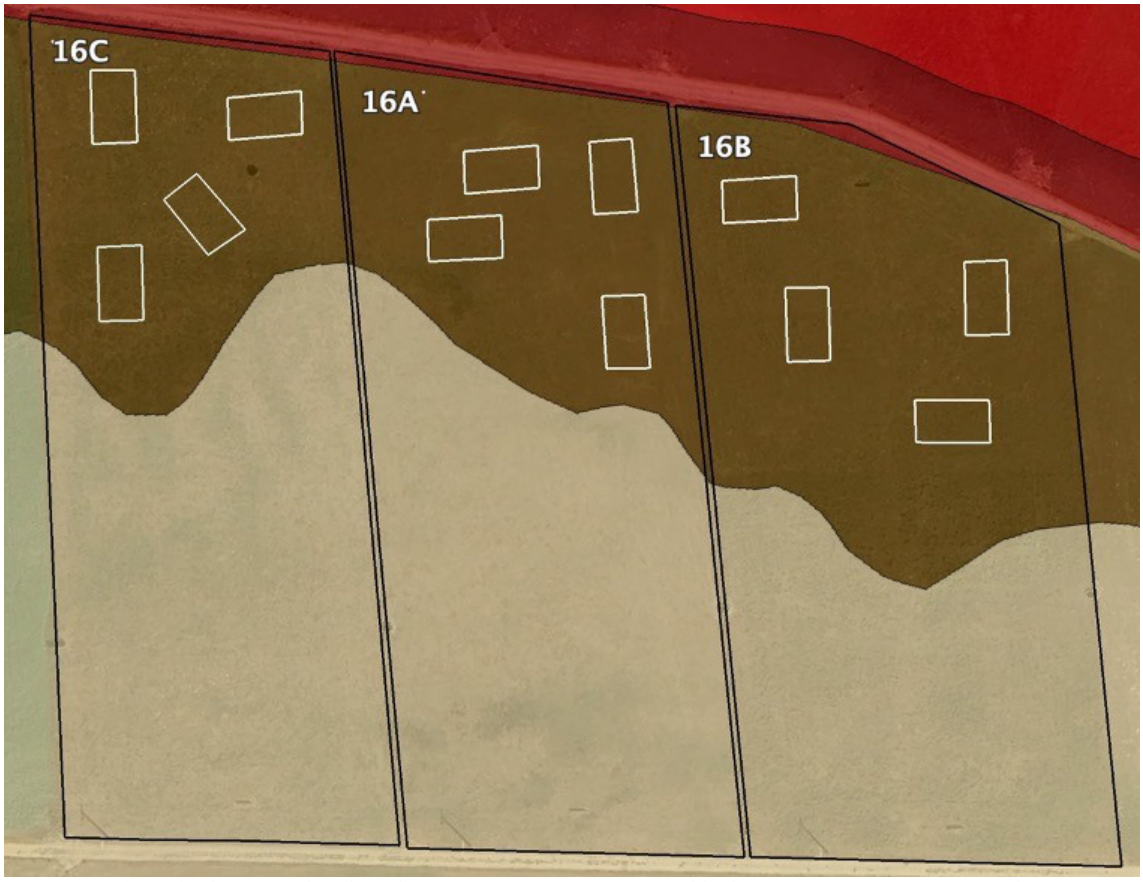

Figure 4.4: Sampling plot layout within the Whenua Haumanu treatment paddocks. Treatments were (16A) StdCon not cropped, (16C) DivCon not cropped and (16B) DivRgn not cropped. Pasture and management strategies are as follows: Std: ryegrass and white clover pastures, Div: diverse pastures, Con: contemporary management and Rgn: regenerative management.

4.1.4 Chemical analysis

4.1.4.1 HWEC and HWEN

Hot water extractable C and N were analysed using the method of Ghani et al. (2003). Three grams of soil was added into 50mL polypropylene centrifuge tubes. Thirty mL of distilled water was then added to the centrifuge tubes, and the soil samples were extracted for 30 minutes on an end-for-end shaker at 30rpm. These samples were then centrifuged at a rate of 3500rpm for 5 minutes at 20 °C to separate the extractant and soil supernatant. Using a 0.45 µm cellulose nitrate NO_3^- membrane filter, followed by centrifugation at 3500 rpm for 5 minutes. The extractant was then filtered using a 0.45 µm cellulose NO_3^- membrane filter.

Thirty mL of distilled water was added to the polypropylene centrifuge tube containing the cold-

water extracted soil supernatants. These supernatants were then resuspended in the solution by inverting by hand. Hot water extraction was then performed by submerging the soil samples in a water bath at 80 °C for 16hr. These were then centrifuged at 3500rpm for 5 minutes. Using a 0.45 µm cellulose NO_3^- membrane filter, the extractant was filtered.

Hot water filtrates are analysed for organic C using a Shimadzu TOC-V_{CSH}/TNM-1 analyser. Fourty µl of the extract was injected into the detection chamber of the analyser. Following a 9-minute analysis, the total C and inorganic C in the samples were measured using non-dispersive infrared (NDIR). The difference between the total C and total inorganic C in the extracts is calculated to determine the organic water-soluble C concentration. Using a SKALAR analyser, ammonium-N (NH_4^+-N) and NO_3^--N was measured in the samples. The difference between these measures determines the quantity of water-soluble organic N in the extracts.

4.1.4.2 Analysis of total C % and total N %

Total C % and total N % was measured using the Dumas method of combustion (Nelson & Sommers, 1996), and a VarioMAX Elementar Analyser. Five hundred g of soil was combusted at 900 °C in a pure oxygen atmosphere, completely decomposing all N containing compounds and converting them to nitrous oxides (NO and NO_2) and all C compounds, converting them to methane (CH_4) and carbon monoxide (CO). These nitrous oxides were then reduced to elemental N (N_2) and C gases were oxidised to carbon dioxide (CO_2) using the catalysts copper oxide and platinum. The percentage of elemental N and CO_2in the sample was measured using thermal conductivity to determine total C % and total N %.

4.1.5 Statistical analysis

The objective of the statistical analysis was to assess changes in HWEC and N in each of the six treatment paddocks to determine temporal changes under a range of tillage and cropping practices. While the lack of paddock replication limited the statistical comparison between treatments, it created a pool of data to examine the changes in HWEC, HWEN, total C and total N pools to identify differences in the rates of change in these two fractions under a range of tillage and cropping practices as well as temporal changes.

R version 4.2.3 (2023-03-15) was used to produce all statistical analysis. Descriptive statistics

(minimum, mean, maximum and IQR) were calculated for all outcome variables (HWEC, HWEN, total C and total N).

Linear regression tests were performed for each treatment individually, to determine any statistically significant overall changes in HWEC, HWEN, total N and total N throughout the study period. Two-sided, two-sample t-test assuming unequal variance were also performed on each sampling group to determine statistically significant differences in HWEC, HWEN, total C and total N between each time of sampling to analyse changes throughout sampling.

Management and climate factors (soil temperature and cattle grazing) were plotted alongside these outcome variables to account for any effects these may have had on the data collected. Correlation tests (Spearman and Pearson) were used to evaluate the relationship between HWEC and HWEN, HWEC and total C and HWEN and total N. Scatterplots were produced to show the correlations between outcome variables within each treatment. Correlation tests were used to evaluate whether soil temperature had any effect on HWEC, HWEN, total C and total N. The amount of HWEC was calculated as a percentage of the total C pool.

4.2 Results

4.2.0 Climate

Soil temperature measured at the nearby weather station at the time of pre-spraying (week 0, 4/10/23) was 7.3 °C and increased by 6.5 °C to 13.8 °C at the time of post-power-harrowing (week 2, 20/10/23). A 0.6 °C decrease in soil temperature to 13.2 °C was measured at the time of post-drilling (week 3, 27/10/23) which increased by 4.0 °C to 17.2 °C, at 6 weeks post-drilling (week 9, 8/12/23). At 16 weeks post-drilling (week 19, 16/02/24), a further increase in soil temperature of 2 °C was measured, with soil temperatures increasing to 19.2 °C (Fig. 4.2).

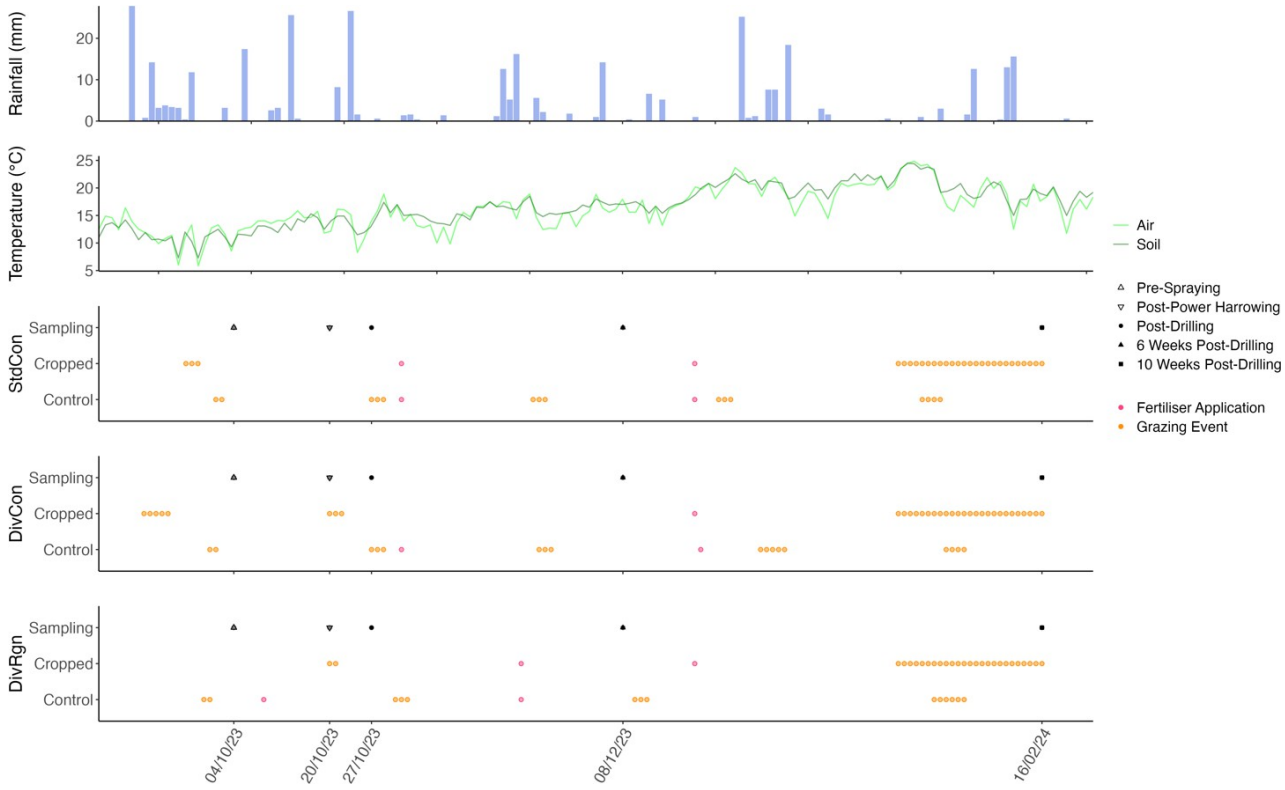

Figure 4.5: Rainfall (mm), soil and air temperature (°C), fertiliser applications, grazing events and sampling events under tillage treatments during the study period. Climate data obtained from the Palmerston North Ews NIWA weather station (number 21963) (NIWA, 2024).

To remind the reader, the standard pasture under contemporary management (StdCon), diverse pasture under contemporary management (DivCon) and diverse pasture under regenerative management (DivRgn) not cropped treatment plots remained in pasture for the duration of the study period and continued to be grazed (Table 4.1). The StdCon cropped treatment plots underwent glyphosate spraying, power-harrowing and drilling and were sown in a monocrop of turnips (Table 4.1). The DivCon and DivRgn cropped treatment plots both underwent glyphosate spraying and direct-drilling and were sown in a diverse summer fodder crop (Table 4.1).

4.2.1 HWEC changes in not cropped and cropped treatments

4.2.1.1 Not cropped

The StdCon, DivCon and DivRgn not cropped treatments showed no significant overall change in HWEC throughout the study period (Fig. 4.6). The HWEC concentrations varied from a minimum of 1723 to a maximum of 2630 µg OC/g for not cropped treatments. An insignificant increase in HWEC concentrations was measured in the DivCon treatment at week 0 compared to week 2. Throughout the rest of the study period (week 2 to week 19), HWEC concentrations showed a decreasing trend, however this wasn't significant. The most notable change in HWEC in the StdCon plots occurred between sampling at week 0 and week 2, where the mean HWEC increased, however this was not significant. Although no cropping, grazing or fertiliser events occurred during this period, a 2-day grazing event occurred 3 days prior to the week 0 soil sampling (Table 4.1, Fig. 4.6). This event may influence the HWEC concentration measured in week 0, affecting the change measured between the first and second soil sampling times.

Throughout the rest of the period (week 2 to 20) the HWEC concentration remained fairly constant, with no significant difference between each sampling time.

4.2.1.2 Cropped

The StdCon cropped treatment plots underwent glyphosate spraying, power-harrowing and drilling and were sown in a monocrop of turnips (Table 4.1, Fig. 4.6). With these interventions, there was no significant overall change in HWEC concentrations throughout the study period. A slight decrease in mean HWEC concentrations was measured at post power-harrowing (week 2), however this was not significant. No grazing or fertiliser events occurred during the period between spraying and power-harrowing (Table 4.1, Fig. 4.6). Concentrations of HWEC remained

relatively constant throughout the rest of the period [post-drilling (week 3), 6-weeks post-drilling (week 9) and 16-weeks post-drilling (week 19)] with a slight increasing trend, but this was not significant. Fertiliser events prior to the 6 and 16-week samplings (week 9 and 19) and a grazing event occurring for the 24 days leading up to and including the 16-week sampling (week 19), did not have any significant effect on the HWEC concentrations (Table 4.1, Fig. 4.6).

The DivCon and DivRgn cropped treatment plots both underwent glyphosate spraying and direct drilling and were sown in a diverse summer fodder crop (Table 4.1, Fig. 4.6). DivCon and DivRgn treatments showed no significant overall change in HWEC throughout the study period. The mean HWEC concentration of the DivRgn treatment plots showed a slight increase at post-drilling (week 3), however this was not significant. The HWEC concentrations varied from a minimum of 1067 to a maximum of 2095 μg OC/g for copped treatments.

4.2.1.3 Comparison between not cropped and cropped plots

The initial week 0 HWEC correlations were similar between contemporary and regenerative plots for each treatment. The time of sampling was shown to have no overall effect on the mean HWEC concentration within both the cropped and not cropped plots of the StdCon treatment (Fig. 4.6). Although not significant, the observable increase in mean HWEC between the pre- spraying (week 0) sampling time and the post-power harrowing (week 2) sampling time coincided with an insignificant, observable decrease in the mean HWEC within the cropped treatment plots. This suggests that power-harrowing may have caused a loss of labile C within the cropped treatment plots, although no definite conclusions can be made due to the lack of significance of this result.

Under the cropped treatment, the mean HWEC appeared to increase from power-harrowing (week 2) onwards while the not cropped treatment plots appeared to decrease from this sampling time onwards (Fig. 4.6). While these differences between individual sampling times were not significant, it appears that HWEC concentrations increased with time following the initial decrease seen at power harrowing. However, as time of sampling had some influence on HWEC within both cropped and not cropped treatment plots, we cannot determine whether these changes were caused by contemporary cropping practices, contemporary pasture management practices or standard pastures because of the absence of true replication. Comparatively, the mean HWEC was not influenced by the sampling time within the cropped and not-cropped DivCon or DivRgn

treatments. This suggests that direct drilling in the absence of power harrowing appeared to cause minimal impacts on soil labile C measured by HWEC test.

Furthermore, the establishment of a diverse summer fodder crop did not show any positive effects on the HWEC concentrations in the soil over the study period.

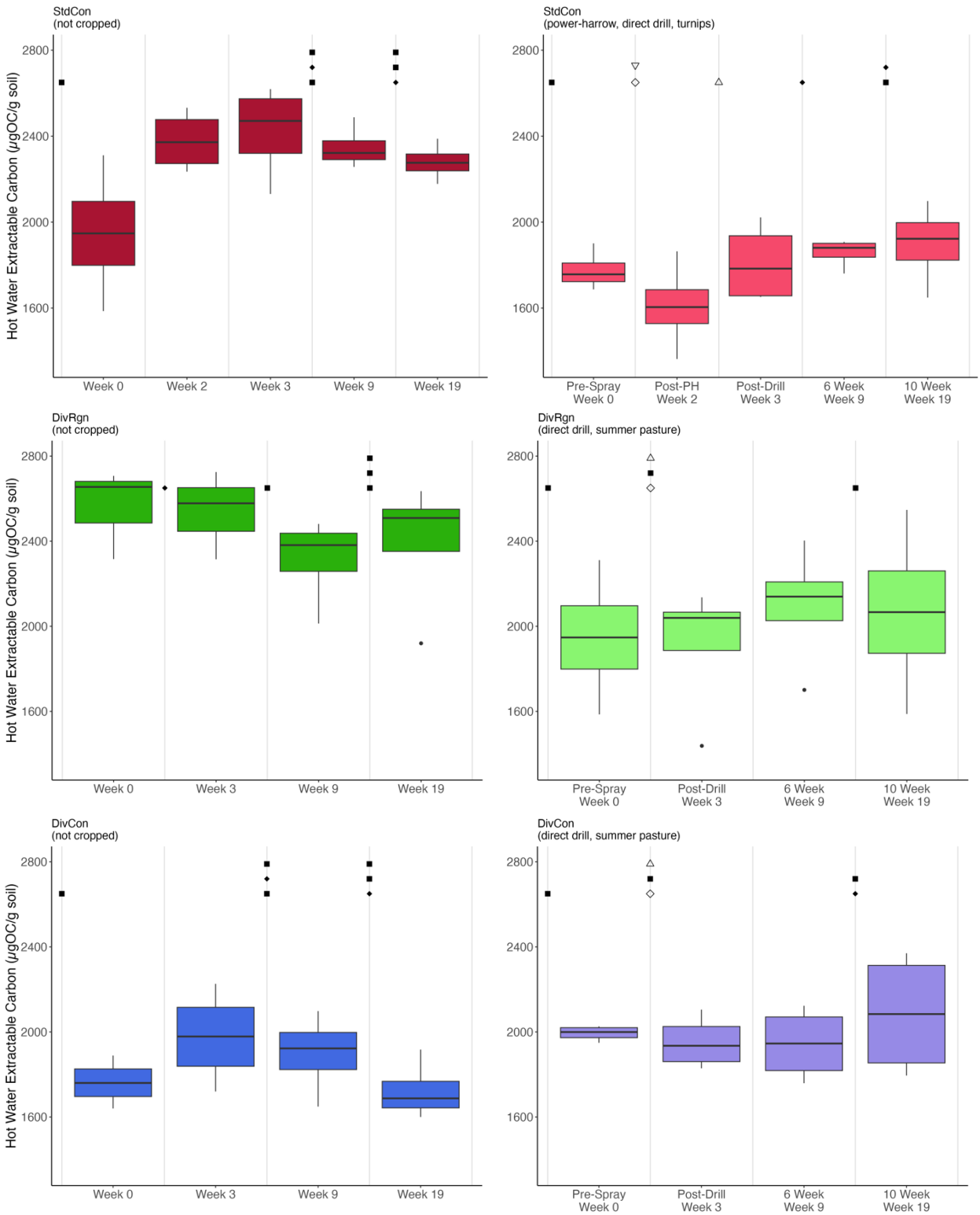

Figure 4.13: Changes in HWEC between treatments including: StdCon not cropped ($p > 0.05$), StdCon power-harrow, direct drilling, turnips (Brassica rapa) ($p < 0.05$), DivCon not cropped ($p > 0.05$), DivCon direct drilling, diverse summer fodder crop ($p > 0.05$), DivRgn not cropped ($p > 0.05$) and DivRgn direct drilling, diverse summer fodder crop ($p > 0.05$) throughout the study period. P-values were generated using linear regression tests performed for each treatment individually.

▽: Power-harrowing, Δ: Direct drilling, ♦: Fertiliser application, ■: Grazing event

4.2.2 Total C % changes in not cropped and cropped treatments

4.2.2.1 Not cropped

StdCon, DivCon and DivRgn treatment plots all showed no significant overall change in total C % (Fig. 4.6).

4.2.2.2 Cropped

The StdCon cropped treatment plots showed a significant overall increase in total C % concentrations ($p < 0.05$) throughout the study period.
The DivCon and DivRgn cropped treatment plots showed no significant overall change in total C % throughout the study period. A notable but not significant increase was observed between post-drilling (week 3) and 6 weeks post-drilling (week 9).

4.2.2.3 Comparisons between not cropped and cropped

Under the not cropped treatment plots of the StdCon treatment, no significant overall change in total C was observed (Fig. 4.7). The slight change in HWEC was not reflected in the total C response over time (Fig. 4.3, Fig. 4.7). However, under the cropped treatment plots of the StdCon treatment, a significant ($p < 0.05$) overall increase in total C was observed. This increase occurred despite a non-significant decrease in total C after power-harrowing occurred. An increasing trend was seen from the time of drilling through until the end of the study period. This reflected changes seen in HWEC within these treatment plots (Fig. 4.6). However, the overall change in total C was of higher significance than that of HWEC. This suggests that greater variability in the HWEC concentrations were measured compared to total C, and therefore may be influenced by other variables such as climate.

Both cropped and not cropped treatment plots of each DivCon and DivRgn treatments measured no significant changes in total C. This lack of change in total C reflects the stability observed in HWEC measures and suggests that total C concentration was not negatively influenced by the sowing of a diverse, summer active pasture. The lack of change in total C measured under the StdCon not cropped treatment supports the finding that total C was influenced by the practice of power-harrowing in the cropped treatment.

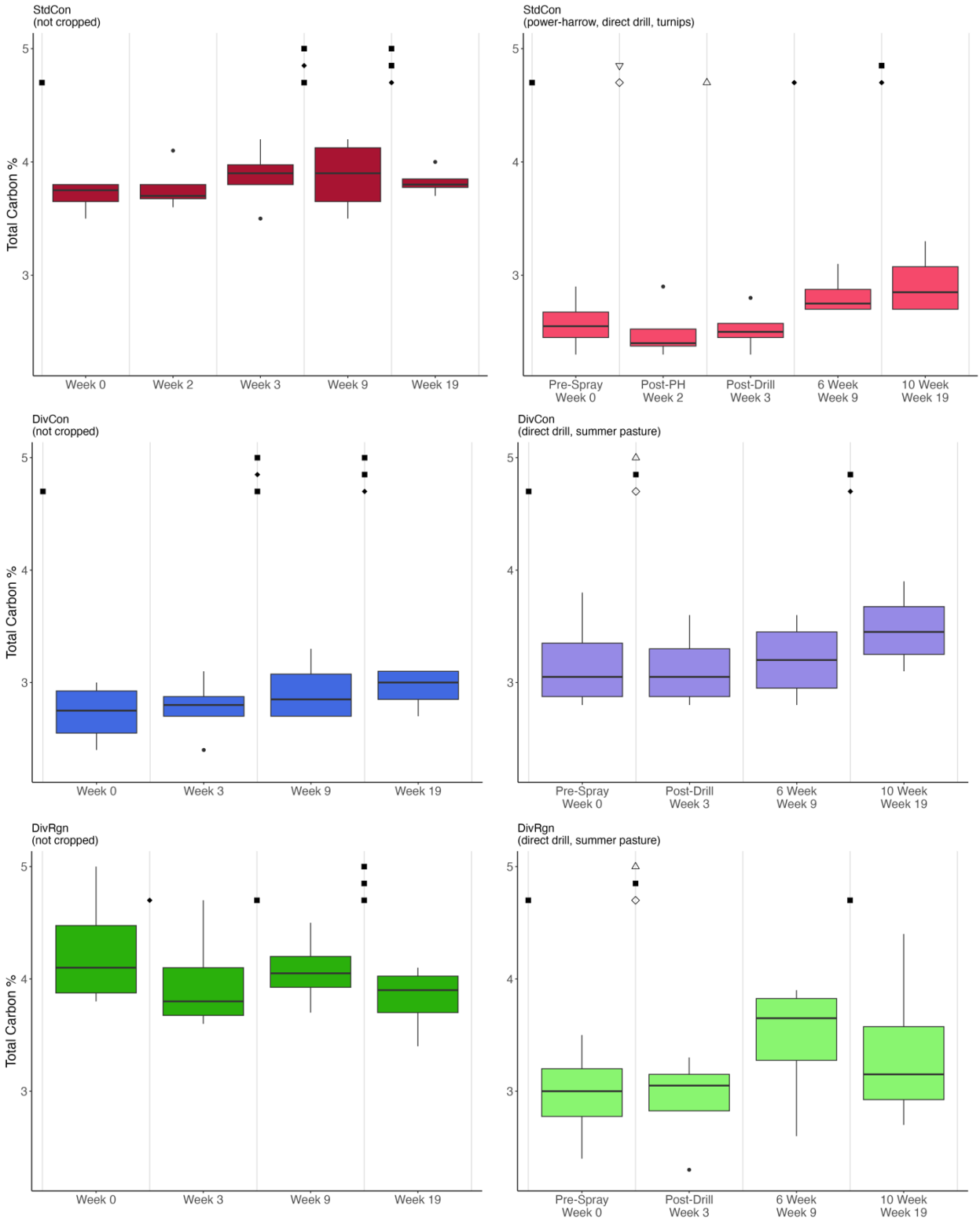

Figure 4.7: Changes in Total C % between treatments including: StdCon not cropped ($p > 0.05$), StdCon power-harrow, direct drilling, turnips (Brassica rapa) ($p < 0.05$), DivCon not cropped ($p > 0.05$), DivCon direct drilling, diverse summer fodder crop ($p > 0.05$), DivRgn not cropped ($p > 0.05$) and DivRgn direct drilling, diverse summer fodder crop ($p > 0.05$) throughout the study period. P-values were generated using linear regression tests performed for each treatment individually.

▽: Power-harrowing, Δ: Direct drilling, ♦: Fertiliser application, ■: Grazing event

4.2.3 HWEN changes in not cropped and cropped treatments

4.2.3.1 Not cropped

The StdCon, DivCon and DivRgn treatments showed no significant overall change in HWEN throughout the study period (Fig. 4.8). The HWEN concentrations varied from a minimum of 179 to a maximum of 303 µg N/g for not cropped treatments. There were no significant changes in HWEN between individual sampling times throughout the study period, despite the occurrence of N additions through both fertiliser (ammonium sulphate) and N return through grazing events (Table 4.1, Fig. 4.8).

4.2.3.2 Cropped

The StdCon cropped treatment plots underwent glyphosate spraying, power-harrowing and drilling and were sown in a monocrop of turnips (Table 4.1, Fig. 4.8). Despite these interventions, there was a significant positive overall change in HWEN concentrations throughout the study period. HWEN concentrations showed a decrease at the post-power harrowing sampling compared to the pre-spraying (week 0) sampling, however this was not significant. An increasing trend in HWEN was observed between post-power harrowing (week 2) through until 16-weeks post-drilling (week 19). This increase was greatest between post- drilling (week 3) and 6-weeks post-drilling (week 9) but not significant. A fertiliser application of N (ammonium sulphate) occurred during this period (Table 4.1, Fig. 4.8). Changes between all other sampling times were insignificant, despite an additional N fertiliser (ammonium sulphate) application prior to the 16-weeks post-drilling (week 19) and a grazing event occurring for the 24 days leading up to and including 16-week post-drilling (week 19) sampling within the treatment plots (Table 4.1, Fig. 4.8).

The DivCon and DivRgn cropped treatments showed no significant overall change in HWEN throughout the study period. No notable or statistically significant changes were observed between sampling times in either of these treatments. The N fertiliser applications and grazing events did not appear to have any influence on the HWEN concentrations under these treatments (Table 4.1, Fig. 4.8). The HWEN concentrations varied from a minimum of 174 to a maximum of 255 µg N/g for cropped treatments.

4.2.3.3 Comparison between not cropped and cropped

The initial week 0 HWEN correlations were similar between contemporary and regenerative plots for each treatment. Only the cropped plots under StdCon treatments showed a slightly significant increase in HWEN throughout the study period (Fig. 4.8). The insignificant decrease in HWEN after power harrowing of the cropped treatment plots coincided with a non significant decrease in the not cropped treatment plots. This suggests a potential loss of labile N from the soil may have occurred, however due to the limitations of the study design, this conclusion cannot be definitively asserted.

The further increasing trend in the StdCon treatment plots from post-drilling onwards was not seen in the cropped treatment plots. None of the cropped or not cropped treatment plots of DivCon or DivRgn treatments showed any overall significant change in HWEN.

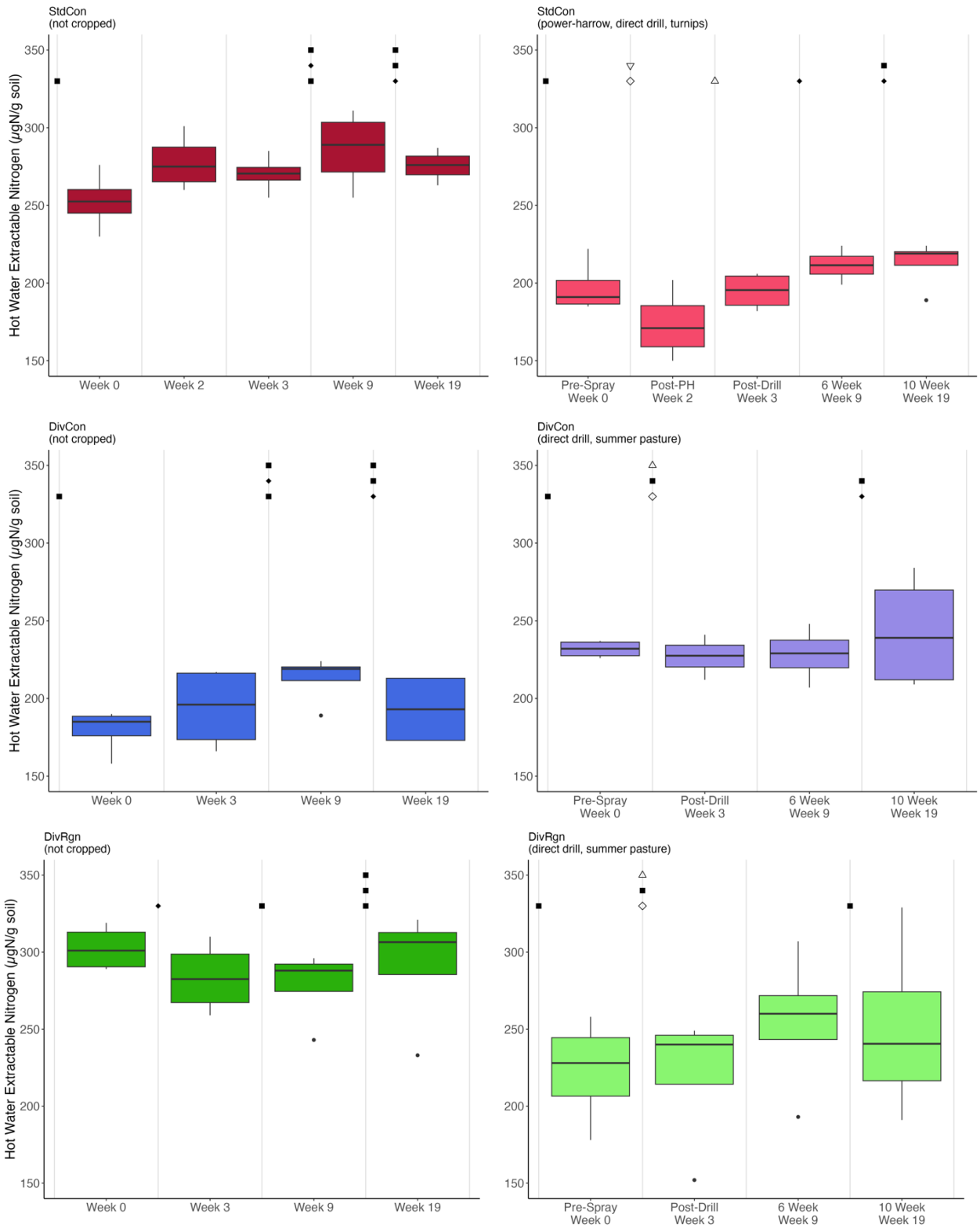

Figure 4.8: Changes in HWEN between treatments including: StdCon not cropped ($p > 0.05$), StdCon power-harrow, direct drilling, turnips (Brassica rapa) ($p < 0.05$), DivCon not cropped ($p > 0.05$), DivCon direct drilling, diverse summer fodder crop ($p > 0.05$), DivRgn not cropped ($p > 0.05$) and DivRgn direct drilling, diverse summer fodder crop ($p > 0.05$) throughout the study period. P-values were generated using linear regression tests performed for each treatment individually.

▽: Power-harrowing, Δ: Direct drilling, ♦: Fertiliser application, ■: Grazing event

4.2.4 Total N% changes in not cropped and cropped treatments

4.2.4.1 Not cropped

The StdCon, DivCon and Div Rgn not cropped treatments showed no significant overall change in total N, (Fig. 4.9).

4.2.4.2 Cropped

A significant increase ($p < 0.05$) in total N throughout the study period occurred under the StdCon cropped treatment (Fig. 4.9). Comparatively, the StdCon not cropped treatment plots did not show any change in total N throughout the study period. N fertiliser applications were equivalent in both of these treatment plots, and the not cropped StdCon treatment plots was grazed more frequently, leading to greater inputs of NO_3^- through animal excreta (Table 4.1, Fig. 4.9). The not cropped treatment plots did not experience any notable declines in total N concentrations, and the cropped treatment plots saw an overall positive increase despite an initial decrease following power-harrowing. This suggests that the establishment of the turnip monocrop led to increases in soil total N.

4.2.4.3 Comparisons of not cropped and cropped

No significant changes in total N were seen under either cropped or not cropped treatment plots of the DivCon and DivRgn treatments (Fig. 4.9). These treatments showed only minor fluctuations throughout this time, none of which were significant. Within the StdCon treatments, only the cropped treatment showed a significant increase ($p < 0.05$) while the not cropped treatment showed no change.

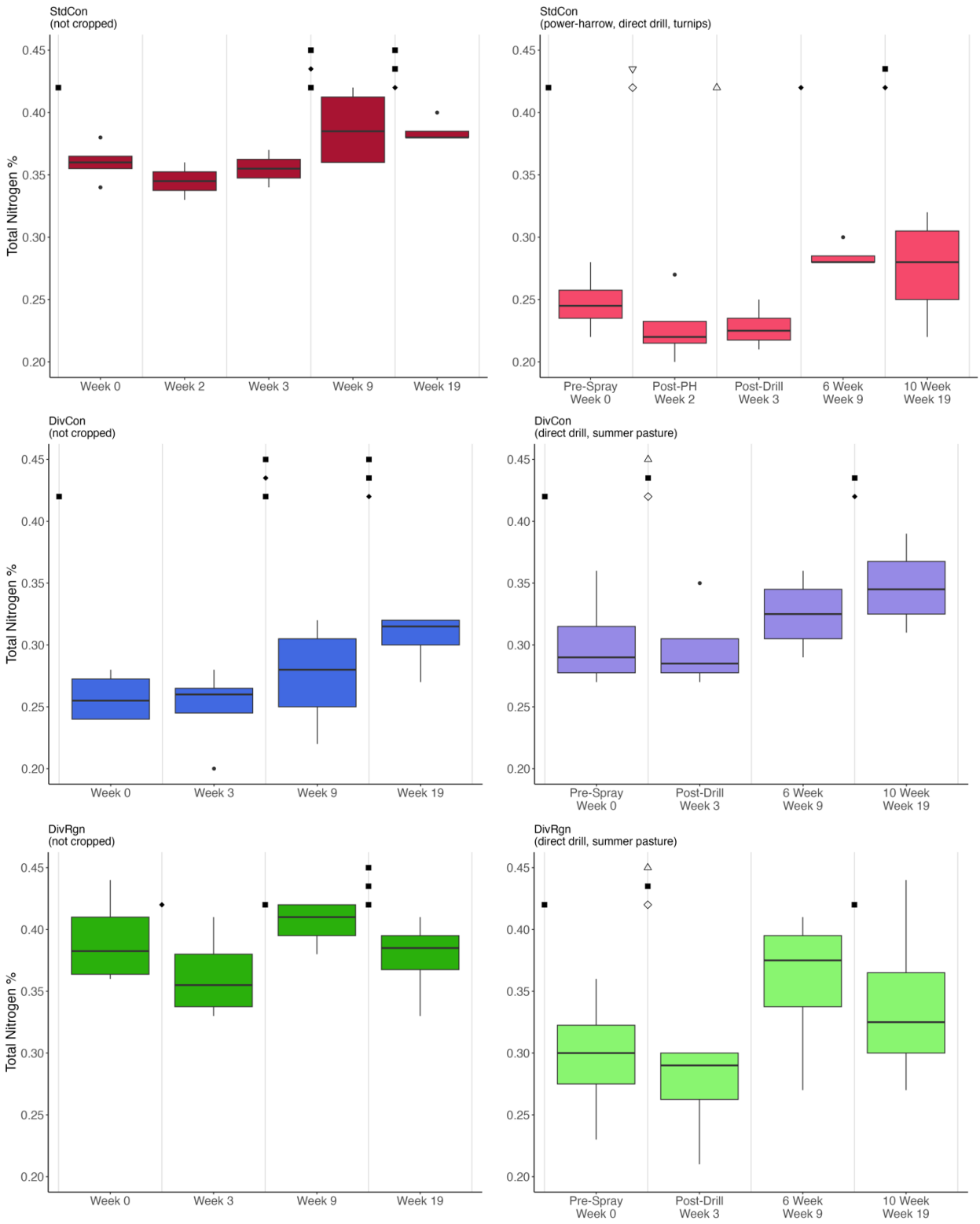

Figure 4.9: Changes in total N % between treatments including: StdCon not cropped ($p > 0.05$), StdCon power-harrow, direct drilling, turnips (Brassica rapa) ($p < 0.05$), DivCon not cropped ($p > 0.05$), DivCon direct drilling, diverse summer fodder crop ($p > 0.05$), DivRgn not cropped ($p > 0.05$) and DivRgn direct drilling, diverse summer fodder crop ($p > 0.05$) throughout the study period. P-values were generated using linear regression tests performed for each treatment individually.

▽: Power-harrowing, Δ: Direct drilling, ♦: Fertiliser application, ■: Grazing event

4.2.5 HWEC and total C % changes with tillage treatments

Correlations between total C and HWEC in soil did not change across all treatments (StdCon, DivCon, DivRgn) (Fig. 4.10). There were no trends in the linear relationship between total C and HWEC concentration caused by sampling time or tillage. This tells us that HWEC was measuring the same quantity of labile C present in the total C pools despite temporal, climatic and management factors such as tillage and N fertiliser applications. Under cropped treatments there was a more pronounced increase in total C with increasing HWEC, than the not cropped.

The correlation was significant for all cropped treatments ($p < 0.05$) but was not significant for StdCon and DivRgn not cropped treatments (Fig. 4.10).

The percentage of HWEC in the total C pool did not change significantly throughout the study period, under either cropped or not cropped treatments. These percentages varied from 6.0 to 8.5% under cropped treatments, and 5.7 to 7.1% under not cropped treatments. Detailed analysis of these proportions can be found in Appendix B.

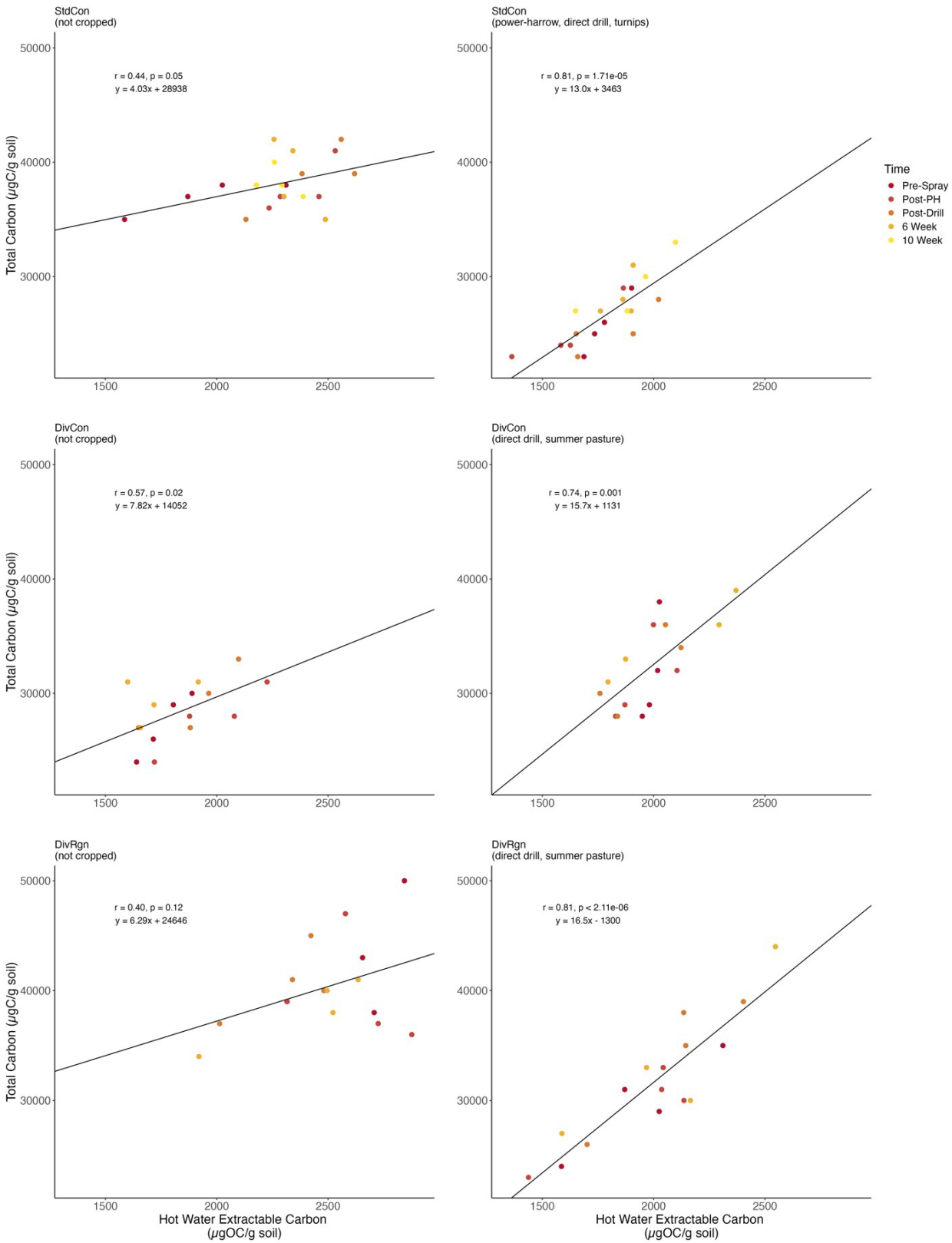

Figure 4.10: Correlations between Total C % and HWEC for both cropped and not cropped plots for the StdCon, DivCon and DivRgn treatments. The linear equation for each correlation and their r values are presented within each graph and correlations were significant when $p < 0.05$.

4.2.6 HWEN and total N % changes with tillage treatments

Linear relationships between total N and HWEN in soil did not change over the study period in both cropped and not cropped treatment plots across all treatments (StdCon, DivCon, DivRgn) (Fig. 4.11). There were not trends in the linear relationship between total N and HWEN concentration caused by sampling time or following tillage treatments. This tells us that HWEN was measuring the same quantity of labile N present in the total N pools despite temporal, climatic and management factors including tillage and N fertiliser applications. Under cropped treatments, the correlation was significant ($p < 0.05$) and there was a more pronounced increase in total N with increasing HWEN than not cropped. Under not cropped treatments, the correlation was not significant.

The percentage of HWEN in the total N pool did not change significantly throughout the period, under either cropped or not cropped treatments. These percentages varied from 6.9 to 8.6% under not cropped treatments, and 6.2 to 8.1% under cropped treatments. Detailed analysis of these percentages can be found in Appendix B.

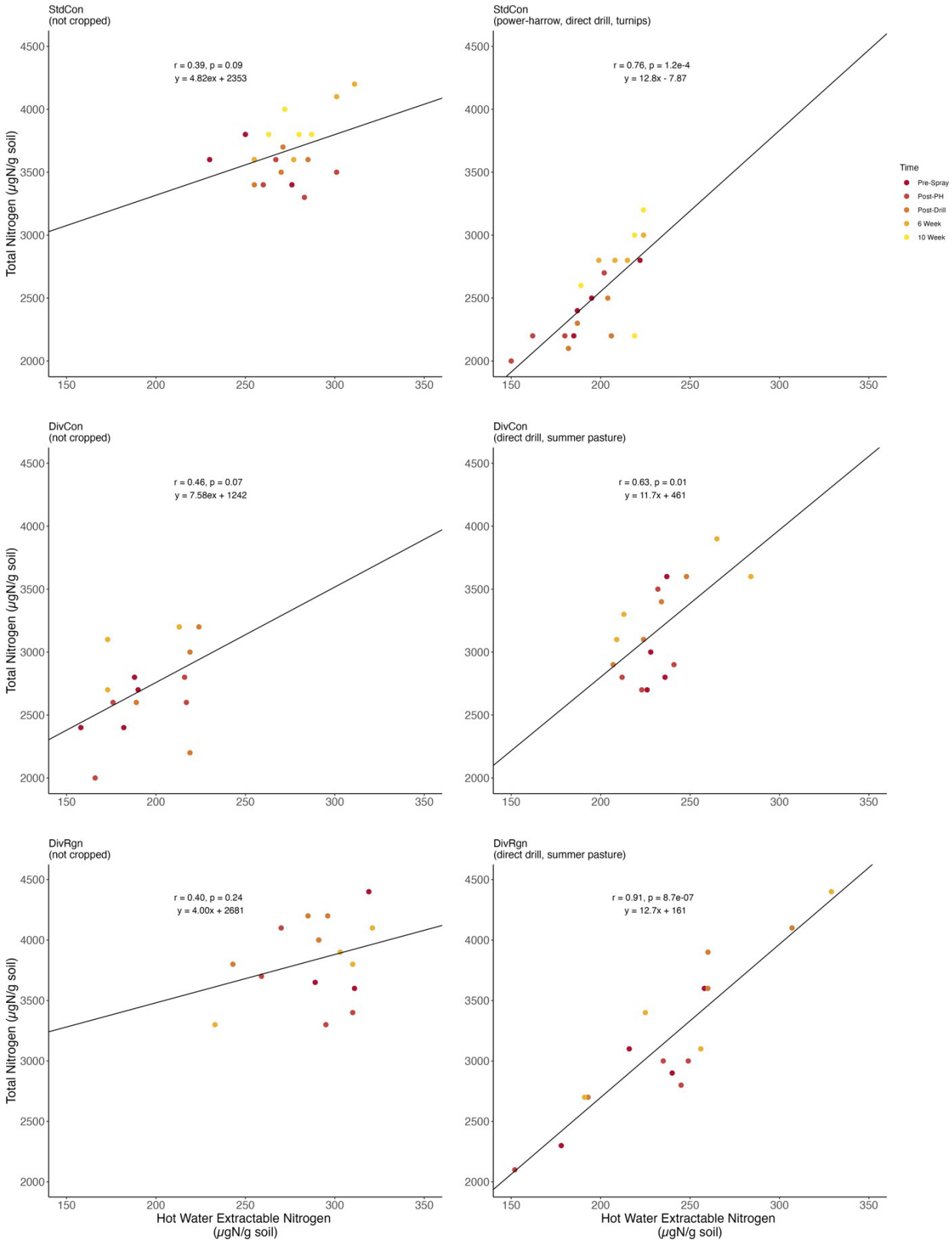

Figure 4.11: Correlations between Total N % and HWEN for both cropped and not cropped plots of the StdCon, DivCon and DivRgn treatments. The linear equation for each correlation and their r values are presented within each graph and correlations were significant when $p < 0.05$.

4.2.7 Correlation of HWEC, HWEN, total C % and total N % within all tillage treatments

The HWEC and HWEN concentrations measured under both the cropped and not cropped treatments showed a strong positive correlation (Fig. 4.12a). This relationship was highly significant ($r = 0.93$, $p < 0.001$). HWEC and HWEN were equally highly related to their respective total C and N pools ($r = 0.87$, $p < 0.001$) (Fig 3.12b, c).

The HWEC concentrations showed a significant strong positive correlation with total C ($r = 0.87$, $p < 0.001$) (Fig. 4.12b). For every unit increase in HWEC, the total C % increased by $1.591e^{-3}$ units, with a negligible constant of $5.968e^{-4}$. The amount of total C present as HWEC across all paddocks averaged 6.2%.

The HWEN concentrations showed a significant and strong positive correlation with total N ($r = 0.87$, $p < 0.001$) (Fig. 3.12). For every unit increase in HWEN, the total N % increased by $1.163e^{-3}$ units, with a negligible constant of $4.315e^{-2}$. The amount of total N present as HWEN across all paddocks averaged 5.6%.

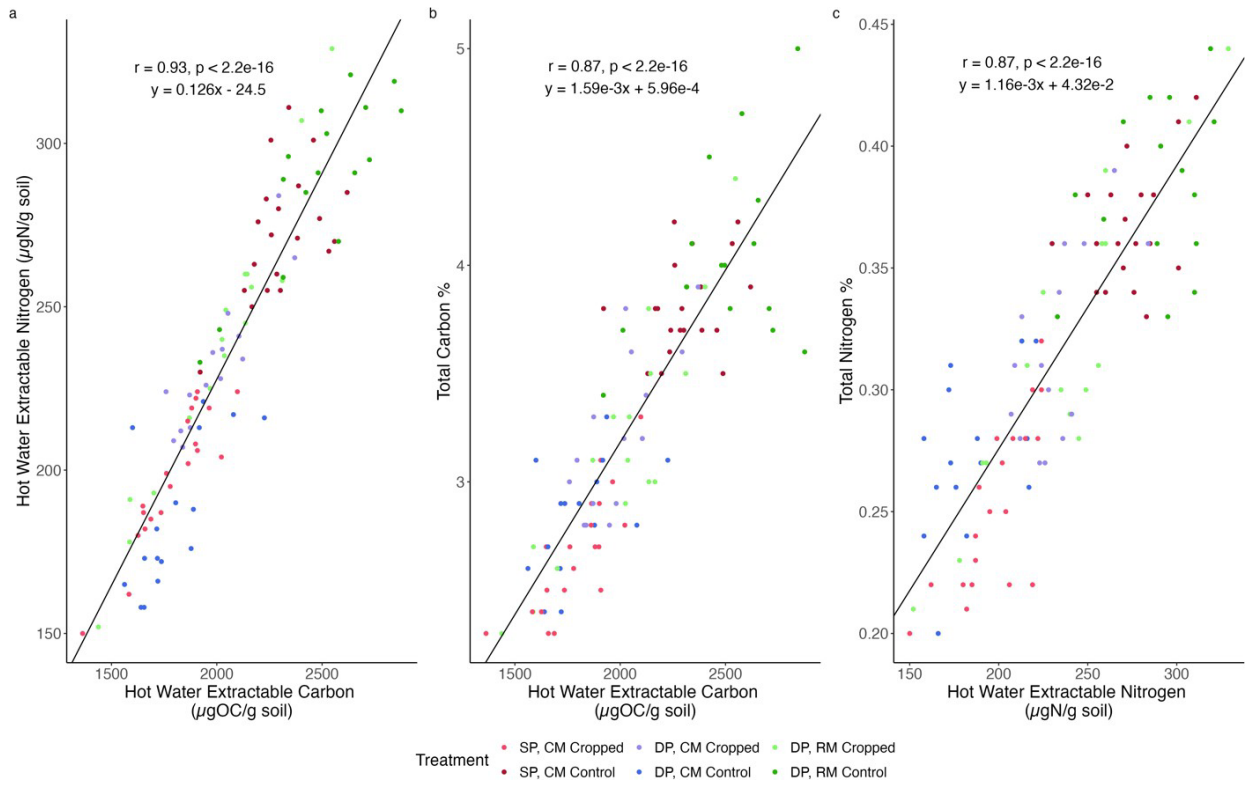

Figure 4.12: Linear relationships between (a) HWEC and HWEN ($p < 0.001$), (b) HWEC and total C % ($p < 0.001$) and (c) HWEN and total N % ($p < 0.001$) for all treatments throughout the study period. The linear equation for each linear relationship and their r values are presented within each graph.

4.3 Discussion

Soil C and N and microbial biomass is recognised as an important aspect of soil health within NZ, as emphasized in the State of Environment report (Ministry for the Environment & Stats NZ, 2022). The use of the HWEC and HWEN tests in NZ provides farmers with an economical monitoring tool of soil C and N and microbial biomass. However, research is required to determine the applicability of these measurements under different management scenarios.

Previous research has investigated HWEC and HWEN measures under different land use systems (e.g., arable systems and sheep, beef and dairy grazing), however there are large gaps in our understanding of how these measures can monitor changes in labile C and N in response to management practices, within the same land use. The research in this chapter aimed to investigate the use of HWEC and HWEN throughout contemporary and regenerative tillage practices and general management practices (fertilisation, grazing and pasture composition) within a dairy system. The objectives (Section 4.0.1) were to assess the impacts of different fodder cropping establishment practices, namely herbicide application and conventional vs no- till cropping on HWEC, total C %, total N % and the relationships between these measures throughout the study period.

4.3.0 Changes in HWEC, HWEN, total C % and total N % under different tillage and management methods

The action of establishing a diverse summer fodder crop using herbicide application and direct drilling (no-till) (DivCon and DivRgn) practices, did not have an influence on the HWEC and HWEN concentrations throughout the study period. No-till practices aim to prevent detrimental effects on labile C and N in the soil (Fonteyne et al., 2022; Rutledge et al., 2017; Whitehead et al., 2018). From this result we can conclude that glyphosate herbicide application and the direct drilling of a diverse summer fodder crop did not negatively affect the labile soil C or N pool over the study period, however there were also no increases in HWEC and HWEN measured.

In contrast, a decrease was seen in HWEC and HWEN following power-harrowing within the StdCon cropped treatment plots in the current study, though this was not significant. As this decrease was not seen in the not cropped StdCon treatment plots, it can likely be attributed to a loss caused by power-harrowing. Previous studies have shown that tillage practices such as power-harrowing have the potential to stimulate microbial activity and subsequently cause a release of CO_2 and N_2O, depleting the soil of labile C and N pools (Rutledge et al., 2017).

Following power-harrowing, the overall increase in HWEN and lack of change in HWEC could be attributed to the incorporation of pasture residues into the soil as a source of labile C and N. Research has found HWEC and labile C can increase with the incorporation of residues due to their contribution to the HWEC fraction under minimal tillage practices (Ghatohra, 2012; Ladoni et al., 2015; Margenot et al., 2017; Sequeira & Alley, 2011). This result suggests that under minimal tillage practices, CO_2 and N_2O emissions may be balanced by the sequestration of soil C and N as a result of decomposition of crop residues. The total C and N measured under the cropped StdCon treatment supports this, with a significant increase in total C and N measured throughout the study period which was not observed under the not cropped StdCon treatment.

The minimally destructive nature of the StdCon power harrowing tillage treatment may also have played a role in the lack of detrimental effects seen on HWEC and HWEN. Gajda et al. (2021) found that conventional tillage resulted in lower HWEC and HWEN concentrations (approx. 1100 µg OC/g and 100 µg N/g, respectively) than reduced tillage (1500 µg OC/g and 130 µg N/g, respectively). The Gajda et al. (2021) study utilized more destructive tillage methods, comparing mouldboard ploughing to no-tillage. Therefore, decreased soil destruction under the standard tillage method of the current study would contribute to the lack of response seen. The HWEC and HWEN concentrations averaged 2024 µg OC/g and 236 µg N/g for all treatments within the current study, which was much higher overall than the study by Gajda et al. (2021).

The treatment paddocks used within this study were established in their current pasture in April 2022, with a long history in pasture. As seen from the results, initial HWEC and HWEN concentrations prior to treatments were not significantly different between contemporary and

regenerative plots. Due to the short time frame since the trial establishment, and the long period since any prior cropping, microbial communities may be more resilient to disruptive cropping practices. Whereas continual cropping of the treatment paddocks may lead to the degradation of microbial biomass and communities as well as a reduction of accumulated OM in the soil (Bongiorno et al., 2019). This is supported by the high proportion of total C present as HWEC, which varied between 6.0 and 8.5%. Previous studies by Bongiorno et al. (2019) found a greater variation of this fraction under different tillage treatments, with values spanning from 1.0 to 6.0% of total C present as HWEC. The fact that this range is much wider than that found under the current study suggests that HWEC may be a valuable indicator of changes in total C, however under the current study, soil and tillage conditions were not significantly detrimental to either labile or total soil C.

Changes in total N were observed under both cropped and not cropped treatment plots of DivCon and the cropped treatment plots of StdCon. This result may be influenced by the application of synthetic N fertiliser during the study, which could directly influence the total N pool (Bankó et al., 2021).

Applications of bases such as lime results in an increase in soil pH. As the solubility of OM is heavily pH dependent, lime applications have been shown to increase the water solubility of OM and thus HWEC and HWEN concentrations (Curtin et al., 2017). Lime applications occurred between week 3 and week 9 for the not cropped treatment plots of StdCon and DivCon, and the cropped treatment plots of StdCon. The action of adding lime does not appear to have an influence on the solubility of OM, with no increases in HWEC and HWEN concentrations measured between these weeks.

4.3.1 Effects of diverse summer fodder crop

The utilisation of a deep-rooted multi-species pasture crop has been shown to increase labile C and N pools and sequestration of C and N in soils (Alemu et al., 2019; Bracken et al., 2020; Cummins et al., 2021; Fornara & Tilman, 2008; Jing et al., 2017; Laubach et al., 2023; McNally et al., 2015). Evidence from the current study suggests little change in HWEC, HWEN, total C or

total N concentrations following the sowing of diverse summer fodder crops. This supports findings of Wall et al. (2024) that increased allocation of C and N to above ground biomass in diverse species pastures can decrease the belowground inputs. Soil samples in this study were only taken to a depth of 7.5cm. It has been well documented that topsoil within NZ are often at or close to C saturation, but there is further potential for these crops to sequester C at greater depths where C is less likely to be at the saturation point of the soil (McNally et al., 2015; McNally et al., 2017; Peixoto et al., 2022). Therefore, while the benefit of these crops was not evident in the top 7.5cm of the soil, C and N pools may have increased at greater depths.

4.3.2 HWEC, HWEN, total C % and total N % correlations

The HWEC and HWEN measures were highly correlated with each other and also with their respective total C and N pools. This supports previous research that HWEC and HWEN are accurate predictors of the larger and more stable C and N pools and C and N sequestration potential (Bankó et al., 2021; Ghani et al., 2003). Linear relationships between HWEC and total C and HWEN and total N were higher under all of the cropped treatment plots than under the not cropped treatment plots. Due to the spatial separation between the cropped and not cropped treatment paddocks, it cannot be concluded whether this change was due to the establishment of crops, or potential climatic and soil variations.

4.3.3 Soil temperature and HWEC and HWEN correlations

Soil temperatures increased steadily over the study period, but this did not correlate with changes in HWEC or HWEN (data not presented). This was surprising as soil temperatures throughout the study period showed large fluctuations, from 7.3 to 19.2 (°C). Microbial biomass generally makes large contributions to both HWEC and HWEN measures, and increasing soil temperatures tends to stimulate microbial respiration and subsequent C loss through turnover of OM (Ghani et al., 2003; Walker et al., 2018; Weigel et al., 2011; Zhao et al., 2013). Therefore, it was expected that changes in soil temperature over the study period might have resulted in a decrease in HWEC and HWEN concentrations in soil (Ghani et al., 2003; Walker et al., 2018; Weigel et al., 2011; Zhao et al., 2013).

4.3.4 Limitations

This research was conducted on sites within an operational farm system research study. Single paddocks of the existing pasture treatments (StdCon, DivCon and DivRgn) were converted to summer fodder crops to provide feed for the grazing dairy cows. Due to the existing farmlet design, it was not possible to have replication with control (not cropped) and cropped plots within the same paddock. Control plots under the same management were established within the same soil type in separate paddocks. This limited the statistical comparison of changes between each of the six treatment paddocks. The difference in location and variability associated with this spatial separation required linear regression to be performed on each paddock separately. Different background HWEC concentrations measured between treatments and differing fertiliser and grazing associated with the crops also made comparisons challenging. As a consequence, great care has been taken in drawing any conclusions from the analysis of each paddock. What has emerged from the study is an increased understanding of the relationship between the HWEC, HWEN, total C and total N pools, under a range of real-world grazed agricultural systems. These limitations are reflective of those which would be met in the incorporation of these tests into other farming systems.

4.3.5 Further research

Further research on the effects of tillage practices should be considered under a range of different conditions. The sowing of equivalent crops using each tillage method and monitoring soil changes over an extended period would isolate the effects of the tillage practise under investigation. Furthermore, the comparison of not cropped and cropped treatment plots was not possible in this study due to the previously mentioned limitations. Therefore, research designed to monitor each of these treatments in replication within the same paddock would be beneficial, to eliminate the effects of any background changes in the C and N pools measured. Sampling HWEC, HWEN and total C and N measures to greater depths would also be beneficial for investigating the impacts of diverse pasture crops on the labile and sequestered C and N pools across the soil profile. Quantification of C and N measured in HWEC and HWEN through methods such as Fourier transform infrared (FTIR) would be useful in further studies to determine the origins of the C and N measured in these pools and therefore determine whether positive or negative impacts were had on microbial biomass communities.

4.4 Conclusions

The results from this research support the use of direct drilling as a method of sowing pastures to help mitigate the detrimental effects of tillage on labile C, N and total C and N pools. The use of power-harrowing as a minimal-till method within this research has shown to positively affect the labile C, N and total C and N pools in the short term. The sowing of diverse summer fodder crops as opposed to a monocrop of turnips did not appear to have an impact on the HWEC or HWEN concentrations to a depth of 7.5cm within this study. Samples taken to a greater depth may provide a more accurate indication of the potential of these different crops to lead to higher C and N sequestration rates within soils.

The HWEC and HWEN measures appeared to be representative of the total C and N pools under both treatments sown in pasture as well as those not sown in pasture over the period. This shows that, despite the implementation of cultivation management practices to establish a monocrop and diverse summer fodder crops, HWEC and HWEN can give an accurate indication of total C and total N in the soil. Overall, the HWEC and HWEN measures were strongly associated with total C and total N pools. This finding supports current research that these measures can be used as accurate indicators the total C and N pools, but not as early indicators of change in these pools.

Chapter 5
Changes in Hot Water Extractable Carbon and Nitrogen through a grazing cycle under differing grazing intensities

5.0 Introduction

Organic carbon (OC) in soils consists of two pools; a stable slowly decomposed fraction and a labile, more rapidly decomposed fraction (Tirole-Padre et al., 2004). Monitoring the status of these two pools is important for several different reasons. Increased soil OC is effective in mitigating carbon dioxide (CO_2) emissions, while higher OC levels in the microbial community indicates better microbial health of the soil (Voltr et al., 2021). The labile pool of C can rapidly change and is sensitive to changes in agricultural soil management practises and is representative of the total soil organic matter (OM) (Lambie et al., 2019; Landgraf et al., 2006). Therefore, monitoring of labile C changes within soils following a change in grazing practices might be a useful approach for establishing if management practices enhance soil C pools and potentially improve overall soil health.

Grazing practices have been proposed as methods of influencing the OM and labile C and N returns to soil. Throughout a grazing event, the grazing management practices have been found to influence the pool of soil labile C depleted and produced. Optimizing grazing practices can be beneficial to soil C and the microbial community (Stanley et al., 2024). While New Zealand (NZ) farmers often practice rotational grazing, high instantaneous stocking densities utilizing short grazing events (e.g., hours rather than days) and longer recovery periods which provide sufficient time for pasture regrowth are being investigated for their potential to promote soil health and C and N stocks (Abagandura et al., 2024; Kim et al., 2023; Ma et al., 2021; Mosier et al., 2021) and are referred to in this study as adaptive grazing. Adaptive grazing decreases the opportunity for preferential grazing by stock through higher grazing pressure, resulting in even distribution of

greater quantities of residual pasture cover. Furthermore, higher stocking densities results in greater incorporation of plant litter into the soil, where it becomes available as substrates for microbial decomposition (Mosier et al., 2021).

Despite the claims which have been made regarding the improvements to soil C and N sequestration and general soil health as a result of this grazing practice, impacts such as physical soil damage may constrain any improvement in soil C and N (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). Greater disturbance of soil aggregates has been shown to expose OM protected within the aggregates to microbial decomposition (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). Specifically, within NZ grazing systems, the benefits of high instantaneous stocking densities may provide no further improvements to soil health when compared to the current practices (Rowarth et al., 2020).

Determining the response of HWEC and HWEN in the soil to a livestock grazing event, by monitoring the changes from grazing throughout the different stages of pasture regrowth may provide insights into the likely changes in the labile soil C pool throughout a grazing cycle.

Studies have investigated the response of HWEC in soil across different livestock types and intensities by comparing the findings under different pastoral land uses (e.g., sheep versus dairying etc.) (Ghani et al., 2003). However, there have been no studies on the effects of grazing practices and intensity within a specific land use or on a short-term scale such as throughout a single grazing event. In this study, opportunity was taken to sample soil in a grazing study comparing two treatments that differed in pre- and post-grazing pasture covers and also in instantaneous stocking density at grazing.

5.0.0 Objectives

The two grazing treatments in the study simulate ‘rotational grazing’ one labelled ‘Control’ and the other ‘Adaptive’. Each grazing treatment consisted of two experimental factors:

Herbage mass: The Adaptive grazing management targeted a pre-grazing mass of 3500 - 4000 kg DM/ha grazed to a post-grazing residual of 2000 - 2500 kg DM/ha, and the Control grazing management consisting of targeting a pre-grazing mass of 2500 - 2800 kg DM/ha grazed to a

post-grazing residual of 1600 - 1800 kg DM/ha.

and

Instantaneous stocking intensity: The Adaptive grazing treatment consists of 'high' intensity, short-duration stocking, with animals moved to a fresh grazing cell 3 times per day, compared with the Control grazing treatment consisting of 'low' intensity, longer-duration stocking with animals moved to a new cell once every three days. This created a nine-fold difference between the two treatments in both instantaneous stocking intensities and liveweight loading.

The grazing study provided an opportunity to explore the following objectives:

- To determine the effects of differing stages of regrowth, instantaneous livestock stocking density (weight per ha), pasture type and grazing duration on HWEC, HWEN, total C % and total N % concentrations in soil over a grazing cycle.
- To investigate the relationship between HWEC and total C % and HWEN and total N % throughout the grazing cycle as influenced by instantaneous livestock stocking density (weight per ha), pasture type and grazing duration to determine if the percentage of total C and N present as HWEC and HWEN is influenced by these management practices.

5.1 Methods

5.1.0 Study site

Samples were collected from Mangarara Station, a 610ha farm within Elsthorpe, Central Hawkes Bay (39.928°S, 176.757°E). Mangarara Station is the host site of an adaptive grazing trial, which goals include researching the potential benefits of adaptive grazing on:

- Increasing photosynthetic C fixation.
- Reduced loss of C and N from excreta patches due to more even spatial distribution under higher instantaneous stocking density.
- Increased soil C inputs through greater trampling and incorporation of litter and increased soil biological activity (Mackay & Cosgrove, in press).

The adaptive grazing trial within this farm consists of 67 cells within a 9ha paddock, with topography ranging from low slope (0-12 degrees) to medium slope (13-24 degrees). The trial paddock is situated on Pallic soils, dominated by Oaklea loam (50%), with Airedale silt (25%) and Hastings loam (25%) (Manaaki Whenua Landcare Research, 2024). Soil information has been obtained from Smap, at a scale of 1:50,000 (Manaaki Whenua Landcare Research, 2024).

5.1.1 Climate

Soil temperatures were taken to 10cm using Watchdog 2000 series weather stations from the top and bottom ends of the paddock. Soil temperatures were 11.8 °C on average for the day of sampling (28/09/23).

5.1.2 Treatments

Of the cells within the paddock, 24 are ‘paired’ cells, located at the North and South end of the paddock with transects set up in each for monitoring. Cells at the North end are dominated by Fescue (*Festuca pratensis*), while cells at the South end are dominated by ryegrass (*Lolium perenne*).

Control paired cells consist of 2 sets of 6 cells at the North and South ends of the paddock. Wagyu cross heifers and steers are grazed in mobs of 6 cattle at a stocking density of 17,000 kg liveweight/ha. Control mobs spent an average of 4.8 days (92 hours) grazing each cell, with a range of 3 days (72 hours) to 4.3 days (102 hours).

Adaptive grazed paired cells consist of 2 sets of 6 cells at the North and South ends of the paddock. Wagyu cross heifers and steers are grazed in mobs of 57 cattle at a stocking density of 162,450 kg liveweight/ha. Adaptive mobs spent an average of 11 hours grazing each cell, with a range of 5 to 15 hours.

5.1.3 Sampling

The 24 paired cells within the adaptive grazing trial, 12 of each control and adaptive grazing, were selected for this study due to spatial proximity, consistency and intensive monitoring for general soil health measures (Fig. 5.1). This allowed access to any relevant historical soil information on soil fertility and pasture composition. Selecting cells on opposite ends of the paddock also allowed for the greatest distinction between pasture types for comparison. Samples were taken from a mixture of both pre- and post-grazing samples. These were categorised as < 9 days post-grazing and > 39 days post-grazing.

These cells were soil sampled in spring on the 28/9/2023 during the tenth grazing cycle of the trial. Sampling was undertaken on a single day to reduce temporal and climatic effects on the measures. Samples were taken from cells stage of grazing which they were at on this day. This created a timeline of grazing throughout the different cells.

Twelve individual soil cores per cell were sampled to a depth of 7.5cm along a W transect and bulked for analysis. Soils were then air dried, ground and sieved to <2mm before laboratory analysis. Pasture dry matter production/ha pre- and post-grazing was estimated using a rising plate meter by taking 20 readings across each cell in a V transect.

Table 5.1: Grazing treatment (adaptive and control), pasture type (ryegrass (Lolium perenne.) and fescue Festuca pratensis)) and time post-grazing (< 9 days and > 39 days) for cells within Mangarara regenerative trial in 2023.

Cell	Grazing treatment	Pasture type	Days post-grazing
4	Adaptive	Fescue	< 9 days
7	Adaptive	Ryegrass	< 9 days
9	Control	Fescue	< 9 days
10	Adaptive	Fescue	< 9 days
13	Control	Ryegrass	< 9 days
14	Adaptive	Ryegrass	< 9 days
18	Control	Fescue	< 9 days
19	Adaptive	Fescue	< 9 days
23	Control	Ryegrass	< 9 days
24	Adaptive	Ryegrass	> 39 days
29	Control	Fescue	> 39 days
30	Adaptive	Fescue	> 39 days
35	Control	Ryegrass	> 39 days
36	Adaptive	Ryegrass	> 39 days
41	Control	Fescue	> 39 days
42	Adaptive	Fescue	> 39 days
46	Control	Ryegrass	> 39 days
47	Adaptive	Ryegrass	> 39 days
51	Control	Fescue	> 39 days
52	Adaptive	Fescue	> 39 days
56	Control	Ryegrass	> 39 days
57	Adaptive	Ryegrass	> 39 days
61	Control	Fescue	> 39 days
66	Control	Ryegrass	> 39 days

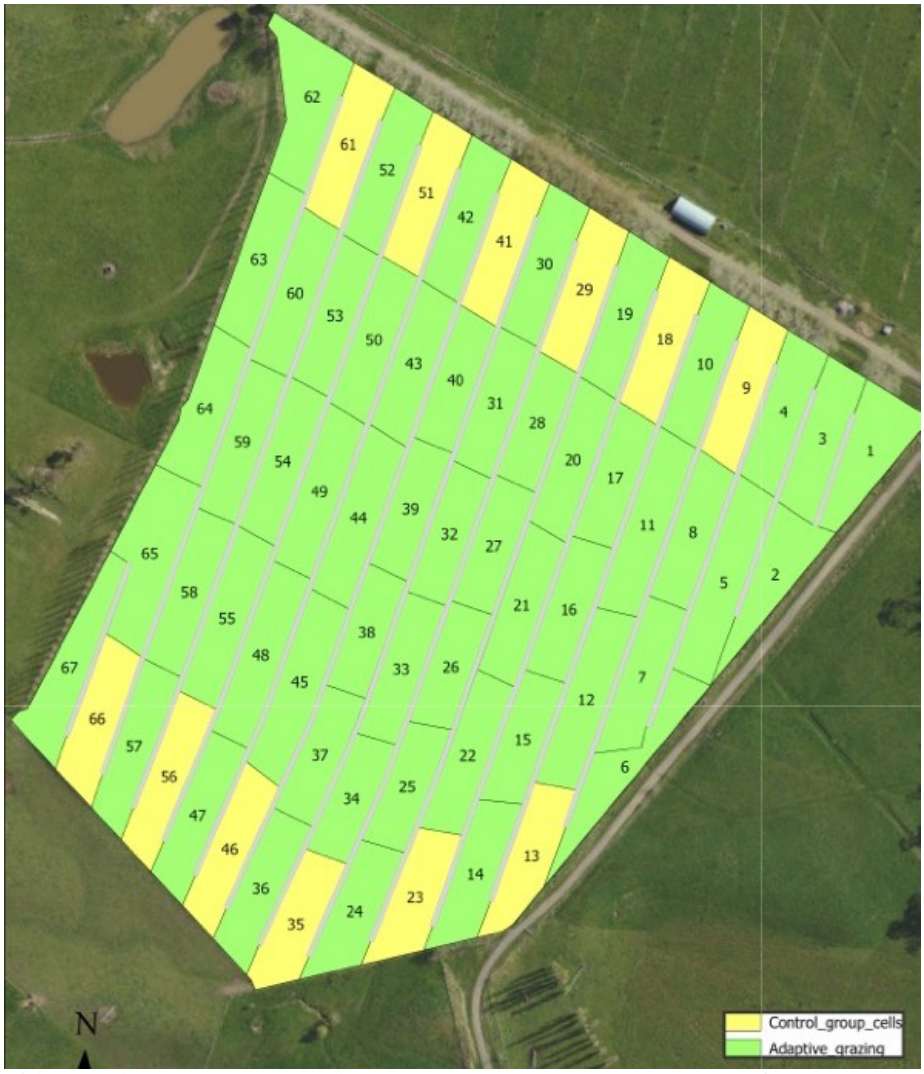

Figure 5.1: Map of the 67 cells under control and adaptive grazing management on Mangarara farm.

5.1.4 Chemical analysis

5.1.4.1 HWEC and HWEN

Hot water extractable C and N were analysed using the method of Ghani et al. (2003). Three grams of soil was added into 50mL polypropylene centrifuge tubes. Thirty mL of distilled water was then added to the centrifuge tubes, and the soil samples were extracted for 30 minutes on an end-for-end shaker at 30rpm. These samples were then centrifuged at a rate of 3500rpm for 5 minutes at 20 °C to separate the extractant and soil supernatant. Using a 0.45 µm cellulose nitrate (NO_3^-) membrane filter, the extractant was filtered for cold-water extractable C analysis. The supernatant was kept for further (hot water extractable) analysis.

Thirty mL of distilled water was added to the polypropylene centrifuge tube containing the cold-water extracted soil supernatants. These supernatants were then resuspended in the solution by inverting by hand. Hot water extraction was then performed by submerging the soil samples in a water bath at 80 °C for 16hr. followed by centrifugation at 3500 rpm for 5 minutes. The extractant was then filtered using a 0.45 µm cellulose NO_3^- membrane filter.

Hot water filtrates are analysed for organic C using a Shimadzu TOC-V_{CSH}/TNM-1 analyser. Fourty µl of the extract was injected into the detection chamber of the analyser. Following a 9-minute analysis, the total C and inorganic C in the samples were measured using non-dispersive infrared (NDIR). The difference between the total C and total inorganic C in the extracts is calculated to determine the organic water-soluble C concentration.

Using a SKALAR analyser, ammonium-N (NH_4^+-N) and NO_3^--N was measured in the samples. The difference between these measures determines the quantity of water-soluble organic N in the extracts.

5.1.4.2 Analysis of total C % and total N %

Total C % and total N % was measured using the Dumas method of combustion (Nelson & Sommers, 1996), and a VarioMAX Elementar Analyser. Five hundred g of soil was combusted at 900 °C in a pure oxygen atmosphere, completely decomposing all N containing compounds and converting them to nitrous oxides (NO and NO_2) and all C compounds, converting them to methane (CH_4) and carbon monoxide (CO). These nitrous oxides were then reduced to elemental N (N_2) and C gasses were oxidised to CO_2 using the catalysts copper oxide and platinum. The percentage of elemental N and CO_2 in the sample was measured using thermal conductivity to determine total C % and total N %.

5.1.5 Statistical analysis

R version 4.2.3 (2023-03-15) was used to produce all statistical analysis. Descriptive statistics (minimum, mean, maximum and standard deviation) were calculated for all outcome variables (HWEC, HWEN, total C % and total N %). Normality was determined for all outcome variables using Shapiro-Wilk tests. The amount of HWEC was calculated as a percentage of the total C pool. Data was grouped by treatment and pasture type to determine which pasture type and

treatment exhibited greater variability in HWEC, HWEN, total C % and total N %. Variance represents the average square deviation of HWEC, HWEN, total C % and total N % values from the mean.

Scatterplots with regression lines were used to determine trends between pasture residue and days post-grazing for each grazing practice. Spearman correlation tests were used to establish any relationships between pasture cover and days post-grazing. Linear regression analysis was used to assess the relationship between the outcome variables (HWEC, HWEN, total C % and total N %) and the explanatory variables (treatment, pasture type, days post-grazing and pasture cover), multivariable linear regression was used to account for the combined effects of these predictors. Correlations between HWEC and HWEN, HWEC and total C % and HWEN and total N % were determined using Pearson and Spearman correlation tests, with correlations considered strong when $r > 0.7$ and significant when $p < 0.05$.

5.2 Results

5.2.0 Pasture cover

Pasture cover increased with time following both control and adaptive grazing events ($p < 0.05$) (Fig. 5.4). Control grazing cells showed a stronger relationship with pasture cover, recovering faster than those under adaptive grazing. Cells with fescue dominated pasture had observable patches of bare ground interspersed with dense clumps of fescue pasture swards (Fig 5.2). Comparatively, ryegrass dominated pastures had a more even distribution of pasture sward (Fig. 5.3).

Immediately after grazing, pasture covers were around 1000 kg DM/ha for both control and adaptive grazing treatments, which corresponds to the point where the linear regression lines intersected. This occurred despite the large difference in instantaneous stocking density between the two treatments, with control cells being grazed by 6 cattle for on average 4 days (92 hours) (17,000 kg liveweight /ha) and adaptive cells being grazed by 57 cattle for on average 11 hours (162,450 kg liveweight/ha).

As days post-grazing increased, the variation in pasture cover increased also. Control grazing showed a faster recovery in pasture cover than adaptive grazing following a grazing event.

Control grazing cells had an average pasture cover of 2605 kg DM/ha for the period > 39 days post-grazing. Comparatively, adaptive grazing cells had an average pasture cover of 1954 kg DM/ha for the period > 39 days post-grazing.

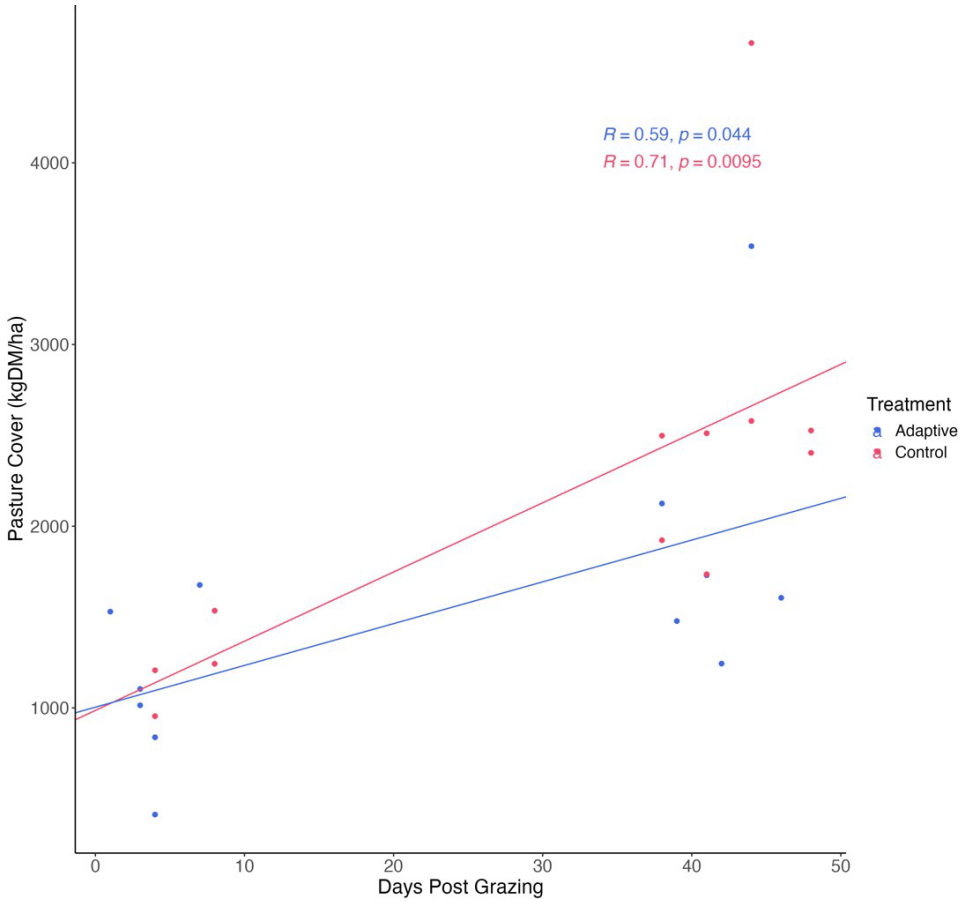

Figure 5.2: Pasture cover (kgDM/ha) following a grazing event under control and adaptive grazing practices.

5.2.1 HWEC and HWEN

The HWEC concentration measured across the 24 cells varied from a minimum of 2848 to a maximum of 4790 µg OC/g and averaged 3406 µg OC/g. This accounted for 7.1% of the total C measured, which varied from a minimum of 87,000 to a maximum of 37,000 µg C/g and averaged 48,000 µg C/g (Table 5.2). The HWEN concentration varied from a minimum of 225 to a maximum of 500 µg N/g across all cells and averaged 332 µg N/g. This accounted for 7.7% of the total N measured, which varied from a minimum of 3200 to a maximum of 8100 µg N/g and averaged 4200 µg N/g (Table 5.2).

Table 5.2: Mean HWEC, HWEN, total C and total N concentrations measured across all (adaptive and control) cells measured from a depth of 7.5cm.

	Mean (± SD)
HWEC (µg OC/g)	3406 (± 494)
HWEN (µg N/g)	332 (± 58)
Total C (%)	4.8 (± 1.0)
Total N (%)	0.42 (± 0.12)

5.2.2 Changes in HWEC and total C % - control and adaptive grazing practices, pasture regrowth stage and different pasture types

The control grazing treatments measured slightly higher HWEC concentrations (3524 µgOC/g), however this was not significantly different from the adaptive treatments (3290 µgOC/g) (Fig. 5.5). The total C % was slightly higher under the control treatments (4.7%), however, this difference was not significantly different to the adaptive treatments (4.8%) (Fig. 5.5). A higher level of variance around the mean was consistently seen for the adaptive cells. There were no major differences in the percentage of HWEC as a fraction of total C between the two treatments.

The ryegrass pasture treatment cells measured slightly higher soil HWEC concentrations (3559 µg OC/g) than the fescue pasture treatment cells (3252 µg OC/g), however, this difference was not significant (Fig. 5.5). Total C % was slightly lower under ryegrass pasture (4.5%) than fescue pasture, (5.0%) however this was not significant (Fig. 5.5). Cells dominated by fescue pasture had higher levels of variance (389,671 µgOC/g and 2.2%, respectively) around the total C and HWEC concentration means than ryegrass pasture cells (69,585 µgOC/g and 0.11%, respectively). The variance was particularly high immediately post-grazing (< 9 days) and reduced with time post-grazing. Due to higher concentrations of HWEC but lower total C concentrations under ryegrass pasture, HWEC accounted for a larger percentage (7.9%) of total C than it did under fescue pasture (7.1%).

Days post-grazing (> 9 days and < 39 days) had a significant impact ($p < 0.05$) on HWEC (3496 µgOC/g and 3342 µgOC/g, respectively) and total C % (5.0% and 4.6%, respectively) (Fig. 5.5).

HWEC decreased with time following a grazing event for all fescue based-pasture cells. Conversely, ryegrass pastures showed either no change or increased following a grazing event. There was no major change in the percentage of total C present as HWEC with time following the grazing event.

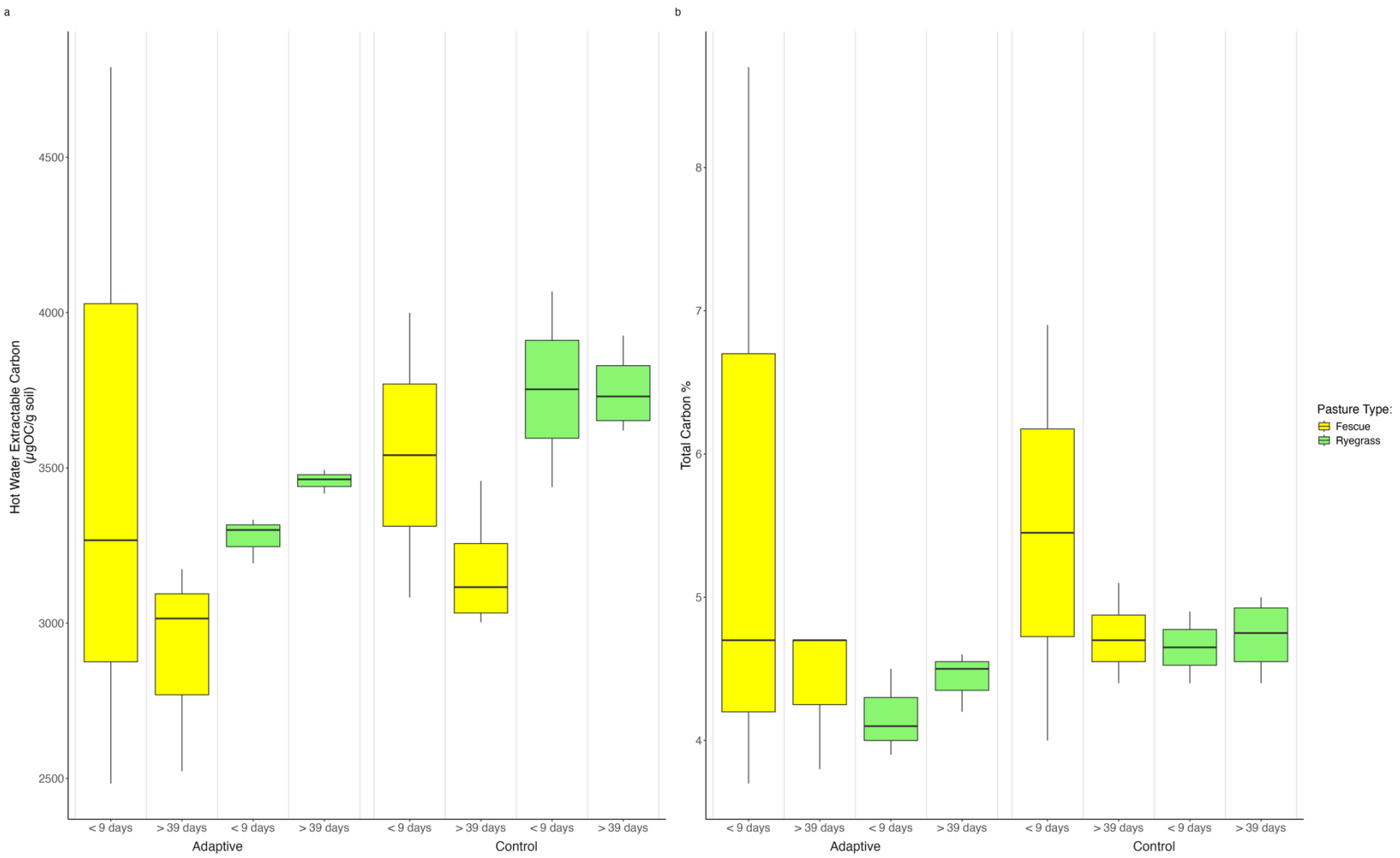

Figure 5.3: Comparison of (a) HWEC, (b) total C %, measured at < 9 days and > 39 days post-grazing under control and adaptive grazing practices with fescue and ryegrass pasture types.

5.2.3 Changes in HWEN and total N % - control and adaptive grazing practices, pasture regrowth stage and different pasture types

The control grazing treatments had slightly higher HWEN concentrations (347 µg N/g) than adaptive treatments (317 µg N/g), however the difference was not significant (Fig. 5.6). Total N % was similar under the control treatments (0.41%) and adaptive treatments (0.42%) (Fig. 5.6). The percentage of HWEN in the total N pool was higher under control treatments (8.3%) than under adaptive treatments (7.7%).

The ryegrass pasture measured slightly higher HWEN concentrations (346 µgN/g) than fescue (317 µgN/g), however this difference was not significant (Fig 5.6). Total N % was slightly lower under ryegrass pasture (0.39%) than fescue pasture (0.44%), again this was not significant (Fig 5.6). Cells dominated by fescue pasture had higher levels of variance (5,242 µgN/g and 0.02%, respectively) around the total N and HWEN concentration means than ryegrass pasture cells (1,494 µgN/g and 0.0008%, respectively). This variance was highest immediately post-grazing (< 9 days) and reduced at > 39 days post-grazing. The percentage of HWEN in the total N pool increased from 7.6% at < 9 days to 8.9% at > 39 days post-grazing.

Days post-grazing (> 9 days and < 39 days) had a significant impact ($p < 0.05$) on HWEN (344 µgN/g and 323 µgN/g, respectively) and total N % (0.45% and 0.39%, respectively) (Fig. 5.6). Both HWEN and total N % decreased with time following grazing in all fescue cells, however within ryegrass cells, HWEN and total N % showed an increasing trend with increased days post-grazing. The percentage of HWEN in the total N pool increased with time post-grazing, from 7.6% to 8.3%.

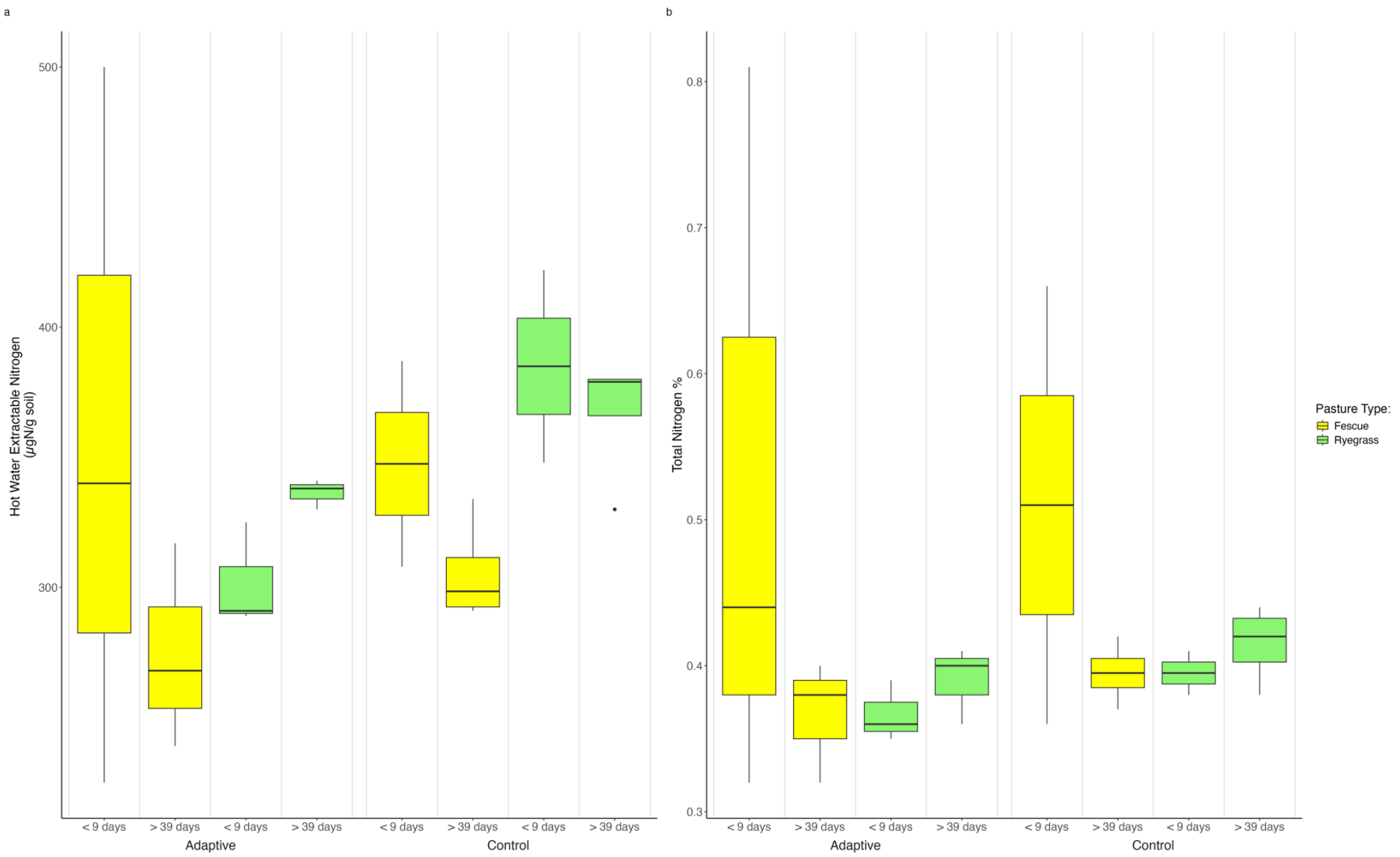

Figure 5.4: Comparison of (a) HWEC, (b) total C %, measured at < 9 days and > 39 days post-grazing under control and adaptive grazing practices with fescue and ryegrass pasture types.

5.2.4 Relationship between HWEC, HWEN, total C % and total N % under grazing treatments

The relationships between HWEC and HWEN, HWEC and total C % and HWEN and total N % as influenced by grazing practice were explored in a combined analysis. HWEC showed a strong significant linear relationship with HWEN (r = 0.91, $p < 0.001$) (Fig. 5.7).

The HWEC concentration was strongly and positively correlated with total C across all cells (r = 0.61, $p < 0.001$) (Fig. 5.7). For every unit increase in HWEC, the total C % increased by $1.65e^{-3}$ units, with a constant of 0.873.

The HWEN concentration was strongly and positively correlated with total N across all cells (r = 0.60, $p < 0.001$) (Fig. 5.7). For every unit increase in HWEN, the total N % increased by $1.39e^{-3}$ units, with a constant of 0.0457.

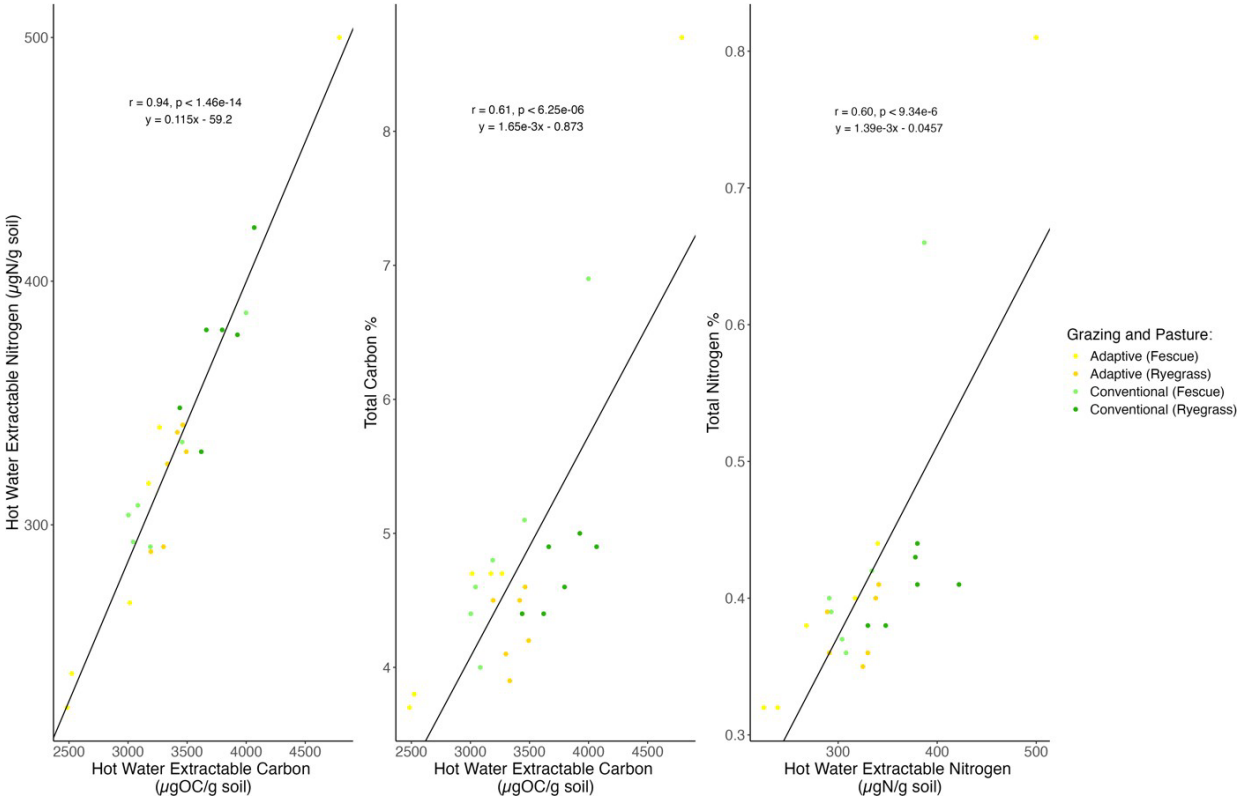

Figure 5.5: Linear relationships between HWEC and HWEN ($p < 0.001$), HWEC and total C % ($p < 0.001$) and HWEN and total N % ($p < 0.001$) for all treatment cells throughout the study period.

5.3 Discussion

5.3.0 Pasture regrowth under control and adaptive grazing treatments

The adaptive grazing practice aimed to utilise higher instantaneous stocking density, longer pasture recovery periods between grazing events and higher pre- and post- grazing residuals to improve pasture and animal production, plant litter return and soil health. However, post-grazing residuals were similar between control and adaptive grazing practices for the grazing cycle investigated, which may reflect the challenge of meeting post grazing targets in spring when growth rates are high. The control cells showed a higher rate of pasture regrowth than the adaptive cells, resulting in a higher pre-grazing cover prior to the next grazing (Fig. 5.2). Studies conducted in Manawatū, NZ, have found that trampling damage caused by increased cattle treading can have detrimental effects on tillers and growing points, reducing the pasture cover and regrowth capacity (Pande et al., 2000). Less frequent defoliation such as that practiced within the adaptive cells can, if left too long, cause shading and supress species such as clover, which is commonly featured in NZ pastures (Rowarth et al., 2020; Stanley et al., 2024). In some cases, this could reduce the overall pasture regrowth and cover (Rowarth et al., 2020).

5.3.1 HWEC, HWEN, total C % and total N % - control and adaptive grazing treatments

There were no significant differences in HWEC, HWEN, total C, or total N measured between the two grazing practices (Fig. 5.3, Fig. 5.4). While the lack of change in the labile C and N pools as measured by HWEC and HWEN may be due to specific conditions of the grazing cycle measured, the similarities in the total C and total N pools between the control and adaptive treatments suggests that there has not been an increase in C and N incorporation and sequestration under adaptive grazing during the 16 months the Mangarara trial has been running. Furthermore, control treatment cells had a slightly higher percentage of total C and total N measured as labile HWEC and HWEN than adaptive grazing. This result does not reflect the expected effect of larger quantities of plant litter as a source of labile C and N being incorporated into the soil under high instantaneous stocking density grazing practices of the adaptive treatment (Abagandura et al., 2024; Khatri-Chhertri et al., 2024). This may be due to mechanisms such as the exposure of intra-aggregate OM to microbial decomposition as a result of physical

damage caused by higher stocking densities (Abagandura et al., 2024; Khatri- Chhertri et al., 2024). Another possible mechanism is the trampling of the plant litter into the soil surface accelerates the decomposition process by ensuring the plant litter is in intimate contact with the soil surfaces that increases the access of litter to microbial attack (Wei et al., 2021). The increased pasture cover within control grazing cells could be expected to have a significant impact on this result. The HWEC, HWEN, total C and total N concentrations in soil were all higher under high pasture cover than under low pasture cover across both treatments (Fig. 5.5).

Additionally, higher instantaneous stocking densities in adaptive grazing cells aims for more even spatial distribution of dung and urine patches. Livestock urine contains large amounts of N and C in forms which are hot water-soluble (Ghani et al., 2003). Areas affected by livestock urine can elevate soil pH, soil NO_3^-, the solubility of OM and increase microbial respiration (Curtin et al., 2017; Curtin et al., 2020). This was not reflected in these results, with higher HWEC and HWEN concentrations measured under the control verse adaptive treatments.

5.3.2 HWEC, HWEN, total C % and total N % - fescue and ryegrass pasture

The cells situated on the northern end of the paddock were dominated by fescue-based pasture, while the cells situated on the Southern end were dominated by ryegrass-based pasture. A large discrepancy in the concentration of HWEC, HWEN, total C and total N was measured between the two pasture types. Ryegrass cells consistently measured higher concentrations of HWEC and HWEN than fescue cells, irrespective of the grazing management and the time after a grazing event. A defining characteristic of the fescue cells was the presence of significant areas of bare ground and dense clumps of fescue swards, particularly notable when compared to the even surface coverage of ryegrass pasture. Bare soil can lead to soil C losses via erosion and provide a less optimal environment for soil microbes (Stanley et al., 2024). A higher concentration of total C was measured in fescue cells. This led to the percentage of total C and N present as HWEC and HWEN being higher under ryegrass pasture. Due to the dense clumps of fescue swards and their roots, sampling from the ryegrass cells may have incorporated greater quantities of root biomass, and associated root exudates, which would have contributed to the HWEC and HWEN pools and led to less variance in measures (Curtin et al., 2022; Ghani et al., 2003; Li et al., 2007).

5.3.3 HWEC, HWEN, total C % and total N % - grazing event

The percentage of HWEC and HWEN in the total C and N measured increased with time post-grazing. Immediately post-grazing we would expect to see higher levels of trampled OM from the plant litter and dung return. However, with time, this OM may have been broken down by increased microbial activity into more labile compounds, such as monomers of compounds like lignin (Balaria et al., 2009). This would increase the quantity of OM present in the highly labile pool of C and N as well as increased microbial activity and subsequent enzymes etc, as the microbial community recovered after a grazing event. Defoliation through grazing has been shown to stimulate root exudation to support pasture regrowth and subsequently increases labile C in the rhizosphere providing substrate for microbial communities (Hamilton et al., 2008).

While it has been shown that grazing practices can decrease root exudation, provided a sufficient recovery period is achieved, grazing can lead to increases in root exudation rates (Sun et al., 2017).

5.3.4 HWEC, HWEN, total C % and total N % linear relationships

The HWEC and HWEN measures were highly correlated with each other and also with their respective total C and N pools. This supports all previous research that HWEC and HWEN are accurate predictors of the larger and more stable C and N pools and C and N sequestration potential (Bankó et al., 2021; Ghani et al., 2003). The percentage of the total pools present as highly labile, hot water extracted pools fluctuated between treatments, days post-grazing and pasture type. However, despite these changes, the HWEC fraction accounted for 7.0-7.9% of total C, which is in the upper range of that typically removed in the hot water extraction in NZ soils (Curtin et al., 2022). The HWEN fraction accounted for 7.2-8.9% of total N. This is also in the upper range (2.6-8.7%) of that which has been observed in other NZ studies (Curtin et al., 2006).

5.3.5 Further research

Analysis of HWEC and HWEN following a grazing event was limited in this study, to one sampling in early spring. Further research into the impacts that different HWEC and HWEN concentrations have on pasture regrowth rates would be beneficial in providing insight into how these pools contribute to plant available nutrients and microbial activity.

5.4 Conclusions

The lack of differences in post-grazing pasture residuals between the adaptive and control treatments in this study, may have contributed to the absence of any difference in the labile C and N pools in the soils in the days after grazing. Adaptive grazing was shown to have lower pasture regrowth rates after a grazing event than control grazing. Both HWEC and HWEN increased with time following a grazing under ryegrass pasture but decreased under fescue pasture. These differences were attributed to the spatial distribution of pasture swards and presence of bare ground within the fescue cells. Furthermore, HWEC accounted for a greater percentage of total C under the ryegrass pasture cells.

The HWEC and HWEN measures appeared to be representative of the total C and N pools, respectively, in both grazing treatments, pasture types and with time, following a grazing event. This shows that, despite changes in management practices and pasture type, HWEC and HWEN can give an accurate indication of total C and total N in the soil.

Chapter 6
General Discussion

6.0 Summary of chapters

The effects of P fertiliser and sheep grazing history on hot water extractable carbon (HWEC) and hot water extractable nitrogen (HWEN) investigated in chapter 3 were not as expected. Increased net primary production (NPP) and associated Olsen phosphorus (P) and carbon (C) and nitrogen (N) cycling with increasing P fertiliser addition did not translate into an increase in HWEC on the HFHF farmlet in this study (Bilotto et al., 2022; Wakelin et al., 2017). There was no significant difference between the HFHF farmlet and the LFLF or LFNF farmlet. This suggests that while more C and N is cycling within the grazing system as a consequence of increased NPP, an increased rate of microbial turnover may mean that the overall microbial biomass remain constant, while C from increased plant litter is more rapidly decomposed and removed from the labile pool (Wakelin et al., 2017). While there is less plant litter returned in the two farmlets (LFNF and HFNF) that have received no P fertiliser for more than 40 years, the quality of that litter is likely to be lower. This would slow decomposition rates, potentially increasing the fraction of C and N extractable by hot water.

The effects of topography were consistent with expectations, with HWEC and HWEN concentrations lowest on high slopes, where the soil was shallow and less well developed and had lower organic matter (OM), biological activity and nutrient fertility, all contributing to low pasture growth rates and C and N cycling. Topsoil erosion and loss of OM is also more prevalent on high slope areas, and these areas are less frequently grazed by livestock. Samples taken from medium slope transects for soil health monitoring were also shown to be representative of HWEC across all slope classes. East and NW aspects showed the highest concentrations of HWEC and HWEN, reflecting enhanced pasture production due to greater solar radiation (Mackay et al., 2021).

Further investigation of physico-chemical factors influencing HWEC when slope and P fertiliser history were considered, yielded the most influential factors to be OM (positive), effective CEC (positive), sulfate sulfur (S) (negative) and microporosity (negative). This finding supported our understanding that the main source of HWEC is OM (Curtin et al., 2020; Ghani et al., 2003). The model to predict HWEC also reinforces the influence of effective CEC on HWEC, due to relationships between effective CEC and OM as well as soil clay content (Curtin et al., 2022; Harrison-Kirk et al., 2014; Tirole-Padre et al., 2004; Voltr et al., 2021).

The adaptive tillage practices and diverse summer fodder cropping management practices investigated in chapter 4 did not appear to have any measurable impacts on the size of the labile C and N pools measured by HWEC and HWEN. The no-till methods of summer cropping did not have any overall detrimental effects on HWEC, nor did the power-harrowing and herbicide treatments. Power-harrowing appeared to have a slight negative impact on HWEC and HWEN concentrations, however this change did not appear to be long-term. Diverse summer fodder crops also did not result in any increases in HWEC and HWEN over the short study period, and these pools remained relatively constant throughout the study.

The adaptive grazing practices, which included a 9-fold difference in the instantaneous stocking density at grazing, investigated in chapter 5 did not appear to have any impact on the size of labile C and N pools measured by HWEC and HWEN. However, the spring grazing event sampled was not successful in achieving the targeted higher pre- and post- grazing residuals under the higher instantaneous stocking density of the adaptive grazing. Our study did, however, suggest that the potential OM build-up, proposed to occur due to greater trampling of plant litter under higher instantaneous stocking density, did not manifest in any improvements in the labile C and N pools as measured by HWEC and HWEN. At the same time, the influence of dominant pasture type on labile C and N was reflected in the HWEC measures.

One key finding from all of the studies in this thesis was the consistency in the proportion of total C and N present as HWEC and HWEN across P fertiliser and sheep grazing histories, slopes

and aspects, tillage practices and grazing treatments (Table 6.1). These proportions remained relatively constant despite the different management practices and strategies that have a substantial influence on the amount of C and N cycling in the systems investigated in the three research chapters. For example, despite a nearly 3-fold difference in the amount of C cycling due to differences in NPP from P fertilisers, that ratio remained relatively unchanged.

Table 6.1: Fraction of total C present as HWEC (%) for chapter 3 (Ballantrae Hill Country Research Station), chapter 4 (Whenua Haumanu) and chapter 5 (Mangarara).

	Fraction of total C present as HWEC (%)	**Fraction of total N present as HWEN (%)**
Chapter 3	4.8 – 6.0	4.4 – 6.0
Chapter 4	6.0 – 8.5	6.2 – 8.6
Chapter 5	7.0 - 7.9	7.2 – 8.9

6.1 Linear relationships between total C, total N and HWEC and HWEN

Linear relationships between HWEC and HWEN fractions and their respective total C and N pools were determined for each study (Chapters 3-5). Despite each research study examining different soil types and management practices, each study consistently showed a positive correlation between HWEC and total C, and HWEN and total N (Figs. 3.4, 4.12, 5.7).

6.1.0 HWEC as a predictor of total C

The relationship between HWEC and total C differed significantly between the study sites, as seen by the highly variable rate of total C change with increasing HWEC. Soils sampled from Ballantrae (Chapter 3) showed the highest rate of total C change with increasing HWEC concentrations (Fig. 3.4). For each unit of HWEC increase, total C increased by the factor of 1.65, minus a negligible negative constant of $1.22e^{-3}$. Predictive equations derived from the samples taken from the Whenua Haumanu (Chapter 4) and Mangarara (Chapter 5) study sites also showed a positive linear relationship between HWEC concentrations and total C, however,

these equations differed significantly to the equation derived from the Ballantrae samples (Fig. 4.12, 5.7). Both of these study sites showed much lower increases in total C with increasing HWEC concentrations. Mangarara showed a much lower baseline total C concentration compared to both the Ballantrae and the Whenua Haumanu study sites. As this was consistently lower across both grazing treatments when compared to all cropped and not cropped treatments, it is likely that this difference could be attributed to soil type.

These linear relationships deviated largely from the findings of Curtin et al. (2021), who found that every unit increase in HWEC (µgOC/g) correlated with a 12.2× increase in total C (µgC/g), with a large positive constant of 9170. The Curtin et al. (2021) study showed a strong correlation ($r = 0.97$) between the two measures which included samples taken from depths to 15cm, and soils from permanent pasture as well as various arable cropping soils. As the linear relationship between HWEC and total C was not affected by soil depth, or treatment type in the Curtin et al. (2021) study, the difference in soil type appears to be the main contributing factor to variations in the relationship.

As suggested by the strong positive linear relationship between total C and HWEC, Ballantrae appeared to have much higher levels of C sequestration, with a higher proportion of the labile C measured in HWEC likely being sequestered to recalcitrant forms measured by the total C method. Substantial applications of P fertiliser to this study site may have increased microbial activity and subsequently the sequestration of labile C over time (Ortas & Bykova, 2020; Poeplau et al., 2016; Wakelin et al., 2017). With an average C:P demand of microbial biomass being 60:1, applications of P fertiliser will overcome the stoichiometric constraints caused by P deficiency (Poeplau et al., 2016; Wakelin et al., 2017). Studies have shown long-term increases in C mineralisation in P fertiliser trials when N is limited, reducing labile C present and stimulating faster turnover of C in soils (Mackay et al., 2021; Poeplau et al., 2016; Wakelin et al., 2017).

6.1.1 HWEN as a predictor of total N

The rate of total N increase per unit of HWEN increase was relatively consistent between each study (Chapters 2-5). Samples taken from the Mangarara study site showed the strongest linear relationship between HWEN and total N (total N increased by $1.39e^{-3}$ units for every unit increase of HWEN), followed by Whenua Haumanu samples (total N increased by $1.16e^{-3}$ units

for every unit increase of HWEN). The weakest linear relationship between HWEN and total N was seen in the Ballantrae samples (total N increased by $1.06e^{-3}$ units for every unit increase of HWEN). Potential reasons for this may include the effect P fertiliser applications may have had on the rate of C and N cycling. Greater rates of microbial activity may have decreased the labile N pool under P fertilised pastures, which have higher total N pools (Bilotto et al., 2022). Therefore, the differences in the linear relationship between total N and HWEN may be influenced by soils which have received P fertiliser input compared to soils that have not had P fertiliser inputs since 1980.

These linear relationships deviated from the findings of Curtin et al. (2021), who found that every unit increase in HWEN correlated with a 10.8 increase in total N, with a large positive constant of 1145. Their study showed a strong correlation ($r = 0.97$) between the two measures which included samples taken from depths to 15cm, and soils from permanent pasture as well as various arable cropping soils. As the linear relationship between HWEN and total N was not affected by soil depth, or treatment type in the Curtin et al. (2021) study, the difference in soil type appears to be the main contributing factor to variations in the relationship.

The fact that the HWEC and HWEN fractions did not change in response to changes in management including P fertiliser use and sheep grazing, tillage and crop management and grazing practices, despite all impacting C and N cycling (e.g., by increasing NPP and causing physical disturbance to the soil), suggests the size of the fraction extracted by these two tests is not impacted by these management factors but regulated by other factors. The differences in the linear relationship between HWEC and total C and HWEN and total N, particularly seen between the current study and the findings of Curtin et al. (2021), stresses the need for further investigation into the effect of soil type on the size of the respective pools and by default the relationship between the two. The strong relationship between HWEC and total C and HWEN and total N suggests that these measures could be used as a substitute to monitor changes in soil C and N cycling, C and N sequestration and general soil environmental monitoring. However, the need for HWEC and HWEN to be used in conjunction with total C and total N measures has been emphasized by the current study. Deviations from what might be considered the normal range of

total C and total N present as the HWEC and HWEN fraction for a certain soil for an extended period of time may be the most informative use of these tests. Such deviations may be indicative of changes in soil OM, microbial biomass and activity and potential C and N sequestration. The monitoring of such would aid in the early detection of soil degradation or improvement and assess the impacts of management practices. As the HWEC and HWEN measures used as standalone tests in in the current study, did not provide any more information than the total C and total N tests used routinely in NZ.

6.2 Soil type effects on HWEC and HWEN

The potential for soil type to have a large influence on the proportion of total C and N represented by HWEC and HWEN, respectively, has been well documented (Curtin et al., 2022; Curtin et al., 2017; Filimonova et al., 2016; Harrison-Kirk et al., 2014; Lambie et al., 2019; Ortner et al., 2021; Ortner et al., 2022; Schipper et al., 2014; Voltr et al., 2021). Ortner et al. (2022) found the interactions of HWEC with the mineral phase of soil highly predicted the stabilization of organic C, and therefore the accumulation of labile HWEC. The findings of Ortner et al. (2022) were supported by the findings in chapter 3 of the current study, with effective CEC being a predictor of HWEC within the multi-level analysis. The soil types examined in each chapter of the current study varied, the effects of which may be reflected in the varying correlations between HWEC and total C and HWEN and total N.

The Pallic soils examined in chapter 5 demonstrated an increase in the stability of OM and reduced bioavailability, as indicated by the smallest increase in HWEC with increasing total C among all the studies. The fine texture of these soils is the most likely factor contributing to this, leading to greater stability of OM within the soil (Curtin et al., 2022; Voltr et al., 2021).

Comparatively, the Recent alluvial soil examined in chapter 4 showed a moderate increase in HWEC with increasing total C. The loam texture of this soil supports moderate levels of OM. Finally, the Brown and Pallic soils examined in chapter 3 are more likely to develop organo-mineral associations which stabilise organic C (Ortner et al., 2022). This process was likely reflected in the highest increase in HWEC with increasing total C on these soils.

6.3 Stability of labile C fraction

Throughout this thesis, the labile C and N fractions measured by the HWEC and HWEN method remained relatively stable despite different management practices across a number of pastoral systems on a range of diverse landscapes. None of the land management factors examined, such as soil tillage, fertilisation and grazing practices caused the proportion of total C and N present as HWEC and HWEN to fall below the expected ranges for the land uses studied. A factor attributing to this may be the characteristics of conventional grazing systems within NZ. Pastures high in legumes such as clover are commonly utilized in these grazing systems and so the soil practices which were studied in this thesis were not majorly destructive. Furthermore, grazing systems within NZ are considered to lean towards the regenerative end of the grazing spectrum (Rowarth et al., 2020). While Curtin et al. (2006) showed the fraction of total organic C present as HWEC to be in the range of 2.6-8.7% for NZ pastoral soils, international studies by Círíc et al. (2016) found the fraction of total organic C present as HWEC to be in the range of 0.73 to 4.01%, supporting the argument that many NZ pastoral soils are potentially close to labile C saturation.

Therefore, the condition of the soils studied here are likely to be close to optimal in terms of highly developed microbial communities and labile and total C and N pools, making them more resilient to changes to slightly less optimal practices. Due to the potential high baselines of labile C and N in soils, the HWEC and HWEN measures may not be sensitive enough to detect changes caused by short term management practices. Furthermore, these higher levels may adapt and enhance the soils ability to buffer and minimise changes in labile C and N due to management practices in the short-term (Luo et al., 2010; Shapiro et al., 2013). High labile C and N pools are indicative of high microbial biomass (Ghani et al., 2003). This may also play a role in the lack of fluctuations in the HWEC and HWEN pools in response to stresses such as tillage and grazing practices. Improved resilience and resistance to detrimental effects on labile C and N pools may be a result of healthy microbial communities in these soils (de Moraes Sá et al., 2014; Shu et al., 2021).

Longer term studies where these practices have been implemented over multiple years would be

required to establish if these regenerative or adaptive practices are likely to result in greater soil C and N in contrast to more conventional practices. Monitoring HWEC and HWEN over a longer period would additionally determine the sensitivity of these measures once the soils have undergone repeated, potentially detrimental stressors.

The HWEC and HWEN fractions of C and N are promoted as accurate and cost effective measures of labile C and N for farmers. It is well established that HWEC and HWEN detect differences in the labile C and N and microbial biomass between land uses and changes resulting from the conversion of one land use to another. Yet, HWEC and HWEN may not be suitable to detect more subtle changes caused by management practices within a land use. With such high correlations with total C and N pools, a more useful application of the HWEC and HWEN tests may be to monitor how soils are tracking with respect to their total pools. An increase in the proportion of total pools present as hot water extractable fractions may indicate the potential for greater C and N sequestration rates in soils in response to optimizing management practices. Conversely, a reduction in this proportion may provide forewarning of potential declines in C and N pools, indicating the need for changes in management practices to arrest this decline.

6.4 Conclusions

The results of each study in this thesis further emphasized the effectiveness of HWEC and HWEN as indicators of changes in their respective total soil C and N pools. The strong relationships between the two C and N pools, support the utility of these measures as indicators of their respective total C and N pools. However, the observed differences in the linear relationships between these pools suggest the need for further research into the effects soil type, soil depth and the length of time particular management have been practiced on the application of the HWEC and HWEN measures. These findings indicate that even subtle changes in soil type can influence the relationship between the HWEC and HWEN fractions and the total C and N pools.

Given these insights, and the lack effectiveness which HWEC and HWEN had in capturing more subtle variations caused by management practices within a single land use, the use of these tests as stand-alone measures may not be highly informative within a land use. A key insight from this research is that an increase in the proportion of total C and total N pools present as hot water extractable fractions may signal a potential for greater C and N sequestration rates in response to optimized management practices.

Therefore, I conclude that while HWEC and HWEN are valuable tools for monitoring soil health, their most effective use may lie in tracking changes in relation to total C and N pools. Incorporating all four measures (HWEC, HWEN, total C and total N) appears to provide a more comprehensive assessment of soil conditions, enhancing our ability to monitor soil changes effectively.

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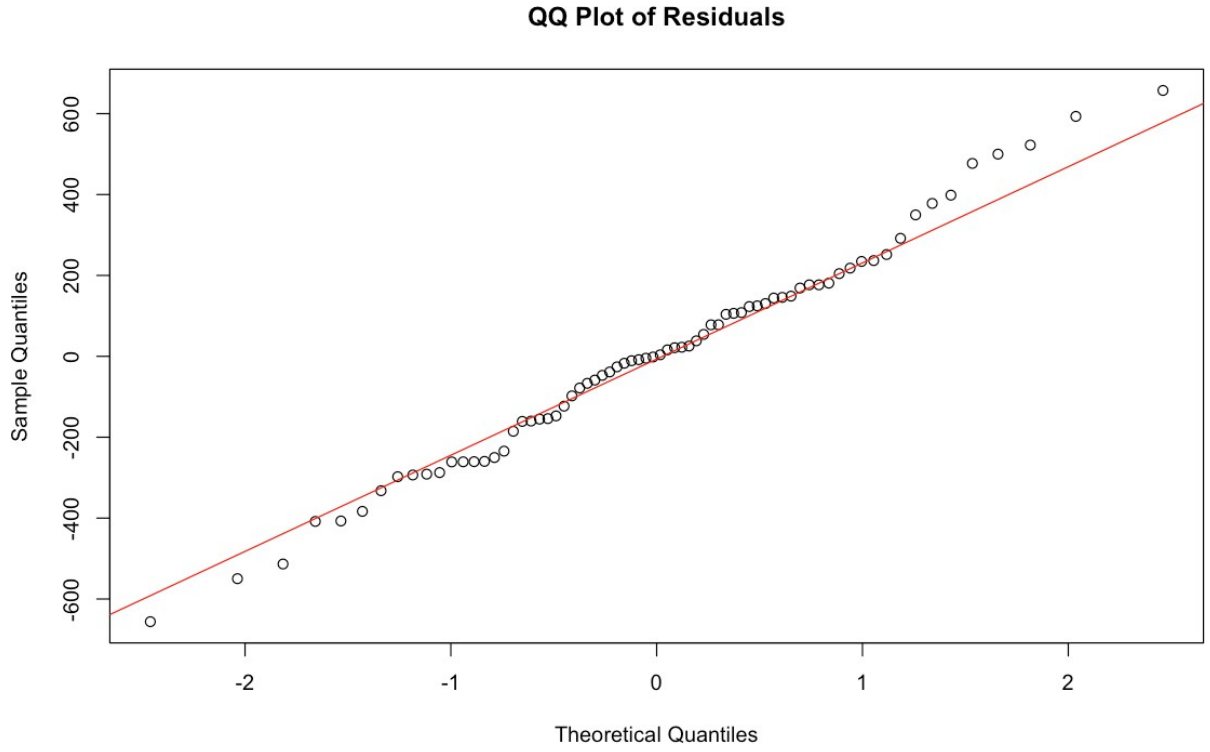
Appendices

Appendix A

Figure A1: Quantile-quantile plot of residuals of predictors in multilevel model used to predict HWEC (lmer(HWEC(µgOC/g soil) ~ OM (%) + Effective CEC (cmol+/kg) + Microporosity (%) + Sulfate S (mg/kg) + (1 + Slope | Farmlet))).



Appendix B

Table B1: Total C (µg C/g) and HWEC (µg OC/g) concentrations and HWEC as a percentage of total C for each treatment plot of Whenua Haumanu throughout the study period.

	HWEC (µg OC/g)	**Total C (µg C/g)**	**HWEC as a percentage of total C (%)**	**HWEC (µg OC/g)**	**Total C (µg C/g)**	**HWEC as a percentage of total C (%)**
SPCM	*Cropped*			*Not cropped*		
Week 0	1776	25759	8.0	2131	37000	5.7
Week 2	1609	25000	7.6	2378	37750	6.3
Week 3	1810	25250	8.5	2423	38750	6.3
Week 9	1856	28250	7.4	2347	38750	6.1
Week 19	1898	29250	7.7	2279	38250	5.9
DPCM	*Cropped*			*Not cropped*		
Week 0	1994	31750	6.3	1762	27250	6.5
Week 3	1951	31250	6.2	1976	27750	7.1
Week 9	1943	32000	6.1	1723	28750	6.0
Week 19	2083	34750	6.0	1723	29500	5.8
DPRM	*Cropped*			*Not cropped*		
Week 0	1948	29750	6.6	2630	42500	6.2
Week 3	1913	29250	6.6	2623	39750	6.6
Week 9	2095	34500	6.1	2314	40750	5.7
Week 19	1067	22500	6.2	2393	38250	6.3

Table B2: Total N (µg N/g) and HWEN (µg N/g) concentrations and HWEN as a percentage of total N for each treatment plot of Whenua Haumanu throughout the study period.

	HWEN (µg N/g)	**Total N (µg N/g)**	**HWEN as a percentage of total N (%)**	**HWEN (µg N/g)**	**Total N (µg N/g)**	**HWEN as a percentage of total N (%)**
SPCM	*Cropped*			*Not cropped*		
Week 0	197	2475	8.0	253	3600	7.0
Week 2	174	2275	7.6	278	3450	8.1
Week 3	195	2275	8.6	270	3550	7.6
Week 9	212	2850	7.4	286	3875	7.4
Week 19	213	2950	7.7	277	3850	7.2
DPCM	*Cropped*			*Not cropped*		
Week 0	232	3025	7.7	180	2575	7.0
Week 3	227	2975	7.6	194	2500	7.8
Week 9	228	3250	7.0	179	2900	6.2
Week 19	243	3475	6.9	193	3050	6.3
DPRM	*Cropped*			*Not cropped*		
Week 0	223	2975	7.5	303	3900	7.8
Week 3	220	2725	8.1	283	3625	7.8
Week 9	255	3575	7.1	279	4050	6.9
Week 19	250	3400	7.4	292	3775	7.7