Are low-producing plants sequestering carbon at a greater rate than high-producing plants?

A test within the genus *Chionochloa*

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Abstract

Plant life and primary production play an important role in the global carbon (C) cycle through the fixing of atmospheric C into the terrestrial biosphere. However, the sequestration of C into the soil not only depends on the rate of plant productivity, but also on the rate of litter decomposition. The triangular relationship between climate, litter quality, and litter decomposition suggests that whilst low-producing plants fix C at a slower rate than high-producing plants, they may release C at an even slower rate, due to the production of a recalcitrant litter.

Here, the relationships between environment, productivity, litter quality and decomposition are investigated to determine their relative influences on C sequestration for taxa in the genus *Chionochloa.* Annual productivity was measured *in situ* for 23 taxa located across New Zealand, whilst litter and soil were collected for analyses and two *ex-situ* decomposition experiments; litter incubation on a common alpine soil, and litter incubation on each taxon's home-site soil.

Plant growth rate was found to be positively correlated with both litter nitrogen and litter fibre content. Litter decomposition on the common soil was instead negatively correlated with lignin content, which showed a strong correlation with phylogeny, as opposed to environment or growth rate. When incubated on home-site soils, litter quality had no influence on decomposition, which was instead positively correlated with the rate of soil C decomposition, and negatively correlated with both soil organic matter and soil water content.

On the common soil there were weak correlations between productivity and decomposition; however the proportional increase in productivity was greater than the corresponding increase in decomposition, resulting in high-producing plants sequestering C at a greater rate than low-producing plants. However, there was no correlation between productivity and decomposition on the home-site soil, with soil water content being a better predictor of C sequestration rate than productivity.

Despite the range of variation in morphology, ecophysiology, productivity and habitat displayed within the *Chionochloa* genus, taxa all produced litter of a very similar quality. Breakdown of that litter is then most strongly influenced by the environment in which decomposition occurs, as opposed to the quality of the litter. Any subsequent differences in rates of C sequestration are therefore most influenced by the environment decomposition occurs in, with wet and cool environments likely to result in increased rates of C sequestration, independent of the rate of productivity.

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Contents

Chapter 1

Introduction

Chionochloa tussock grassland, Mt Burns, Fiordland – M. Dickson

Introduction

Carbon Cycling and Carbon Sequestration

The long term storage of atmospheric carbon dioxide (CO_2) into the soil carbon (C) pool is an important part of the global C cycle. This transfer of C from the atmosphere to the biosphere is made possible through photosynthesis, resulting in net production by primary producers (Pregitzer *et al.*, 2007). In general, a large proportion of this C is released back into the atmosphere through biotic respiration by C consumers, but a small proportion of it is stored long-term in the soil C pool (Wigley and Schimel, 2005). This storage of C in the soil is called C sequestration, and occurs when the ratio of the total C fixed to total C released, within a system over a set time period, is greater than one (Krna and Rapson, 2013). Thus, C sequestration within an ecosystem is equal to the balance between productivity and decomposition (Bradley and Pregitzer, 2007). Determining this balance between productivity and decomposition will provide an insight into the factors driving C sequestration, and may help to identify biotic and abiotic characters that increase C sequestration.

The importance of C sequestration has been accentuated by an imbalance in the C cycle as a result of increased anthropogenic CO_2 emissions. Cycling of C between oceanic, terrestrial, and atmospheric pools has historically, prior to anthropogenic interference, had long time periods of near net balance (Wigley and Schimel, 2005). Anthropogenic influences, especially those associated with the eighteenth century's industrial revolution, have disrupted this balance through a number of ways, with the most significant being the destruction of existing vegetation, land use change, fossil fuel burning, and cement production (Houghton, 1991; Watson *et al.*, 1996; Vitousek *et al.*, 1997; Wigley and Schimel, 2005). This trend continues to occur, with anthropogenic CO_2 emissions increasing dramatically over the last century, resulting in an increase in the atmospheric C pool (Friedlingstein *et al.*, 2010).

CO_2 and Global Warming

The global atmospheric CO_2 concentration has increased dramatically since the industrial revolution from approximately 285 parts per million to now exceeded 400 parts per million (Pachauri *et al.*, 2014; Gall and Nazaroff, 2015). One of the lead-on effects from an increase in atmospheric CO_2 has been a change in climate, due to the

green-house effect of CO_2 and other green house gases. The Intergovernmental Panel on Climate Change (IPCC) has reported an unequivocal rise in average global temperature over the past 50 years, associated with an increase in anthropogenic green-house emissions, with further increases predicted (Pachauri *et al.*, 2014). Of these anthropogenic emissions, CO_2 is by far the most prevalent. In 2010 anthropogenic CO_2 emissions made up 76% of the total equivalently weighted anthropogenic green-house gas emissions (with Methane at 16%, Nitrous oxide at 6.2%, and Fluorinated gases at 2% making up the remainder) (Pachauri *et al.*, 2014). Hence, reducing this imbalance in the carbon cycle has become an issue of significance worldwide, both for ecosystem preservation and anthropoic reasons.

The long term sequestering of atmospheric C into the soil pool in the form of potentially stable humus to reduce atmospheric CO_2 is a possible solution for the mitigation of climate change. Long term storage of C in the soil is considered a better solution than the temporary sequestration of C into biomass through afforestation and reforestation (Batjes, 1998; Krna and Rapson, 2013). Hence, understanding relationships between productivity and decomposition is vital for accurate C budgeting and decision-making in the mitigation of global climate change.

Litter Quality, Productivity, and Decomposition

The decomposition of organic matter ultimately determines how much C is emplaced in the soil and how much C is released (Berg and McClaugherty, 2008). This process is controlled by a number of factors including temperature, moisture, litter quality, and the microbial community (Aerts, 1997; Zhang *et al.*, 2008; Prescott, 2010). Whilst climate is thought to be the primary determinant of decomposition rate, there are suggestions litter quality may play an equally important role, particularly in the formation of stable humus in the latter stages of decomposition (Couteaux *et al.*, 1995).

The physical and chemical characteristics of plant litter play an important role in its rate of decomposition (Aerts, 1997). These characteristics can be generally summarised as litter quality. High quality litters contain metabolites and constituents that are readily metabolised and broken down by microorganisms, resulting in rapid rates of decomposition and C release (Cadisch and Giller, 1997). Conversely, poor quality litters contain metabolites and constituents that are recalcitrant to decomposition. Thus, the quality of litter is an important determinant of the amount of C stored and released within the soil.

A plants litter quality is thought to be related to the growth strategy implemented by that plant and growth rate at which the material is produced (Coley *et al.*, 1985). The influence of environmental and resource stress on plant growth often results in trade-offs between productivity and survival (Grime, 2006). Adaptations for survival and persistence not only influence the rate of growth, but also the underlying plant physiology, morphology, and resource use strategy, which in turn influences the chemistry of the plant material produced (Poorter and Villar, 1997). Environments that contain favourable conditions and ample resources for growth tend to be dominated by plants with characters that allow them to exploit these conditions (Darwin, 1991; Grime, 2006). These characters, such as rapid rates of growth and a large photosynthetic area, tend to result in the production of a high quality litter that is readily decomposed. In contrast, environments that contain conditions and levels of resources that are limiting for growth, tend to favour plants with characters than allow them to endure and persist in that environment (Grime, 2006). Characters associated with this strategy, such as a slow rate of growth and production of tough leaf material, tend to result in the production of a poor quality litter, recalcitrant to decomposition (Coley, 1988).

Theory and Hypotheses

The relationships between environment, productivity, litter quality, and decomposition are likely to influence C sequestration. The productivity to decomposition ratio (P:D), as used by Kirschbaum (2000) and Krna (2015) indicates that C sequestration increases when the ratio of productivity to decomposition increases. Where C is added to the surface of the soil at a great rate, as occurring under high producing plants, C sequestration may be zero if C is lost from the soil at an equivalent rate, as occurs in readily decomposing litter (Figure 1; red dashed lines).

Underlying the assumption that higher-producing plants are sequestering more carbon than lower-producing plants (Figure 1;b), is the assumption that productivity has little influence on the rate of litter decomposition, assuming the rate litter of decomposition remains relatively constant, independent of the rate at which the litter is produced (Figure 1; a).

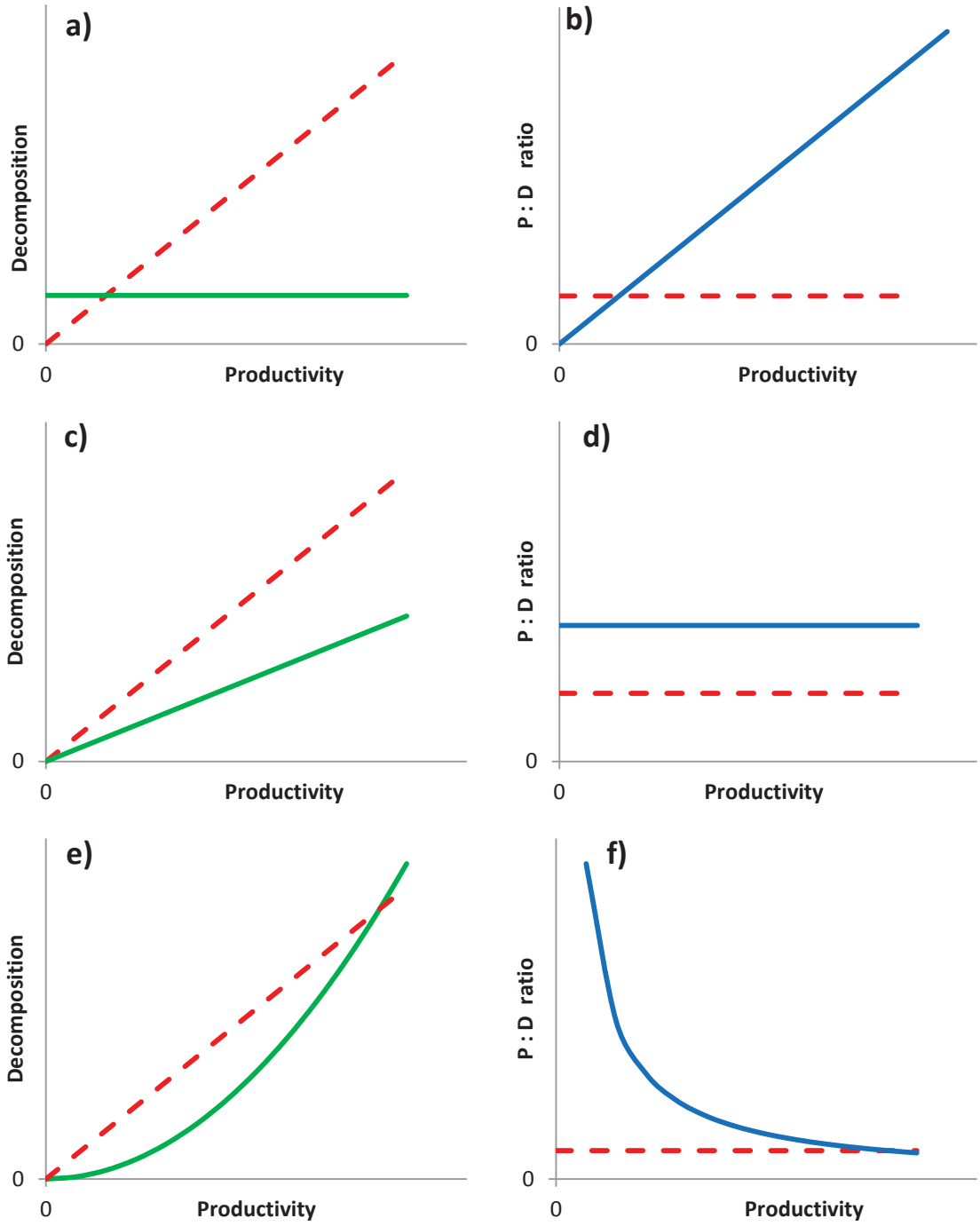


Figure 1: Hypothetical relationships between productivity and decomposition (green lines), and the corresponding relationships between productivity and C sequestration (P:D ratio; blue lines). The Red dashed line indicates a 1:1 relationship between productivity and decomposition, resulting in no C sequestration where the P:D ≤ 1. **a & b)** rate of decomposition remains constant independent from rate of productivity. **c & d**) rate of decomposition increases linearly with rate of productivity. **e & f)** rate of decomposition is relatively lower in low-producing plants compared to high producing plants.

However, according to the relationships introduced above, there is evidence that the rate of litter decomposition is not independent of the rate of productivity at which that litter was produced. Where the rate of decomposition increases at a constant rate relative to productivity (Figure 1; c), low-producing plants can be expected to sequester approximately equal amounts of C per gram of productivity (Figure 1; d). Due to the tendency of stress-tolerating and low-producing plants to produce litter recalcitrant to decomposition, it could be possible that the relationship between productivity and decomposition is not linear (Figure 1; e), where the rate of decomposition is much lower for low-producing plants. If this decrease in decomposition associated with low-producing plants, is proportionally greater than the associated reduction in productivity, C sequestration per gram of productivity produced may be greater in low-producing plants (Figure 1; f).

Chionochloa *as a Suitable Study System*

An appropriate system to test the relationships between productivity, plant litter, and decomposition would require the following: *i*) ease and accuracy in measurement of annual productivity; *ii*) variation in productivity, morphology, climate, habit, and subsequent variation in litter quality; and *iii)* minimal phylogenetic dissimilarities between taxa that may alter litter quality and productivity. Testing for relationships in C sequestration within a congeneric group will allow for the detection of factors influencing C sequestration, independent of major genotypic variation.

The genus *Chionochloa* provides such a suitable system for investigating C sequestration. An Australasian genus of 25 species, of which 23 species are endemic to New Zealand, these tussock grasses predominantly occur in native grassland and scrubland, where they are often the dominant species (Connor, 1991; Connor and Lloyd, 2004). *Chionochloa* tussocks are long-lived perennials grasses, made up of modular tiller units, which allow for ease in productivity measurements (Mark, 1965b; Williams, 1977), and in addition produce little somatic C sequestration, as woody species do, which complicates the measurement of productivity (Krna and Rapson, 2013).

The distribution of *Chionochloa* ranges from localised to widespread, with species occurring throughout the North, South, Stewart, and offshore Islands, with variation in habitat and niche occurring (Connor, 1991). This, in combination with an altitudinal range from sea-level to high-alpine grasslands, provides a vast range in environmental conditions and resulting productivity

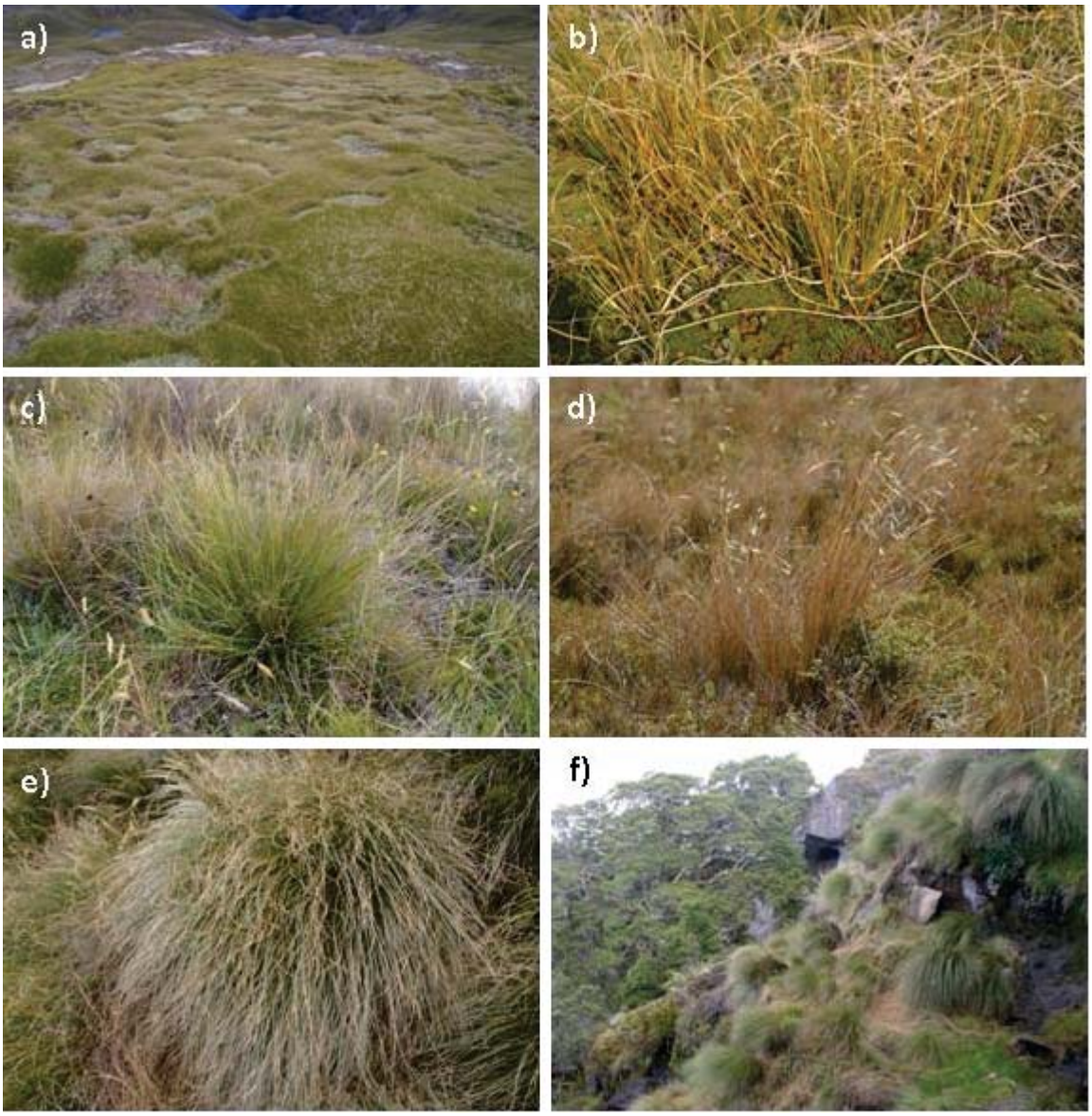


Figure 2: Some of the variation in morphology and habit present in smaller taxa in the *Chionochloa* genus. Taxa are displayed in order of increasing size (height and width) from *a* – *k*. **a)** *C. australis*, Mt Robert, **b)** *C. crassiuscula* ssp. *torta*, Mt Eldrig, **c)** *C. vireta*, Richardson Mts., d) *C. juncea*, Denniston Plateau, e) *C. teretifolia*, Mt Burns, f) *C. spiralis*, Mt Luxmore. Figure 2 (*g* – *k*) continues below.

(Mark, 1965a; Mark, 1965b; Mark, 1969). *Chionochloa* also has a variety of growth forms and habits, ranging from less than 15 cm in height to over 130 cm in height, and shows variation in leaf morphology and tiller size (Connor, 1991) (Figure 2).

Relationships between litter quality and decomposition have been tested before (Melillo *et al.*, 1982; McClaugherty *et al.*, 1985; Trofymow *et al.*, 2002). However, these studies often test for relationships between plant functional groups and unrelated taxa, which can result in large differences in plant physiology and morphology due to differences in phylogeny. In support of this, Cornelissen *et al.* (2004) and Cornwell *et al.* (2008) found the greatest differences in litter quality and rates of litter

Figure 2 continued: Larger *Chionochloa* tussock taxa. **g)** *C. pallens*, ssp. *pilosa*, Poplars Range, **h)** *C. defracta*, Red Hills, **i)** *C. rigida* ssp. *amara*, Mt Anglem, **j)** *C. rubra* spp. *cuprea*, Pukerau, **k)** *C. flavescens* ssp. *lupeola*, Mt Rochfort.

decomposition between taxa were due to differences in plant functional group and plant species traits, as opposed to environment. However, there is evidence that environment and climate do influence the traits of green leaves and resulting litter quality (Aerts, 1997; Wright *et al.*, 2005), though these relationships may be hidden if phylogenetic differences occur between the taxa studied. From this it is proposed that to accurately detect the relationships between environment, productivity, and decomposition, any phylogenetic differences between taxa need to be minimal, and-or taken into account.

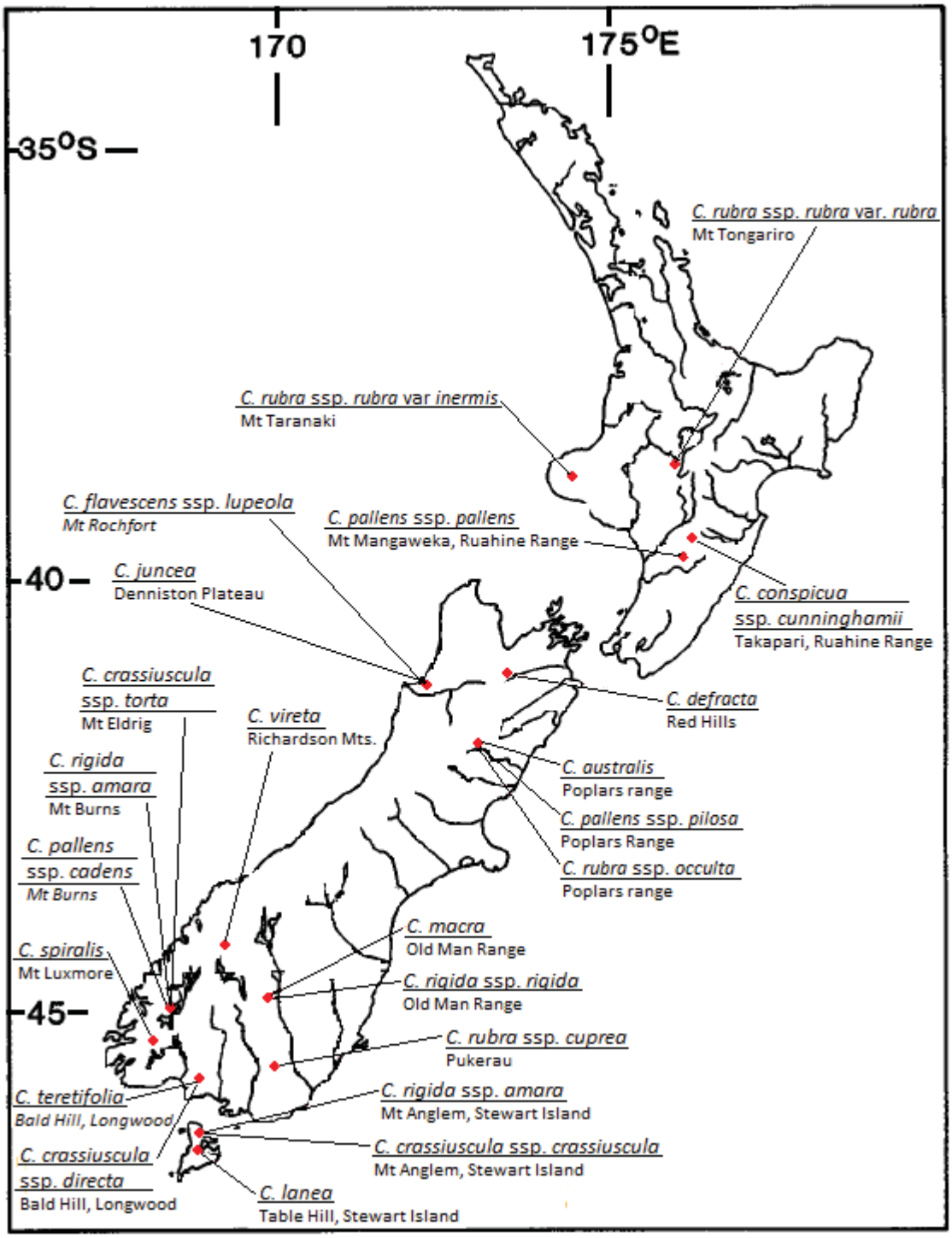


Figure 3: *Chionochloa* taxa sampled and sampling locations.

The *Chionochloa* genus should provide sufficient variation in environment, productivity, morphology, and consequent litter quality, without any critical genetic differences between taxa to distort this relationship. Relationships between leaf litter traits and decomposition have been found to occur between congeneric species (Geng *et al.*, 1993), supporting the notion that a congeneric group may be a good system to test for the relationships above. This research tests for relationships between a plant's

environment, leaf productivity, leaf litter quality, and leaf litter decomposition, principally focusing on C sequestration of aboveground plant tissues.

Research Sites

For the 23 species of *Chionochloa* endemic to New Zealand, as many taxa as possible were located within their natural geographic range. A total of 23 taxa were located, comprising 14 species (61% of genus) and 13 subspecies and or varieties (72% of genus subspecies and varieties), resulting in a wide representation of the different growth forms present in the *Chionochloa* genus. Taxa occurring in indigenous grassland or the alpine zone were selected for predominantly, although some taxa sampled do occur in montane herbfield-scrubland, with one taxon on montane forest margins, and another under open montane forest canopies. A wide geographic sampling range was also achieved, with species occurring throughout the North Island, South Island, and Stewart Island (Figure 3). Due to the wide geographic range and altitude range, sites differed in their vegetation composition and environmental characters, but at these sites *Chionochloa* tended to be the dominant vegetation

Objectives

The aim of this research is to investigate the relationships between productivity, litter quality, and decomposition, and their subsequent roles in C sequestration. It is hypothesised that low-producing plants may be sequestering equal or greater amounts of C per gram of productivity when compared to high producing plants, due to the production of litter recalcitrant to decomposition.

Chapter two investigates the range in litter quality and chemistry occurring in the *Chionochloa* genus, with the aim of determining how climate and environment may be influencing these. It is hypothesised that taxa under greater environmental and climatic stress will produce a poorer quality litter.

Chapter three investigates the range of productivity occurring in the *Chionochloa* genus, with the aim of testing for relationships between productivity and environment, and productivity and litter quality. It is hypothesised that litter quality will be higher in plants with greater productivity, due to a reduction in the concentration of leaf secondary plant metabolites and constituents, and an increase in concentration of leaf nutrients.

Chapter four investigates the influence of litter quality on the rate of litter decomposition. The influence of soil characteristics on litter decomposition are also considered, and compared with the influence of litter quality. It is hypothesised that litter decomposition will be lower in taxa with a poorer quality litter, due to the recalcitrant nature of secondary plant metabolites and constituents.

Chapter five investigates the relationships between productivity and decomposition, providing a synthesis of the previous chapters. P:D ratios are created to test for relationships between productivity and C sequestration, and ultimately test the hypothesis; are low-producing plants, per gram of productivity, sequestering C at an equal or greater rate than high-producing plants?

References

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos*, 439-449.

Batjes, N. 1998. Mitigation of atmospheric CO2 concentrations by increased carbon sequestration in the soil. *Biology and Fertility of Soils,* 27**,** 230-235.

Berg, B. & Mcclaugherty, C. 2008. Plant litter. *Decomposition, humus formation, carbon sequestration. 2nd Ed Springer*.

Bradley, K. L. & Pregitzer, K. S. 2007. Ecosystem assembly and terrestrial carbon balance under elevated CO 2. *Trends in Ecology & Evolution,* 22**,** 538-547.

Cadisch, G. & Giller, K. E. 1997. *Driven by nature: plant litter quality and decomposition*, CAB international.

Coley, P. 1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia,* 74**,** 531-536.

Coley, P. D., Bryant, J. P. & Chapin, F. S. 1985. Resource availability and plant antiherbivore defense. *Science,* 230**,** 895-899.

Connor, H. 1991. Chionochloa Zotov (Gramineae) in New Zealand. *New Zealand journal of botany,* 29**,** 219-282.

Connor, H. & Lloyd, K. 2004. Species novae graminum Novae-Zelandiae II. Chionochloa nivifera (Danthonieae: Danthonioideae). *New Zealand Journal of Botany,* 42**,** 531-536.

Cornelissen, J. H. C., Quested, H. M., Gwynn-Jones, D., Van Logtestijn, R. S. P., De Beus, M. a. H., Kondratchuk, A., Callaghan, T. V. & Aerts, R. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology,* 18**,** 779-786.

Cornwell, W. K., Cornelissen, J. H., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., Hobbie, S. E., Hoorens, B., Kurokawa, H. & Pérez-Harguindeguy, N. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology letters,* 11**,** 1065-1071.

Couteaux, M.-M., Bottner, P. & Berg, B. 1995. Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution,* 10**,** 63-66.

Darwin, C. 1991. On the origin of species by means of natural selection. 1859. *Murray, London,* 502.

Friedlingstein, P., Houghton, R., Marland, G., Hackler, J., Boden, T. A., Conway, T., Canadell, J., Raupach, M., Ciais, P. & Le Quere, C. 2010. Update on CO2 emissions. *Nature Geoscience,* 3**,** 811-812.

Gall, E. T. & Nazaroff, W. W. 2015. New directions: Potential climate and productivity benefits from CO2 capture in commercial buildings. *Atmospheric Environment*.

Geng, X., Pastor, J. & Dewey, B. 1993. Decay and nitrogen dynamics of litter from disjunct, congeneric tree species in old-growth stands in northeastern China and Wisconsin. *Canadian Journal of Botany,* 71**,** 693-699.

Grime, J. P. 2006. *Plant strategies, vegetation processes, and ecosystem properties,* John Wiley & Sons.

Houghton, R. A. 1991. Tropical deforestation and atmospheric carbon dioxide. *Tropical Forests and Climate.* Springer.

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

Krna, M. A. 2015. *Productivity, decomposition and carbon sequestration of Chionochloa species across altitudinal gradients in montane tussock grasslands of New Zealand.* PhD, Massey Univeristy, New Zealand.

Krna, M. A. & Rapson, G. L. 2013. Clarifying 'carbon sequestration'. *Carbon Management,* 4**,** 309-322.

Mark, A. 1965a. Ecotypic differentiation in Otago populations of narrowleaved snow tussock, Chionochloa rigida. *New Zealand journal of botany,* 3**,** 277-299.

Mark, A. 1965b. The environment and growth rate of narrow-leaved snow tussock, Chionochloa rigida, in Otago. *New Zealand journal of botany,* 3**,** 73-103.

Mark, A. 1969. Ecology of snow tussocks in the mountain grasslands of New Zealand. *Vegetatio,* 18**,** 289-306.

Mcclaugherty, C. A., Pastor, J., Aber, J. D. & Melillo, J. M. 1985. Forest litter decomposition in relation to soil nitrogen dynamics and litter quality. *Ecology***,** 266-275.

Melillo, J. M., Aber, J. D. & Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, 63**,** 621-626.

Pachauri, R. K., Allen, M., Barros, V., Broome, J., Cramer, W., Christ, R., Church, J., Clarke, L., Dahe, Q. & Dasgupta, P. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Poorter, H. & Villar, R. 1997. The fate of acquired carbon in plants: chemical composition and construction costs.

Pregitzer, K. S., Zak, D. R., Loya, W. M., Karberg, N. J., King, J. S., Burton, A. J., Cardon, Z. & Whitbeck, J. 2007. The contribution of root–rhizosphere interactions to biogeochemical cycles in a changing world. *The rhizosphere: An ecological perspective***,** 155-178.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry,* 101**,** 133-149.

Trofymow, J., Moore, T., Titus, B., Prescott, C., Morrison, I., Siltanen, M., Smith, S., Fyles, J., Wein, R. & Camiré, C. 2002. Rates of litter decomposition over 6 years in Canadian forests: influence of litter quality and climate. *Canadian Journal of Forest Research,* 32**,** 789-804.

Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. 1997. Human domination of Earth's ecosystems. *Science,* 277**,** 494-499.

Watson, R. T., Zinyowera, M. C., Moss, R. H. & Dokken, D. J. 1996. Climate change 1995. Impacts, adaptions and mitigation of climate change: scientific-technical analyses.

Wigley, T. M. & Schimel, D. S. 2005. *The carbon cycle*, Cambridge University Press.

Williams, P. 1977. Growth, biomass, and net productivity of tall-tussock (Chionochloa) grasslands, Canterbury, New Zealand. *New Zealand journal of botany,* 15**,** 399-442.

Wright, I. J., Reich, P. B., Cornelissen, J. H., Falster, D. S., Groom, P. K., Hikosaka, K., Lee, W., Lusk, C. H., Niinemets, Ü. & Oleksyn, J. 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography,* 14**,** 411-421.

Zhang, D., Hui, D., Luo, Y. & Zhou, G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology,* 1**,** 85-93.

Chapter 2

Congeneric variation in litter quality:

Is litter quality more related to environment or to phylogeny?

Chionochloa rubra ssp. *rubra* var. *rubra*, Mt Tongariro – M. Dickson

Introduction

Litter Quality

Litter quality is one of the key factors influencing the rate of litter decomposition (Couteaux *et al.*, 1995; Aerts, 1997; Zhang *et al.*, 2008), and subsequently the sequestration of carbon (C) in the soil (De Deyn *et al.*, 2008). Determining correlations between litter quality and decomposition is thus important for accurately calculating and estimating C sequestration. Research into the influence of increased atmospheric carbon dioxide on litter quality (Coûteaux *et al.*, 1999; Norby *et al.*, 2001) has highlighted the importance of the relationship between the environment and leaf litter quality. Leaf litter can be described by the quality and quantity of resources it contains, and how easily those resources are utilised by other organisms, including animals, soil animals, soil microorganisms, and plants (Cadisch and Giller, 1997).

In addition, leaf litter can generally be described by is chemistry. Poor quality litters generally comprise low quality resources, and/or a low abundance of resources, as well as potentially containing constituents or characters that prevent consumers from accessing to those resources within the litter. Alternatively, high quality litter is comprised of either high quality resources, and/or an abundance of resources, which in turn are easily accessible to consumers. As a result, differences in initial litter chemistry between species directly influences the process of microbial litter decomposition in the soil (Meier and Bowman, 2008). Here, the relationships between plant litter quality and plant environment are explored within a congeneric group to determine the relative influences of the environment and phylogeny on litter quality.

Factors Influencing Litter Quality

The resource quality of plant material is broadly defined by a species' plant functional group, and by the environmental conditions under which that plant material is grown (Grime, 1988; Cornelissen *et al.*, 2004). The ecological strategies used by plants to allocate C and N in the growth of live material, as well as the production of plant protective compounds and other secondary metabolites, have obvious implications for the litter once that material has been shed by the plant. Many of the physiological and protective characters that occur in the live green leaf of a plant persist in discarded litter due to incomplete re-absorption of nutrients during senescence. As a result, there is a

strong correlation between live leaf chemistry and the chemistry of leaf material discarded as litter (Aerts, 1996; Killingbeck, 1996).

Plants can be separated into functional groups based on the resource acquisition strategy they implement for survival, growth, and development. As a result, variation in litter quality seen between species can be attributed to variation in growth strategy used produce leaf material (Cornwell *et al.*, 2008a) and references therein). Plants growing in a resource-rich environment with low-stress tend to have higher productivity, and produce a litter that is more readably decomposed compared to plants growing in a resource-poor and high-stress environment (Coley *et al.*, 1985; Grime, 2006; Ordoñez *et al.*, 2009) (See *Chapter 3*). Therefore, it can be hypothesised that the environment plays a large part in a plant's growth strategy, which in turn determines the makeup of live leaf material and consequent litter quality.

Measures of Litter Quality

Litters with a high nutrient content tend to decompose rapidly as they provide microorganisms with resources that allow for rapid rates of metabolism and growth. In a review of the literature Enriquez *et al.* (1993) found litter nitrogen (N) and phosphorus (P) to both have strong positive linear relationships with rates of litter decomposition, which are attributed to the high nutrient requirements of microorganisms. Where nutrients are readily available, microbial activity and population growth is greatly increased resulting in increased decomposition of substrate, whilst where limiting, decomposition can be greatly reduced (Gulis and Suberkropp, 2003). The C:N ratio has been commonly used to describe this effect, with C rich and N poor litters found to decompose slowly (Cadisch and Giller, 1997).

Secondary plant metabolites also greatly influence the rate of litter decomposition through 'afterlife' effects on decomposers (Cornelissen *et al.*, 2004). Secondary plant metabolites are most simply defined as plant constituents not essential or necessary for basic plant functioning (Seigler, 2012). However, these metabolites may enhance plant growth and development through structural support, protection from stress, herbivory, and disease, as well as by allowing plant signalling (Karlovsky, 2008). As a result, it can be expected that plants under greater stress are likely to contain greater concentrations of secondary plant metabolites.

Plant material can be separated broadly into two components, soluble compounds able to be leached from the litter, and insoluble components constituting

plant fibre. The water soluble fraction of litter is generally made up of simple sugars, lower fatty acids, proteins and peptides (Cadisch and Giller, 1997). Most of these components are easily taken up and metabolized by microorganisms, providing high quality litter which is rapidly and entirely decomposed within the first phase of decomposition. Plant fibre on the other hand, including cellulose, hemicellulose, and lignin, are not as rapidly decomposed. Structural carbohydrates cellulose and hemicellulose provide a good energy source for decomposers, though tend to decompose more slowly than the soluble fraction, decomposing at a steady rate and remaining at a constant concentration in the litter throughout the decomposition (Berg and McClaugherty, 2008).

The secondary metabolite lignin is also a structural component found in plant cell walls and has the main functions of mechanical support, aiding water transport in xylem vessels, and chemical defence against predators and microorganisms (Moura *et al.*, 2010). Lignin content is well known to be an indicator of poor litter quality and reduced rates of decomposition (Meentemeyer, 1978; Melillo *et al.*, 1982; Taylor *et al.*, 1989) due to decomposition being restricted to limited types of fungi and bacteria, such as 'white rot fungi' (Guerriero *et al.*, 2016).

Whilst high N levels are known to increase initial rates of decomposition, in later decomposition where litter lignin concentration is greater, N may inhibit litter decomposition. Berg and Matzner (1997) found N to reduce lignin decomposition, which is attributed to N inhibition of synthesis of ligninolytic enzymes (Carreiro *et al.*, 2000; Sinsabaugh *et al.*, 2002), as well as the formation of recalcitrant humic compounds when N reacts within degraded lignin products (Dijkstra *et al.*, 2004; Berg and McClaugherty, 2008). These humic compounds may act as barriers, preventing microorganisms from accessing more readily decomposable structures such as cellulose and hemicellulose (Nommik and Vahtras, 1982; Liu *et al.*, 1985). As a result, the Lignin: N ratio of litter is used as a key indicator of litter quality for long term decomposition. Therefore, these ratios were derived in this study.

Phenols and tannins also can be used as measures of litter quality. A high tannin content can indicate a poor litter quality, as tannins can form recalcitrant complexes with many other compounds during decomposition (Horner *et al.*, 1988). Similarly, phenolic content can also indicate a poor litter quality, as phenols tend to condense into less decomposable forms over time (Berg and McClaugherty, 2008). Phenols can inhibit the growth or function of decomposing organism by binding to enzymes, or

chemical binding to N leaving it unusable to decomposers (Martin and Haider, 1980; Waterman and Mole, 1994).

Plant Reponses to Stress

Plant responses and adaptations to environmental stress include both phenotypic and genotypic changes, including changes in biochemical systems, which allow survival and growth in environments that do not have favourable conditions (Yordanov *et al.*, 2000). At the plant level, environmental and climatic stress often result in a reduction in growth and photosynthesis rates (Grime, 2006). Environment and climate stress also influencing the chemical composition of plant leaves due to the production of secondary metabolites, which functions often aid in the tolerance of this stress (Gershenzon, 1984; Akula and Ravishankar, 2011).

The quality and quantity of plant lignin is known to be regulated by developmental and environmental cues, with environmental stress thought to increase lignin production (Campbell and Sederoff, 1996). Stresses known to increase lignin content include decreases in temperature, mineral deficiency, drought, increased solar radiation, as well as disease and herbivory (Moura *et al.*, 2010). An increase in fibre is also known to occur in cold-stressed plants, with increased cold-stress thought to result in the production of tougher leaves, and other mechanisms including thickening of the cell wall (Huner *et al.*, 1981; Stefanowska *et al.*, 1999).

Climate has also been shown to influence leaf litter N concentrations. In a meta-analysis Lui *et al* (2006) found significant positive relationships between leaf litter N concentrations in temporal and boreal forest, and temperature and precipitation. Similarly, along a altitudinal gradient, Craine and Lee (2003) found high altitude grasses to have lower N concentrations when compared to low altitude grasses in New Zealand, though tissue density remained constant. Production of phenols appears linked to UV light protection (Kefeli *et al.*, 2003) as well as protection against herbivory and disease (Hättenschwiler and Vitousek, 2000).

Genetic Distance and Functional Group

There is mixed opinion in the literature as to whether litter quality is predominantly controlled by genotype and functional group, or by environment. In a meta-analysis of live leaf traits, climate was found to have a modest relationship with leaf traits (Wright *et al.*, 2004). However, the analysis comprised a wide range of

function groups, with over 2500 species sampled at over 170 sites worldwide, which may indicate that functional groups are primarily responsible for variation in litter quality, as is indicated by other studies (Cornwell *et al.*, 2008a; Cornwell *et al.*, 2008b; De Deyn *et al.*, 2008; Freschet *et al.*, 2012). In addition, a large proportion of the variation in leaf traits was found to occur between co-existing species, suggesting a range of function plant types are likely to occur at a site independent of climate.

A functional group approach is thus unlikely to detect changes in litter quality resulting from differences in environmental conditions (Bradley and Pregitzer, 2007). In this experiment, the use of a congeneric group containing closely related species allows for testing of the relationship between the environment and litter quality, without major phylogenetic differences in litter quality occurring.

Hypotheses and Aims

In this study, the litter quality of taxa in the genus *Chionochloa* is assessed using some of the key litter chemistry variables described above to determine if litter quality in the genus varies with environmental conditions. Litter quality is correlated against the climatic and environmental conditions with which the litter was produced in, as well as the genetic distance between taxa. It is hypothesised that a range of litter qualities will occur within the genus, due to a range of environmental and climatic conditions experienced between taxa. Poor quality litters are hypothesised to correlate with increased environmental stress, whilst high quality litters are hypothesised to correlate with environmental conditions that favour rapid rate of growth.

Methods

Experimental Design

Sites were located for a total of 23 taxa, comprising 14 species (61% of genus) and 13 subspecies and varieties (72% of subspecies and varieties within the genus), resulting in a large representation of the different growth habits present in the *Chionochloa* genus. At each taxon location a 4x4m sampling plot was set up using restricted randomisation (Figure 1a). Plots were restricted to (i) a common location within the known species' range, (ii) the approximate median altitude within the species' altitudinal range at that location, (iii) a homogenous environment with

predominantly uniform indigenous vegetation, (iv) a site with the flattest available topography or on a ridge line. Sampling occurred over two visits made to these plots, approximately one year apart, with sampling Time 1 in February 2013 and sampling Time 2 in February 2014.

Environmental Data

Environmental data parameters were recorded during the first visit in February 2013. Parameters recorded included aspect, measured as degrees of deviation from true north, slope (measured along steepest side of plot using a clinometer), altitude, latitude and longitude measured in degrees, and maximum standing height of *Chionochloa* taxa in the plot.

An estimate of annual climate data for the year February 2013 to February 2014 was provided by the National Institute of Water and Atmospheric Research (NIWA) using their modelled Virtual Climate Stations (VCS's). Over 12,000 VCS's are generated over a 5km grid across New Zealand, with modelling based on spatial interpolation of actual data observations made at over 6000 climate stations located around the country. A thin-plate smoothing spline model is used for the spatial interpolations (Tait *et al.*, 2006; Tait and Woods, 2007). VCS's were selected for each site in order of preference for (i) the least distance from each field site, and (ii) least difference in altitude from the field site. Where two VCS's were equally distant from a site, the VCS with the most similar altitude to the site was chosen. The VCS's climatic parameters used were mean maximum summer temperature, mean minimum winter temperature, mean summer potential evapotranspiration, average annual wind speed, mean summer solar radiation, mean summer soil moisture deficit, and total annual rainfall.

Soil Collection and Preparation

Soil samples were collected from the top 10cm of the soil profile (n=10) using a soil corer (diameter 2.8cm), haphazardly from within and directly adjacent to the plot. Any litter, vegetation, and stones >1cm diameter were removed, and samples bulked. As soon as possible (within 4 days), soil samples were stored intact in a dark deep freeze (-20˚C) until analysis. After thawing, a subsample of each soil was sieved to 4mm, and water holding capacity calculated from saturation on a ceramic pressure plate by means of a water bubble tower with 50cm of suction at 5kpa (Klute, 1986). Soil

samples were spread out and air dried at 25˚C for 72hrs, then sieved to 2mm to remove any further vegetative material and stones. Larger soil particles were finely ground with a mortar and pestle prior to chemical analyses. Organic soil C and total N content for all soils were analysed by flash combustion using Leco TruMac Analyzer (Leco, 2003), analysed at Landcare Research, Palmerston North, New Zealand.

Litter Collection and Preparation

Recently dead leaves, including the lamina and sheath, that were still attached to the plant were collected for each of the 23 taxa at its home plot in February 2014. This litter was collected from a minimum of 10 plants within each plot. Where not enough litter was available within the plot, litter was collected from plants directly adjacent to the plot. Between 50-150 recently dead leaves, depending on the leaf size, were bulked for each taxon and stored in the dark at -20˚C as soon as possible thereafter (within 4 days). In the lab, leaf litter was thawed before being oven dried at 30˚C for 72hrs to standardise moisture content, and then impact-ground to pieces less than 1mm in size (Figure 1b).

Litter Chemical Analyses

Chemical analyses were performed on the impact-ground litter for each of the 23 taxa. Litter was dried again, this time at 60C° for 48hrs before plant tissue carbon (C) and nitrogen (N) were analysed by flash combustion using a Leco TruMac Analyzer (Leco, 2003) at Landcare Research, Palmerston North, New Zealand. Plant C and N are expressed as a percentage of the oven dry weight of litter.

Cellulose, hemicellulose, and lignin occurring in plant litter were analysed by sequential treatment of the dried impact-ground litter with a neutral detergent to separate the neutral detergent fibre (NDF) and neutral detergent solution (NDS). Then the sample is washed with an acid detergent to isolate the acid detergent fibre (ADF) from the NDF, and subsequently with 72% H_2SO_4, before ashing to isolate the lignin content from the ADF. Next NDF, ADF, and Lignin were analysed by a Tecator Fibertec^{TM} system (Mertens, 2002) at the Nutrition Laboratory, IAE, Massey University, Palmerston North, New Zealand. NDS is equivalent to the reciprocal of NDF, whilst ADF is equivalent to cellulose plus lignin. Total phenolic content in the impact ground plant litter was analyzed by Folin-Ciocalteau reagent according to Isabelle *et al.* (2010), at the Nutrition Laboratory, IAE, Massey University, Palmerston

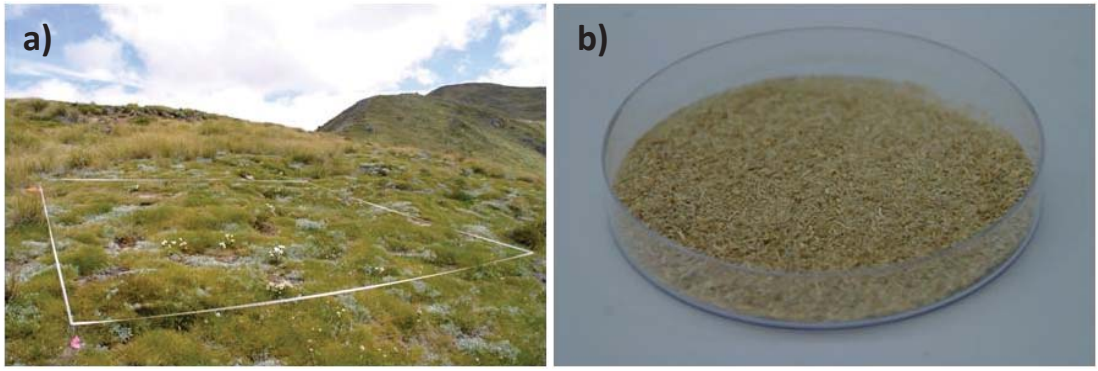


Figure 1: a) 4x4m sampling quadrate for *Chionochloa australis*, Poplars Range. b) Impact ground litter from attached recently dead litter collected from *Chionochloa rubra*, Mt Tongariro.

North, New Zealand. Total phenolic content is expressed as milligrams of gallic acid equivalents per gram of oven dry plant litter.

Analysis

General linear modelling was used to predict the influence of environmental and climatic variables on litter quality. To allow for parsimony and prevent over-fitting of models, explanatory variables were reduced down to only the strongest predictors (Anderson and Burnham, 2002). Firstly, one variable out of highly correlated pairs (R^2 >0.8) were removed using Pearson's correlation, as the pair closely reflect each other. Next the remaining variables were plotted in a PCA ordination using the statistical program *Canoco* (Ter Braak and Smilauer, 2012) (Figure 2). To further reduce the number of explanatory variables, only four variables in the PCA ordination, those with the greatest explanatory strength, were selected as well as soil organic C to reflect soil fertility. Explanatory variables included in the model were mean minimum winter temperature, altitude, total annual rainfall, mean summer potential evapotranspiration, and soil organic C.

To correlate environment against litter quality, the strongest explanatory variables listed above were used to create general linear models in *Systat* (Wilkinson, 1992), with a forward stepwise logistic regression (Tabachnick *et al.*, 2001) used to determine the strongest predictors. For each measure of litter quality, a total of seven candidate models were created, including single variable models for each of the five environmental variables, one combined model including linear interactions between all environmental variables, and one further combined model allowing non-linear interactions between altitude, temperature, and rainfall variables.

To indentify the strongest and most likely models for each litter quality parameter, models within the candidate set for each litter quality measure were compared and ranked using Akaike's information criteria (AIC). The variation AICc was used to account for a small sample size of less than 40 taxa (Symonds and Moussalli, 2011). Models were ranked according to their AICc score and evaluated based on their score relative to the lowest AICc score (AICc Δi) for that litter quality parameter. Models with an AICc Δi of <2 can be considered essentially as good as the best candidate model (Richards, 2005), whilst models with an AICc Δi of >10 were rejected as implausible models and are not reported (Anderson and Burnham, 2002). Akaike weights (AICc Wi) were also calculated, indicating the probability that a model is the best out of the candidate set (Symonds and Moussalli, 2011). Models were also assessed for goodness of fit using R^2 (Symonds and Moussalli, 2011), with models with an R^2 of <0.1 not reported due to extremely poor fit.

To test for relationships between taxa and their litter quality variables, PCA ordinations of litter quality variables for each taxon were created using the statistical program *Canoco* (Ter Braak and Smilauer, 2012). A Mantel test was performed in the statistical program R (Team, 2014) using the *Vegan* package to test for correlations between genetic similarity and litter quality. First, a genetic similarity matrix was calculated by extracting phylogenetic branch distances for paired taxa using the *Ape* package in R. A similarity matrix for each litter quality measure was calculated using the absolute value of the difference between paired taxa. Mantel tests were performed in R using Pearson's correlation and 999 permutations per test.

Results

Litter Chemistry

Quantities of litter structural carbohydrates, including neutral detergent fibre (NDF), hemicellulose, and cellulose, were variable between taxa (Table 1). The percent NDF ranged from 77.1% (*C. vireta*) to 85.7% (*C. crassiuscula* spp. *crassiuscula*), with a generic mean of 82.1% (*SE* = 0.44). Hemicellulose showed similar variability ranging from 35.3% (*C. australis*) to 43.6% (*C. pallens* spp. *pilosa*), with a generic mean of 40.2% (*SE* = 0.43). There was less variation between taxa in cellulose content, ranging

Table 1: Summary of chemistry measures for *Chionochloa* leaf litter. NDF, hemicellulose, cellulose, and lignin are expressed as a percentage of oven dry weight. Phenolic content is expressed as mgg $^{-1}$ of gallic acid equivalent. SI = Stewart Island. Table 1 is continued below.

Taxa	NDF (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	Phenolic content (mg/g)
C. australis	81.8	35.3	34.6	11.9	1.97
C. conspicua ssp. *cunninghamii*	84.4	36.8	37.7	9.8	1.59
C. crassiuscula ssp. *crassiuscula*	85.7	41.5	35.7	8.4	2.44
C. crassiuscula ssp. *directa*	83.1	40.1	34.7	8.3	1.85
C. crassiuscula ssp. *torta*	82.2	39.4	34.4	8.5	2.32
C. defracta	77.9	36.2	34.3	7.3	2.25
C. flavescens ssp. *lupeola*	82.8	40.1	36.9	5.8	1.97
C. juncea	82.8	41.9	32.6	8.3	2.53
C. lanea	84.1	41.2	36.5	6.5	1.94
C. macra	78.9	41.9	33.1	3.9	2.70
C. pallens ssp. *cadens*	82.6	41.1	36.1	5.4	2.38
C. pallens ssp. *pallens*	81.9	43.0	32.8	6.1	1.57
C. pallens ssp. *pilosa*	81.4	43.7	32.8	4.9	1.86
C. rigida ssp. *amara*	82.1	40.1	36.3	5.6	1.58
C. rigida ssp. *amara* (SI)	84.6	41.3	37.0	6.4	1.88
C. rigida ssp. *rigida*	81.2	40.6	36.1	4.5	3.15
C. rubra ssp. *cuprea*	84.6	42.4	35.6	6.6	2.36
C. rubra ssp. *occulta*	82.5	40.2	36.3	6.0	1.90
C. rubra ssp. *rubra* var. *inermis*	81.5	40.8	34.9	5.8	2.15
C. rubra ssp. *rubra* var. *rubra*	79.6	39.2	35.5	4.9	2.13
C. spiralis	83.5	38.6	36.8	8.1	1.37
C. teretifolia	81.1	38.9	35.5	6.7	2.29
C. vireta	77.1	39.5	33.0	4.6	2.58
Mean	82.1	40.2	35.2	6.72	2.12
SE	0.44	0.43	0.32	0.40	0.09

from 32.5% (*C. defracta*) to 37.8% (*C. conspicua* spp. *cunninghamii*), with a generic mean of 35.2% (*SE* = 0.32).

Lignin content was low compared to structural carbohydrates, but showed a greater range, from 3.9% (*C. macra)* to 11.9% (*C. australis)*, with a generic mean of 6.7% (*SE* = 0.40). Total phenolic content was also variable between taxa, ranging from 1.3 mg g^{-1} (*C. spiralis*) to 3.1 mg g^{-1} (*C. rigida* spp. *rigida*), with a generic mean of 2.2 (*SE* = 0.09). C content of litter was similar between taxa (Table 1 continued), ranging from 45.2% (*C. defracta*) to 49.7% (*C. crassiuscula spp. directa*) with a generic mean of 48.0% (*SE* = 0.21). Litter N content was particularly low across the genus, ranging

Table 1 continued: Summary of chemistry measures of *Chionochloa* leaf litter. Phenolic content is expressed as mgg^{-1} of gallic acid equivalent. Concentrations of carbon (C) and nitrogen (N) are expressed as a percentage of oven dry weight. Also included are the corresponding carbon to nitrogen ratio, and lignin (L) to nitrogen ratio. SI = Stewart Island

Taxa	Phenolic content (mg/g)	C (%)	N (%)	C: N	L: N
C. australis	1.97	48.9	0.30	165	40.2
C. conspicua sp. *cunninghamii*	1.59	48.3	0.31	157	31.8
C. crassiuscula sp. *crassiuscula*	2.44	49.0	0.24	207	35.7
C. crassiuscula sp. *directa*	1.85	49.7	0.30	166	27.8
C. crassiuscula sp. *torta*	2.32	49.2	0.30	166	28.6
C. defracta	2.25	45.2	0.27	169	27.4
C. flavescens sp. *lupeola*	1.97	48.4	0.24	200	23.8
C. juncea	2.53	49.0	0.31	160	27.0
C. lanea	1.94	48.3	0.30	158	21.2
C. macra	2.70	46.7	0.31	153	12.8
C. pallens sp. *cadens*	2.38	47.7	0.24	200	22.7
C. pallens sp. *pallens*	1.57	48.2	0.27	180	22.7
C. pallens sp. *pilosa*	1.86	47.9	0.26	188	19.3
C. rigida sp. *amara*	1.58	47.6	0.34	140	16.6
C. rigida sp. *amara* (SI)	1.88	48.2	0.25	192	25.3
C. rigida sp. *rigida*	3.15	47.4	0.28	170	16.3
C. rubra sp. *cuprea*	2.36	49.0	0.40	123	16.6
C. rubra sp. *occulta*	1.90	47.9	0.18	268	33.9
C. rubra sp. *rubra* var. *inermis*	2.15	47.8	0.25	190	23.0
C. rubra sp. *rubra* var. *rubra*	2.13	46.8	0.34	139	14.6
C. spiralis	1.37	47.7	0.31	152	25.7
C. teretifolia	2.29	49.0	0.43	114	15.6
C. vireta	2.58	47.3	0.31	154	15.0
Mean	2.12	48.0	0.29	170	23.6
SE	0.09	0.21	0.01	6.70	1.53

from 0.18% (*C. rubra* spp. *occulta*) to 0.43% (*C. teretifolia*), with a generic mean of 0.29% (*SE* = 0.01).

The litter C:N ratio was markedly different between taxa, more so than C and N individually, and ranged from 114 (*C. teretifolia*) to 268 (*C. rubra* spp. *occulta*), with a generic mean of 170 (*SE* = 6.7). A linear regression between C and C:N displayed no relationship (R^2 = 0.0005), whilst N and C:N displayed a strongly negative linear relationship (R^2 = 0.89). Similarly, taxa were markedly different in Lignin: N ratio,

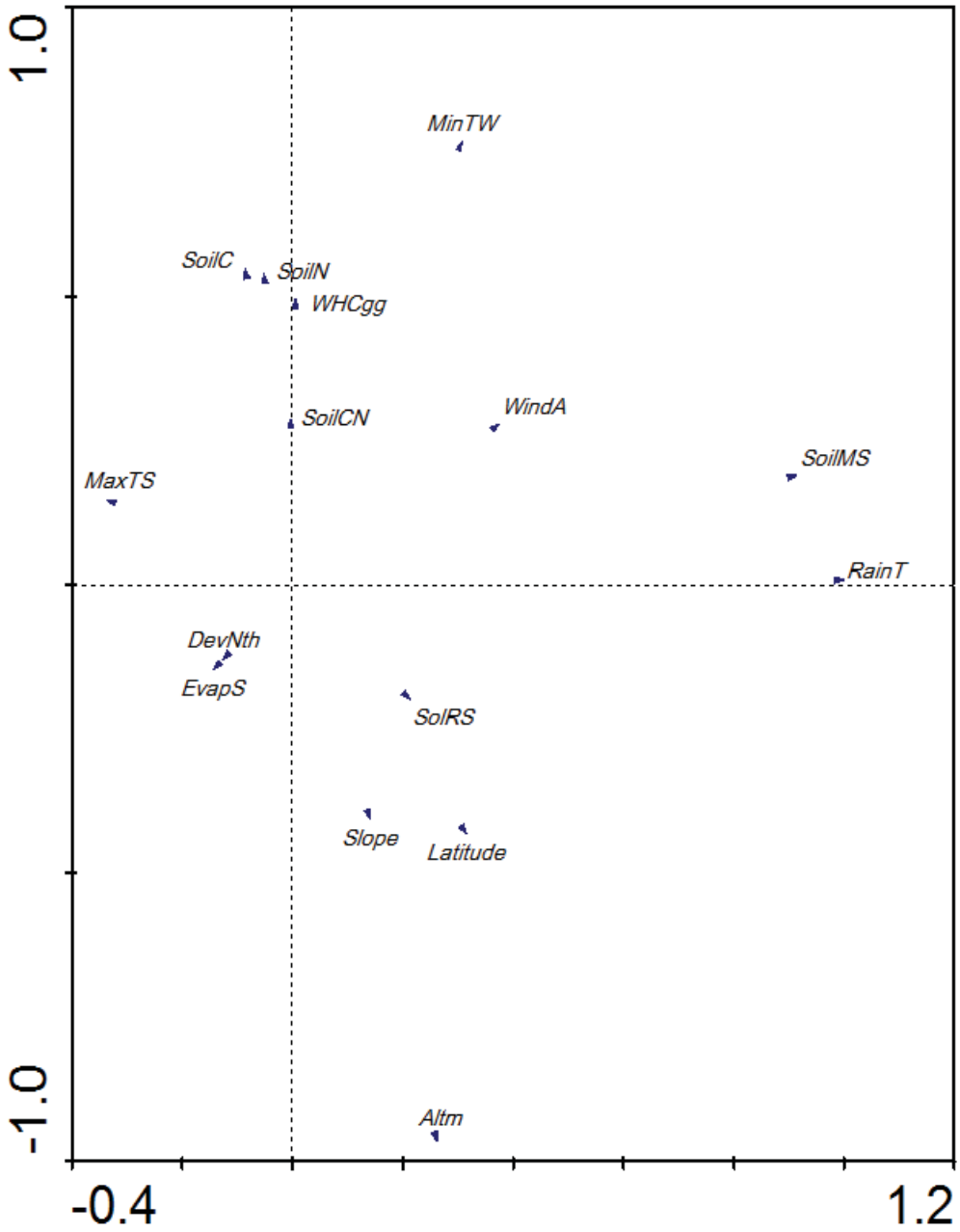


Figure 2: PCA ordination of environmental parameters that may cause plant stress and influence litter quality. Physical parameters are aspect (*DevNth*), slope (*Slope*), latitude (*Latitude*) and altitude (*Altm*). Soil parameters are Soil organic C (*SoilC*), Soil total N (*SoilN*), Soil C to N ratio (*SoilCN*), Soil water holding capacity (*WHCgg*), and mean summer soil moisture deficit (*SoilMS*). Climate parameters are mean maximum summer temperature (*MaxTS*), mean minimum temperature winter temperature (*MinTW*), total annual precipitation (*RainT*), mean daily wind speed (*WindA*), mean summer solar radiation (*SolRS*), and mean potential summer evapotranspiration (*EvapS*).

ranging from 12.8 (C. macra) to 40.2 (*C. australis*), with a mean of 23.6 (*SE* = 1.5) for the genus. A linear regression between N and Lignin: N showed a moderate negative relationship (R^2 = 0.23), whilst Lignin and Lignin: N showed a strongly positive linear relationship (R^2 = 0.70).

Table 2: Comparison of general linear models predicting the influence of environmental stress on litter quality. K= number of parameters in the model including the constant. AICc = Akaike's Information Criterion. AICc Δi = difference in AICc score between model *i* and the best model out of the candidate model set. AICc W*i* = Akaike model weight. Only credible models are presented, with models with an AICc Δi < 10 and R^2 > 0.1 accepted as credible models. Strongest models within a candidate set (AICc Δi <2) are shown in bold. * Bonferroni Significance to 0.10. ** Bonferroni Significance to 0.05.

Litter Parameter	Model	K	P	r	R^2	AICc	AICc Δi	AICc W*i*
NDF	**MinTW**	**2**	**0.0001****	**0.721**	**0.520**	**21.78**	**0.94**	**0.384**
	EvapS + MinTW	**3**	**0.0001****	**0.767**	**0.589**	**20.84**	**0.00**	**0.613**
Cellulose	**MinTW**	**2**	**0.053**	**0.408**	**0.166**	**18.59**	**0.00**	**0.446**
	MinTW*Alt	3	0.043	0.425	0.181	20.86	2.27	0.143
C	**Soil C**	**2**	**0.014****	**0.506**	**0.256**	**-3.70**	**1.85**	**0.179**
	Alt	2	0.134	-0.322	0.104	0.58	6.13	0.021
	EvapS	2	0.037*	-0.436	0.190	-1.76	3.79	0.068
	MinTW	**2**	**0.009****	**0.532**	**0.283**	**-4.56**	**0.99**	**0.275**
	Soil C + MinTW	**3**	**0.007****	**0.623**	**0.388**	**-5.55**	**0.00**	**0.451**
N	**Alt**	**2**	**0.027***	**-0.460**	0.212	**-136.42**	**0.00**	**0.683**
C:N	**Alt**	**2**	**0.081**	**0.372**	**0.138**	**159.78**	**0.00**	**0.510**

General Linear Models

A PCA ordination of the climatic and environmental variables thought to influence litter quality (Figure 2), showed an extremely strong gradient along axis 1, explaining 96.9% of the variation. Strongest parameters along this axis were total annual rainfall and mean summer moisture deficit, opposed by mean maximum summer temperature. Axis 2 explained a further 3% of the variation, with mean minimum temperature in the winter opposed by altitude. Variables selected for inclusion in general linear models were mean minimum winter temperature (*MinTW*), altitude (*Altm*), total annual rainfall (*RainT)*, mean summer potential evapotranspiration (*EvapS*), and soil organic C (*SoilC*).

Of the general linear models tested (Table 2), *MinTW*, and *MinTW + EvapS*, were the strongest predictors of litter NDF, with both being equally suitable models (AICc Δi <2). Out of these two models, the combined model of *MinTW + EvapS* showed a stronger Akaike weight than *MinTW* (0.61 and 0.38 respectively), indicating a 61% probability, given the data used, that this is the strongest model. Individually, NDF

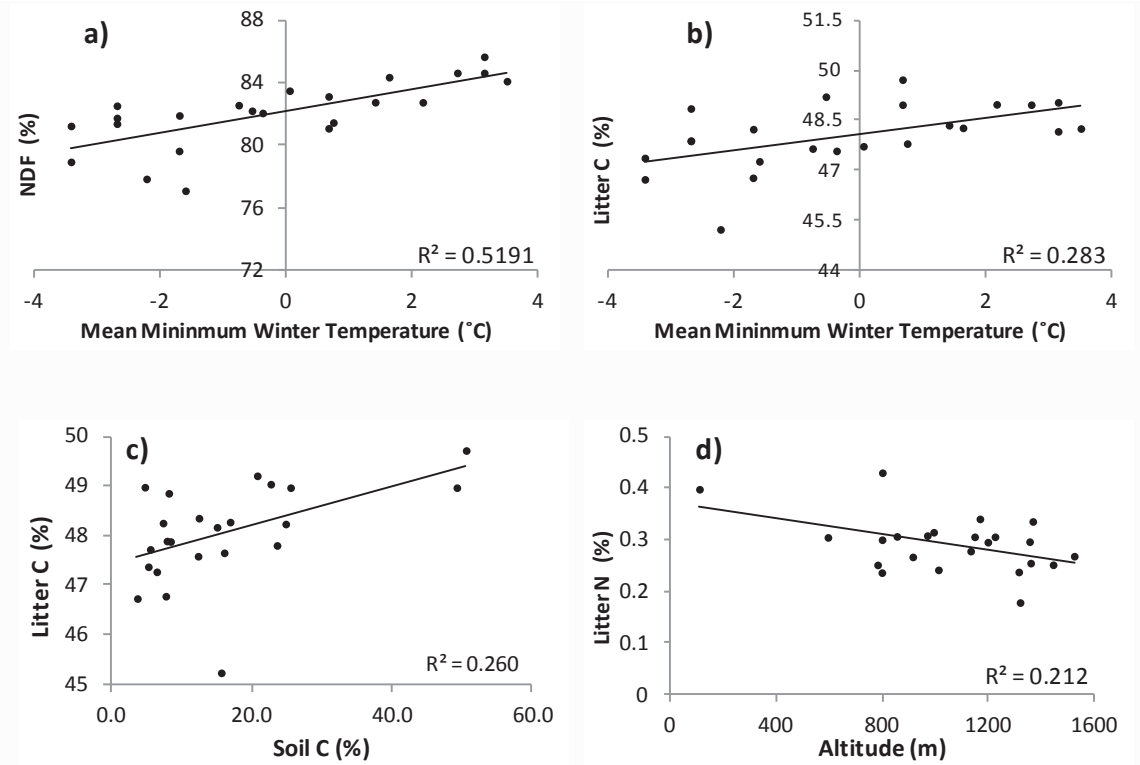


Figure 3: Correlations ions between measures of litter quality and environmental/climatic stress.

shows a strong positive relationship with *MinTW* (Figure 3), and a weak negative correlation with *EvapS* (R^2 = 0.21). Similarly, cellulose was best predicted by *MintTW*, displaying a positive relationship, though this was not strong or significant. Modelling of litter C resulted in three equally good predictors, with moderate to strong positive relationships with *SoilC* (Figure 3), *MinTW*, and *SoilC + MinTW*. Litter N and C:N were best predicted by altitude, with a moderate to weak negative relationship occurring between Litter N and altitude (Figure 3), and a moderate to weak positive relationship between Litter C:N and altitude. Other litter parameters, hemicellulose, lignin, lignin:N, and total phenolics could not be predicted by environment or climate, displaying not credible models..

Genetic Relatedness

The PCA ordination of litter chemistry measures (Figure 4) displayed a strong leaf nitrogen gradient along axis 1, explaining 73.9% of the variance, with total N opposed against the C:N ratio. Axis 2 explained a further 22.6% of variance, and was dominated by a strong gradient between leaf structural components, with the strongest opposing variables being lignin and hemicellulose. Groups of related subspecies showed

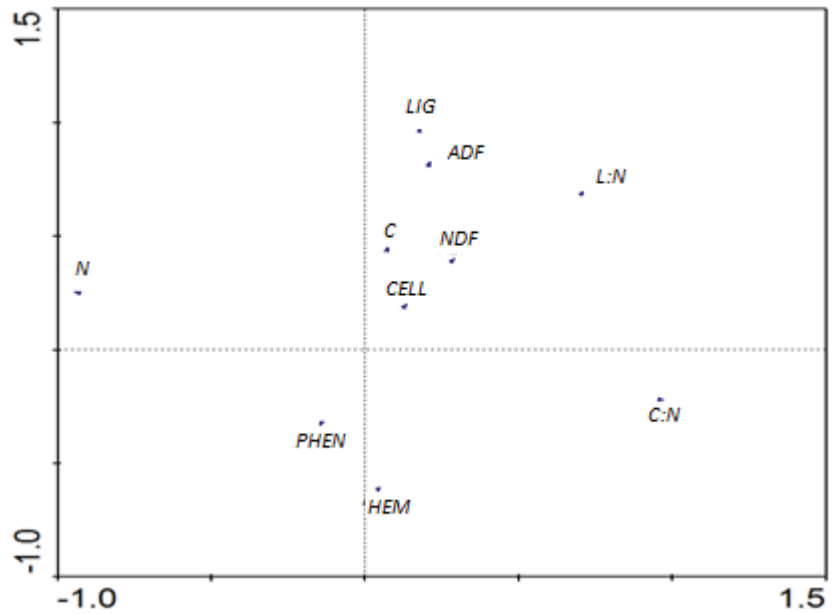


Figure 4: PCA ordination of litter chemistry variables measured in *Chionochloa*. Labels are as follows: Total nitrogen (N), total phenolics (PHEN), hemicellulose (HEM), carbon (C), cellulose (CELL), Lignin (LIG), acid detergent fibre (ADF), neutral detergent fibre (NDF), Lignin to nitrogen ratio (LN), and carbon to nitrogen ratio (CN). CN data have been square-root transformed to dampen down the influence of CN on the ordination.

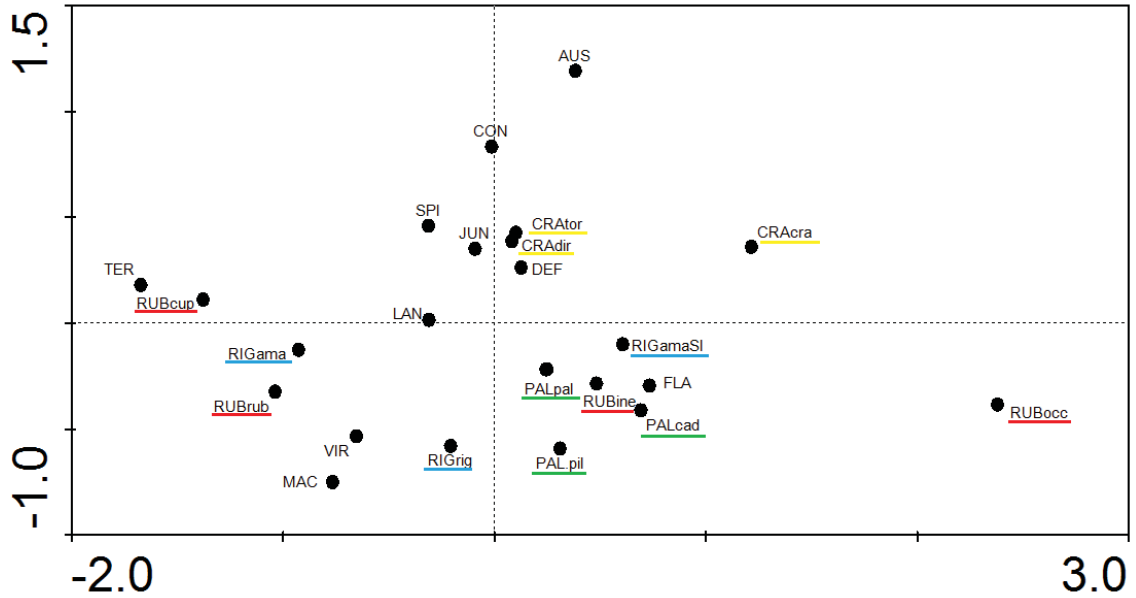


Figure 5: PCA ordination of *Chionochloa* taxa based on their litter chemistry (as per Figure 3). The C:N ratio data have been transformed by square-root to dampen down its influence on the ordination. Labels are as follows: *C. australis* (AUS), *C. conspicua* ssp. *cunninghamii* (CON), *C. crassiuscula* ssp. *crassiuscula* (CRAcra), *C. crassiuscula* ssp. *directa* (CRAdir), *C. crassiuscula* ssp. *torta* (CRAtor), *defracta* (DEF), *C. flavescens* ssp. *lupeola* (FLA), *C. juncea* (JUN), *C. lanea* (LAN), *C. macra* (MAC), *C. pallens* ssp. *cadens* (PALcad), *C. pallens* ssp. *pallens* (PALpal), *C. pallens* ssp. *pilosa* (PALpil), *C. rigida* ssp. *amara* (RIGama), *C. rigida* ssp. *amara* Stewart Island (RIGamaSI), *C. rigida* ssp. *rigida* (RIGrig), C. *rubra* ssp. *cuprea* (RUBcup), *C. rubra* ssp. *occulta* (RUBocc), *C. rubra* ssp. *rubra* var. *inermis* (RUBine), *C. rubra* ssp. *rubra* var. *rubra* (RUBrub), *C. spiralis* (SPI), *C. teretifolia* (TER), and *C. vireta* (VIR). Species subgroups are underlined in colour: *crassiuscula* (Yellow), *pallens* (Green), *rigida* (Blue), and *rubra* (Red).

Table 3: Mantel test results for correlations between genetic similarity and litter quality. Number of permutations used = 999. Significant (< 0.10) correlations highlighted in bold.

Litter quality variable	Mantel *r*	*p*-value
NDF	0.024	0.369
ADF	**0.215**	**0.057**
Cellulose	0.090	0.150
Hemicellulose	**0.434**	**0.001**
Phenolics	0.002	0.461
C	0.062	0.310
N	-0.166	0.927
C:N	-0.171	0.955
Lignin	**0.163**	**0.098**
Lignin:N	0.022	0.351

strong grouping along axis 2, though were less related along axis 1 (Figure 5). A Mantel test between genetic similarity and litter quality measures showed significant correlations between genetic distance and hemicellulose, ADF, and lignin, with no detectable relationship occurring for the other litter quality measures (Table 3).

Discussion

Whilst taxa in the genus *Chionochloa* were distinctly similar in some measures of litter quality, in other measures they showed a greater variability. Taxa tended to be similar in measures of fibre, including NDF, ADF, hemicellulose, cellulose, and C, displaying 1.1, 1.3, 1.2, 1.2, and 1.1 fold differences respectively. The greatest differences between taxa in litter chemistry were seen in measures of total phenolics, nitrogen, and lignin, with 2.4, 2.4, and 3.1 fold differences respectively. Similarly, the C:N and Lignin:N ratio also showed a large range between taxa, with 2.4 and 3.2 fold differences respectively.

Leaf Nitrogen Content

Craine and Lee (2003) suggest that the N content of New Zealand's indigenous grasses may be among the lowest recorded for C3 grasses anywhere. In support of this,

the *Chionochloa* litter in this study was found to contain very low levels of N, which may indicate a poor litter quality. These measures were also similar to those reported in other studies of *Chionochloa.* In *C. rubra* and *C. rigida*, Young *et al.* (1994) found recently fallen litter to have approximately 0.25% N per dry weight, whilst Lee and Fenner (1989) found slightly higher levels (0.35% - 0.71%) in the attached dead material of a number of *Chionochloa* taxa shared with this study. Connor *et al.* (1970) found mean live leaf N concentrations in *C. macra*, *C. rigida*, *C. rubra*, and *C. flavescens* to range from 0.51% to 0.89% of dry weight, with concentrations rarely exceeding 1%. When these results are compared to those of pastoral grasses in New Zealand, *Chionochloa* can be seen to contain much lower N levels. N in the recently dead leaves of a New Zealand perennial ryegrass have been reported to range from 1.5% - 3.2% on unfertile and fertile soils respectively, with live leaf N ranging from 2.9% - 4.2% on unfertile and fertile soils respectively (Hunt, 1983).

Concentrations of live leaf N in *Chionochloa* are not only low compared to other grasses, but are also low when compared to other indigenous montane vegetation. Live leaf N concentrations in New Zealand montane trees were found to range from 1.04% to 1.73% of oven dry weight, Shrubs from 0.92% to 1.25%, and forbs from 1.39% to 2.55% (Körner *et al.*, 1986), suggesting that *Chionochloa* tussocks may have some of the most N poor litter in New Zealand's montane environment. According to positive correlations between litter N and litter decomposition (Köchy and Wilson, 1997; Berg and McClaugherty, 2008), these results initially suggest generically *Chionochloa* litter may be slow to decomposition, not just relative to other grasses, but also relative to other montane vegetation. However, there is a range in litter N content occurring between taxa in the genus *Chionochloa*, which may also result in differential rates of decomposition within the genus.

Leaf Structural Components

Leaf structural components, i.e. hemicellulose, cellulose, and lignin, were found in generally similar concentrations to other studies of *Chionochloa* (Connor *et al.*, 1970; Bailey and Connor, 1972). However, the hemicellulose content measured here was notably higher, with a mean of 40% of dry weight, compared to mean measures of 24% to 30% of dry weight reported in Connor *et al.* (1970) and Bailey and Connor (1972). This difference is most likely due the above studies sampling from the leaf laminal portion only, whereas this study includes both the leaf lamina and the leaf sheath.

Connor and Bailey (1972) found sheaths of *Chionochloa* to contain increased concentrations of hemicellulose and decreased concentrations of cellulose relative to laminae. The above studies also used live green leaves in contrast to recently dead leaves sampled in this study. Hemicellulose and cellulose may therefore be expected to have a greater relative percentage in dead leaves due to the re-absorption of other plant compounds during senescence.

Lignin content was similar to other findings for *Chionochloa,* though a greater range in lignin content was found here. Connor *et al.* (1970) found lignin in the tall *Chionochloa* tussocks, *C. macra*, *C. rigida*, *C. rubra*, and *C. flavescens,* to range on average from 6.4% of dry weight to up to 8.6%. The greater range in lignin content occurring in this study is most likely due to the wider range of taxa sampled, particularly the due to the inclusion of smaller taxa such as *C. australis* and *C. crassiuscula* which tended to have a higher lignin content than tall tussocks. The lignin content of *Chionochloa* litter appears on average to be intermediate between pastoral grasses (2% to 5% lignin) and cereal grasses (12 -14% lignin) (Connor *et al.*, 1970; Allison *et al.*, 2009), though *Chionochloa* has a much larger range compared to pastoral and cereal grasses. Thomas and Asakawa (1993) found leaf Lignin content and lignin:N ratio to be significant predictors of leaf decomposition in tropical grasses and legumes, where lignin was found to be negatively correlated with decomposition. The wide range in leaf lignin, and N content, found here in the *Chionochloa* genus may result in varying rates of litter decomposition between taxa.

Phenolics, C:N, and Soluble Compounds

Measures of NDF and NDS appear to be relatively constant between *Chionochloa* taxa, which may translate to minimal differences in decomposition. *Chionochloa* appears to have much greater NDF, and hence reduced NDS, compared to other pastoral grasses in New Zealand (Bailey and Ulyatt, 1970), resulting in a poorer quality litter and possibly result reduced rates of litter decomposition. Variation in phenolic content between taxa could result in variable rates of decomposition, with higher phenolic content associated with reduced rates of decomposition (Martin and Haider, 1980; Waterman and Mole, 1994).

Chionochloa C:N levels were high compared to other grasses and forbs. Cadisch and Giller (1997) report ratios of 20 or less as comprising rapidly decomposable litter, with green leaves having ratios between 25 - 75 decomposing relatively quickly, whilst

ratios above 100 result in greatly reduced rates of decomposition. *Chionochloa* taxa appear to be approaching C:N ratios of bark and soft wood (200 to 500) (Cadisch and Giller, 1997), with ratios ranging from 114 to 268. The range in C:N between taxa also suggests varying litter quality within the genus.

Environmental Control of Litter Quality

Not all measures of litter quality displayed a response to apparent environmental or resource stress. Plant structural compounds were expected to be greater in taxa experiencing lower temperatures. Thickening of the cell wall and greater concentrations of structural components such as fibre, cellulose, hemicellulose and lignin, have been reported as a plant response to cold stress (Huner *et al.*, 1981; Stefanowska *et al.*, 1999). However, in this study mean winter temperature (MinTW) displayed a positive correlation with structural components C, NDF, and cellulose. The greater concentrations of C, NDF, and cellulose occurring in plants at warmer temperatures is likely due to an increase in plant height associated with increased productivity, where more productive and taller plants require greater structural components to support a tall up-right stature. This is supported by a moderate positive correlation between cellulose and plant height ($R^2 = 0.38$).

Leaf C is generally considered to be relatively constant in plants (Cadisch and Giller, 1997); however the results here suggest that combined with warmer winter temperatures, leaf litter C is also greater on soils with greater soil organic C. Leaf litter C:N ratios in trees have been reported to influence the underlying soil C and soil N contents through litter turnover rates (Vesterdal *et al.*, 2008), suggesting that plants can influence their underlying soil conditions through inputs. It is possible that greater organic C in the soil here correlates with greater leaf C:N ratios due to the negative correlation between C:N and decomposition, though this is more likely due to low leaf N, as opposed to greater leaf C.

Live leaf N content, expressed per unit leaf area, has been shown to increase with increasing in altitude (Körner *et al.*, 1986; Friend *et al.*, 1989; Hultine and Marshall, 2000), however, this relationship is not so clear for leaf N content when expressed by unit dry weight, as is relevant for measuring N available to decomposers. The results found in this study show leaf litter N per dry weight to be negatively correlated with altitude. In support, Körner *et al.* (1986) found live leaf N per dry weight to be negatively correlated with altitude for some New Zealand montane taxa.

Live leaf N per dry weight for both indigenous and introduced grasses in montane New Zealand have also been found to be negatively correlated with altitude (Craine and Lee, 2003).

This negative correlation between leaf litter N and altitude may be explained by soil nitrogen limitation occurring at higher altitudes, as a result of lower rates of N mineralization associated with colder temperatures (Klingensmith and Cleve, 1993). In addition, taxa at low altitude sites in New Zealand are more likely receive N fertilization from livestock or agricultural application. This reduction in leaf N content may also be attributed to the strong relationship between leaf photosynthetic rate per unit mass and leaf N concentration per unit mass (Hikosaka, 2004). If so, this would suggest that the lower leaf N occurring in plants at higher altitudes is linked to a reduction in growth rate associated with increased stress at higher altitudes.

Genotypic Control of Litter Quality

The grouping of related subspecies seen in the PCA ordination of *Chionochloa* taxa litter quality measures (Figure 5) indicates there may be some genetic control of litter quality. The subspecies groups *C. pallens* and *C. crassiuscula* showed the strongest grouping, whilst subspecies groups *C. rigida* and *C. rubra* were less closely related, particularly along axis 1. Grouping for all subspecies groups is strongest along axis 2, which displays a strong structural components gradient, suggesting some structural components may have a strong genetic component. In contrast, subspecies show poorer grouping along axis 1, which displays a strong litter N gradient, suggesting litter N may be influence more by environment than genetic relatedness.

In a study of relationships between leaf nutrients, environment, and phylogeny, He *et al.* (2008) found leaf N concentrations in the taxa of Chinese grasslands to be explained by both phylogeny and environment. However, this study investigated these trends using a large range of unrelated taxa, with over 41 different families sampled. Thus, the strong influence of phylogeny on leaf N reported is most likely due to major differences in plant growth strategy and plant functional group, as highlighted earlier. Where taxa are closely related, as occurring in *Chionochloa*, leaf N is proposed to be predominantly determined environment, due to the relationships between environment, photosynthetic rate, and leaf N, as discussed above.

This is supported by the Mantel test performed here, which reported strong relationships between genetic similarity and the structural components lignin, ADF and

hemicellulose, but no relationships for leaf N and other litter quality variables. Campbell and Sederoff (1996) report lignin synthesis to be influenced by both genetic and environmental cues; however the findings in this study suggest that genotype is a greater determinate of lignin content in *Chionochloa*. The same probably applies to hemicellulose and ADF, which also display strong relationships with phylogeny, and no apparent relationship with environment or resource stress.

Conclusions and Implications

Congeneric *Chionochloa* taxa were shown to be variable in litter quality, which is attributable to both differences in environment and genotype. *Chionochloa* litter quality appears to be generically poor due to low N content, high structural components, and presence of significant lignin. However, there is variability between taxa in lignin, total phenolics, N, and C:N concentrations which is likely to result in variable rates of litter decomposition. Greater N content is likely to result in greater rates of decomposition, whilst greater lignin, total phenolics, and C:N ratios are likely to result in lower rates of decomposition.

The relationship between litter N content and altitude suggests that poorer quality litters may be related to environments with increased stress. However, some measures of litter quality also appear more related to genotype, which may have an influence on apparent relationship between litter quality and an environment. These results show there is potential for differential rates of decomposition between congeneric taxa, and imply the environment and genotype both have strong controls over litter quality, but operate differentially across the genus. This suggests plant growth rate, a combination of genotype and environment, may have merit as a predictor of litter quality, as is tested for in the following chapter.

References

Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology***,** 597-608.

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos***,** 439-449.

Akula, R. & Ravishankar, G. A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant signaling & behavior,* 6**,** 1720-1731.

Allison, G. G., Thain, S. C., Morris, P., Morris, C., Hawkins, S., Hauck, B., Barraclough, T., Yates, N., Shield, I. & Bridgwater, A. V. 2009. Quantification of hydroxycinnamic acids and lignin in perennial forage and energy grasses by Fourier-transform infrared spectroscopy and partial least squares regression. *Bioresource technology,* 100**,** 1252-1261.

Anderson, D. R. & Burnham, K. P. 2002. Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management***,** 912-918.

Bailey, R. & Connor, H. 1972. Structural polysaccharides in leaf blades and sheaths in the Arundinoid grass Chionochloa. *New Zealand journal of botany,* 10**,** 533-544.

Bailey, R. & Ulyatt, M. 1970. Pasture quality and ruminant nutrition: II. Carbohydrate and lignin composition of detergent-extracted residues from pasture grasses and legumes. *New Zealand Journal of Agricultural Research,* 13**,** 591-604.

Berg, B. & Matzner, E. 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environmental Reviews,* 5**,** 1-25.

Berg, B. & Mcclaugherty, C. 2008. Plant litter. *Decomposition, humus formation, carbon sequestration. 2nd Ed Springer*.

Bradley, K. L. & Pregitzer, K. S. 2007. Ecosystem assembly and terrestrial carbon balance under elevated CO 2. *Trends in Ecology & Evolution,* 22**,** 538-547.

Cadisch, G. & Giller, K. E. 1997. *Driven by nature: plant litter quality and decomposition,* CAB international.

Campbell, M. M. & Sederoff, R. R. 1996. Variation in Lignin Content and Composition (Mechanisms of Control and Implications for the Genetic Improvement of Plants). *Plant physiology,* 110**,** 3.

Carreiro, M., Sinsabaugh, R., Repert, D. & Parkhurst, D. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology,* 81**,** 2359-2365.

Coley, P. D., Bryant, J. P. & Chapin, F. S. 1985. Resource availability and plant antiherbivore defense. *Science,* 230**,** 895-899.

Connor, H. & Bailey, R. 1972. Leaf strength in four species of Chionochloa (Arundineae). *New Zealand journal of botany,* 10**,** 515-532.

Connor, H. E., Bailey, R. & O'connor, K. F. 1970. Chemical composition of New Zealand tall-tussocks (Chionochloa). *New Zealand journal of agricultural research,* 13**,** 534-554.

Cornelissen, J. H. C., Quested, H. M., Gwynn-Jones, D., Van Logtestijn, R. S. P., De Beus, M. a. H., Kondratchuk, A., Callaghan, T. V. & Aerts, R. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology,* 18**,** 779-786.

Cornwell, W. K., Cornelissen, J. H., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., Hobbie, S. E., Hoorens, B., Kurokawa, H. & Pérez-Harguindeguy, N. 2008a. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology letters,* 11**,** 1065-1071.

Cornwell, W. K., Godoy, Ó. & Westoby, M. 2008b. The leaf economic spectrum drives litter decomposition within regional floras worldwide.

Couteaux, M.-M., Bottner, P. & Berg, B. 1995. Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution,* 10**,** 63-66.

Coûteaux, M.-M., Kurz, C., Bottner, P. & Raschi, A. 1999. Influence of increased atmospheric CO2 concentration on quality of plant material and litter decomposition. *Tree Physiology,* 19**,** 301-311.

Craine, J. & Lee, W. 2003. Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand. *Oecologia,* 134**,** 471-478.

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

Dijkstra, F. A., Hobbie, S. E., Knops, J. M. & Reich, P. B. 2004. Nitrogen deposition and plant species interact to influence soil carbon stabilization. *Ecology Letters,* 7**,** 1192-1198.

Enriquez, S., Duarte, C. M. & Sand-Jensen, K. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C: N: P content. *Oecologia,* 94**,** 457-471.

Freschet, G. T., Aerts, R. & Cornelissen, J. H. 2012. A plant economics spectrum of litter decomposability. *Functional Ecology,* 26**,** 56-65.

Friend, A., Woodward, F. & Switsur, V. 1989. Field measurements of photosynthesis, stomatal conductance, leaf nitrogen and δ 13 C along altitudinal gradients in Scotland. *Functional Ecology***,** 117-122.

Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. *Phytochemical adaptations to stress.* Springer.

Grime, J. P. 1988. The CSR model of primary plant strategies—origins, implications and tests. *Plant evolutionary biology.* Springer.

Grime, J. P. 2006. *Plant strategies, vegetation processes, and ecosystem properties*, John Wiley & Sons.

Guerriero, G., Hausman, J. F., Strauss, J., Ertan, H. & Siddiqui, K. S. 2016. Lignocellulosic biomass: Biosynthesis, degradation, and industrial utilization. *Engineering in Life Sciences,* 16**,** 1-16.

Gulis, V. & Suberkropp, K. 2003. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology,* 45**,** 11-19.

Hättenschwiler, S. & Vitousek, P. M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in ecology & evolution,* 15**,** 238-243.

He, J.-S., Wang, L., Flynn, D. F., Wang, X., Ma, W. & Fang, J. 2008. Leaf nitrogen: phosphorus stoichiometry across Chinese grassland biomes. *Oecologia,* 155**,** 301-310.

Hikosaka, K. 2004. Interspecific difference in the photosynthesis–nitrogen relationship: patterns, physiological causes, and ecological importance. *Journal of plant research,* 117**,** 481-494.

Horner, J. D., Gosz, J. R. & Cates, R. G. 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. *American Naturalist***,** 869-883.

Hultine, K. & Marshall, J. 2000. Altitude trends in conifer leaf morphology and stable carbon isotope composition. *Oecologia,* 123**,** 32-40.

Huner, N., Palta, J., Li, P. & Carter, J. 1981. Anatomical changes in leaves of Puma rye in response to growth at cold-hardening temperatures. *Botanical Gazette*, 55-62.

Hunt, W. 1983. Nitrogen cycling through senescent leaves and litter in swards of Ruanui and Nui ryegrass with high and low nitrogen inputs. *New Zealand journal of agricultural research,* 26**,** 461-471.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W.-P., Huang, D. & Ong, C. N. 2010. Antioxidant activity and profiles of common vegetables in Singapore. *Food Chemistry,* 120**,** 993-1003.

Karlovsky, P. 2008. *Secondary metabolites in soil ecology,* Springer.

Kefeli, V. I., Kalevitch, M. V. & Borsari, B. 2003. Phenolic cycle in plants and environment. *J. Cell Mol. Biol,* 2**,** 13-18.

Killingbeck, K. T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology,* 77**,** 1716-1727.

Klingensmith, K. & Cleve, K. V. 1993. Patterns of nitrogen mineralization and nitrification in floodplain successional soils along the Tanana River, interior Alaska. *Canadian Journal of Forest Research,* 23**,** 964-969.

Klute, A. 1986. Water retention: laboratory

methods. *Methods of soil analysis. Part 1. Physical and mineralogical methods.* 2nd Edition ed.

Köchy, M. & Wilson, S. D. 1997. Litter decomposition and nitrogen dynamics in aspen forest and mixed-grass prairie. *Ecology,* 78**,** 732-739.

Körner, C., Bannister, P. & Mark, A. 1986. Altitudinal variation in stomatal conductance, nitrogen content and leaf anatomy in different plant life forms in New Zealand. *Oecologia,* 69**,** 577-588.

Leco 2003. Carbon and Nitrogen in plant tissue. *LECO Corporation.* St. Joesph, MO: Organic Application Note 203-821-171.

Lee, W. & Fenner, M. 1989. Mineral nutrient allocation in seeds and shoots of twelve Chionochloa species in relation to soil fertility. *The Journal of Ecology*, 704-716.

Liu, S.-Y., Freyer, A. J., Minard, R. D. & Bollag, J.-M. 1985. Enzyme-catalyzed complex-formation of amino acid esters and phenolic humus constituents. *Soil Science Society of America Journal,* 49**,** 337-342.

Martin, J. & Haider, K. 1980. Microbial degradation and stabilization of 14C-labeled lignins, phenols, and phenolic polymers in relation to soil humus formation. *Lignin biodegradation: microbiology, chemistry, and potential applications.*

Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecology,* 59**,** 465-472.

Meier, C. L. & Bowman, W. D. 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proceedings of the National Academy of Sciences,* 105**,** 19780-19785.

Melillo, J. M., Aber, J. D. & Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology,* 63**,** 621-626.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC international,* 85**,** 1217-1240.

Moura, J. C. M. S., Bonine, C. a. V., De Oliveira Fernandes Viana, J., Dornelas, M. C. & Mazzafera, P. 2010. Abiotic and biotic stresses and changes in the lignin content and composition in plants. *Journal of integrative plant biology,* 52**,** 360-376.

Nommik, H. & Vahtras, K. 1982. Retention and fixation of ammonium and ammonia in soils. *Nitrogen in agricultural soils***,** 123-171.

Norby, R. J., Cotrufo, M. F., Ineson, P., O'neill, E. G. & Canadell, J. G. 2001. Elevated CO2, litter chemistry, and decomposition: a synthesis. *Oecologia,* 127**,** 153-165.

Ordoñez, J. C., Van Bodegom, P. M., Witte, J. P. M., Wright, I. J., Reich, P. B. & Aerts, R. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography,* 18**,** 137-149.

Richards, S. A. 2005. Testing ecological theory using the information-theoretic approach: examples and cautionary results. *Ecology,* 86**,** 2805-2814.

Sinsabaugh, R., Carreiro, M. & Repert, D. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry,* 60**,** 1-24.

Stefanowska, M., Kuras, M., Kubacka Zebalska, M. & Kacperska, A. 1999. Low temperature affects pattern of leaf growth and structure of cell walls in winter oilseed rape (Brassica napus L., var. oleifera L.). *Ann. Bot,* 84**,** 313-319.

Symonds, M. R. & Moussalli, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology,* 65**,** 13-21.

Tabachnick, B. G., Fidell, L. S. & Osterlind, S. J. 2001. Using multivariate statistics.

Tait, A., Henderson, R., Turner, R. & Zheng, X. 2006. Thin plate smoothing spline interpolation of daily rainfall for New Zealand using a climatological rainfall surface. *International Journal of Climatology,* 26**,** 2097-2115.

Tait, A. & Woods, R. 2007. Spatial interpolation of daily potential evapotranspiration for New Zealand using a spline model. *Journal of Hydrometeorology,* 8**,** 430-438.

Taylor, B. R., Parkinson, D. & Parsons, W. F. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology,* 70**,** 97-104.

Team, R. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-0.

Ter Braak, C. J. & Smilauer, P. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0.

Thomas, R. J. & Asakawa, N. 1993. Decomposition of leaf litter from tropical forage grasses and legumes. *Soil Biology and Biochemistry,* 25**,** 1351-1361.

Vesterdal, L., Schmidt, I. K., Callesen, I., Nilsson, L. O. & Gundersen, P. 2008. Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management,* 255**,** 35-48.

Waterman, P. G. & Mole, S. 1994. *Analysis of phenolic plant metabolites*, Blackwell Scientific.

Wilkinson, L. 1992. *SYSTAT for Windows: statistics, graphics, data, getting started, version 5*, Systat.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. & Diemer, M. 2004. The worldwide leaf economics spectrum. *Nature,* 428**,** 821-827.

Yordanov, I., Velikova, V. & Tsonev, T. 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica,* 38**,** 171-186.

Young, R. G., Huryn, A. D. & Townsend, C. R. 1994. Effects of agricultural development on processing of tussock leaf litter in high country New Zealand streams. *Freshwater Biology,* 32**,** 413-427.

Zhang, D., Hui, D., Luo, Y. & Zhou, G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology*, 1**,** 85-93.

Chapter 3

Investigating relationships between environment, plant growth rate, and litter quality:

Can litter quality be determined from plant growth rate?

Chionochloa teretifolia, Mt Burns, Fiordland - M. Dickson

Introduction

The sessile nature of plants and their inability to seek out or escape from certain abiotic and biotic conditions has serious consequences for plant growth and survival. This has generally led to the ability of plants to ameliorate the conditions they live in through physiological and morphological adaptations, as well as adaptations in resource use strategies (Ahuja *et al.*, 2010). These adaptations and strategies result in tradeoffs between productivity and survival, with an increase in a plants ability to tolerate and survive in adverse conditions often resulting in a reduction in productivity (Chapin *et al.*, 1987; Grime, 2006). The resulting changes in plant physiology, morphology, and resource use strategy not only affect plant growth rate, but also influence the chemistry of the plant material produced (Poorter and Villar, 1997). However, most studies investigating relationships between environment, plant growth rate, and litter quality include taxa from vastly different phylogenies and functional groups, resulting in genotypic differences that may have a greater influence than environment (Bradley and Pregitzer, 2007). To reduce the influence of genotype on plant growth rate and litter quality, here, a congeneric group of tussocks is used to test for relationships between environmental stresses and plant growth rate, and their consequent influence on plant leaf litter chemistry.

Plant Productivity

Productivity in vegetation is subject to a variety of environmental constraints, commonly including shortages and excesses in supply of solar energy, water ,and mineral nutrients (Grime, 2006). However, even when resources are in excess, plant growth rate is not always equal among different taxa (Chapin, 1991). The growth rate and productivity of a plant can broadly be defined by its resource use strategy adapted for its environment, as Grime (1988) proposed through expansion on the *r*/*K* selection model proposed by MacArthur and Wilson (1967). The *r*/*K* selection model suggests that where there are productive habitats with continuous resource replenishment, dominance is achieved by rapid rates of resource capture, which translates to rapid rates of growth and/or reproduction (*r* selection). Conversely, where there are systems with limited resources, high environmental stress, and low productivity, dominance is achieved through tolerance of these conditions and the protection of captured resources, translating to efficiency and longevity (*K* selection). Grimes C-S-R theory (Grime,

1988) expands on this by grouping plants in to three categories, *competitors*, *stress-tolerators*, and *ruderals*, based on the environmental conditions and disturbance regimes they experience. This study of long lived perennials tussocks in the genus *Chionochloa* focuses on the environmental stress gradient between *competitors* and *stress-tolerators* in the absence of site disturbance, aiming to investigate the relationship between environment, growth rate, and litter quality.

The nature of competitors leads them to dominate in sites with low environmental stress and low disturbance. Ample resources in the absence of disturbance allow for plants with the highest growth rates to produce greater quantities of photosynthate. This allows competitors to have dominating traits such as tall structures, extensive lateral spread, build-up of large perennating organs, and rapid expansion of the surface areas of leaves and roots (Grime, 2006). Due to available resources, highly competitive plants are able to quickly replace and replenish any damaged leaf material, resulting in a high leaf-turnover rate (Coley, 1988). In addition, highly productive plants generally do not invest in the types of plant compounds and secondary metabolites that aid in leaf protection and longevity, as it is more cost effective to replace lost or damaged material than to produce protective compounds, the cost of which results in a reduction in growth rate (Coley *et al.*, 1985).

In contrast, the nature of stress-tolerators leads them to dominate sites with high environmental stress and low disturbance. High environmental stress favours traits that allow the retention and protection of plant resources, relative to fast-growing plants, due to the increased relative cost of damage owing to slow rates of recovery associated with slow growth rate and difficulty in replacing resources (Coley *et al.*, 1985; Chapin, 1991). The production of secondary plant metabolites and associated compounds aids in leaf protection from physical environmental stress, herbivory, disease, as well as aiding in leaf longevity, which prevents resource loss through litter shedding (Coley *et al.*, 1985). As a result, stress tolerators are comparatively long-lived, and tend to have features which relate to the capacity for endurance. Features include inherently slow growth rates, evergreen habit, long lived organs, slow turnover of carbon (C) and mineral nutrients, and efficient water use (Grime, 2006). This begs the question; do differences in growth rate translate to predictable patterns in litter quality?

Influence of Growth Rate on Litter Quality

The physiological and morphological changes associated with plant growth rate have obvious implications for the chemistry of the plant material produced and corresponding plant litter. The leaves of perennial plants capable of rapid rates of growth have a distinctive chemical composition, due to the allocation of C to photosynthetic structures and a greater photosynthetic capacity (Aerts and Chapin III, 2000; De Deyn *et al.*, 2008). The result of this is the production of material that is low in cellular density and high in nutrient content. In addition leaf nitrogen (N) and phosphorus (P) levels in fast-growing perennial plants tend to correlate with low investment in cell wall structural components and other chemical constituents related to leaf thickness and toughness, as well as an increase in protein content (Lambers and Poorter, 1992; Niemann *et al.*, 1992). High leaf N also tends to coincides with high concentrations of ribulose and rubisco, which appear to confer rapid rates of carbon fixation, whilst P also tends to be greater in leaves of fast-growing species compared to relatively slow-growing species (Grime, 2006). As a result the foliage of fast-growing plants is thought to be relatively more palatable and resource-rich than that of slow-growing plant, not just for herbivores, but also for decomposers.

In contrast, the leaves of plants of low productivity tend to be of poorer litter quality compared to more highly productive plants with lower nutrient content (Poorter *et al.*, 1990), and increased quantities of structural compounds, such as lignin, cellulose, and hemicellulose (Niemann *et al.*, 1992). In addition, slower growing plants tend to be more efficient at stripping nutrients-, particularly N, from older leaves before senescence, further reducing the nutrient content in leaf litter (Chapin *et al.*, 1987). These secondary plant metabolites and associated compounds produced in slow growing plants tent to be more recalcitrant, resulting in reduced rates of litter decomposition (Cornelissen *et al.*, 2004).

Environmental and Resource Stresses

The alpine altitudinal gradient creates a natural stress gradient for montane plants through changes in climate and resource availability. Increases in altitude result in decreases in temperature, changes in precipitation regimes, increased solar radiation, increased wind, and changes in soil fertility and geology (Körner, 2007). Abiotic stress tends to increase with elevation due to a decrease in ambient and soil temperatures, resulting in physical cold stress and a reduced growing season, mineral deficiencies for

both plants and microbes, intense solar radiation, and an increase in windiness resulting increased mechanical and desiccation stress (Callaway *et al.*, 2002; Grime, 2006).
Along with leaf thickening and increased leaf toughness, plant adaptations for stressful alpine environments include a reduction in height and stature, leaf form, and potential growth rate (Grime, 2006).

Aims and Hypothesis

In this study, the growth rate and productivity of taxa in the genus *Chionochloa* are calculated by field growth increment measurements, and then correlated against site environmental stresses to see if growth rate and productivity respond to an environment gradient. It is hypothesised that growth rate and productivity will be negatively correlated with environmental stress, particularly reduced temperature and soil fertility. Growth rate and productivity measures are then correlated against litter quality measures assessed in *Chapter 2* to test growth rate can be used to predict litter quality. It is hypothesised that slow growth rates and low productivities will be correlated with measures of poor litter quality, whilst faster growth rates and higher rates of productivity will be correlated with measures of high litter quality.

Methods

Species Sampled and Locations

Taxa and site sampling locations are the same as described in methods in *Chapter 2*, with the exception of *Chionochloa lanea* collected from Table Hill on Stewart Island. Productivity was unable to be calculated for this taxon as only one visit to the sampling site was possible.

Experimental Design

Establishment and selection of the 4x4m plots were previously described *in Chapter 2.* Within each of these 22 plots, six mature tussock plants of the desired species were randomly selected and tagged for identification. Within each tussock, three tillers were selected at random for growth measurements and non-destructively marked with a large coloured loop (Figure 1b), resulting in 18 marked tillers per plot.

Plant Growth Measurements

Plant growth measurements were recorded for 22 taxa by measurement of changes in leaf length over time (Mark, 1965; Williams, 1977) (Figure 1a). Due to the intravaginal production of leaves in *Chionochloa*, leaf order and age were able to be assigned based on the position of the leaf in the tiller. The youngest leaf occurs at the centre of the tiller with leaves increasing in age moving from the centre of the tiller outwards. The youngest leaf able to hold a small loop was marked (Figure 1b), allowing the identification of individual leaves at a later date. Total lamina length, live lamina portion, dead lamina portion, and tip loss were measured for each individual leaf in the tiller at both Time 1 and Time 2 in the field. Lamina length was recorded from the leaf ligule to the tip of the lamina. Where no ligule was present as occurs on young leaves, the position of the ligule on the nearest leaf was used. The number of tillers per plant was also recorded, estimated from a subsample for large tussocks with numerous tillers.

Measurements at Time 1 occurred in February 2013 with measurements at Time 2 occurring approximately one year later in February 2014. At Time 2, entire marked tillers were harvested from each tussock by excision at the callus and sealed in a polyethylene tube for transport to Massey University in Palmerston North within four days. There they were stored in a dark deep freeze (-20˚C). Once thawed, each tiller's leaves were separated, indentified, and placed in order according to age and loop position. Living leaves were then separated into living lamina, dead lamina and sheath segments (Figure 1c), with each segment length measured. Tissues were then oven dried at 60˚C for 72 hours and weighed.

Annual Productivity

Annual productivity per tiller and per plant was calculated according to the change in the length of lamina segments in individual leaves between Time 1 and Time 2. Several steps were needed, and a number of assumptions had to be made to determine which lamina segments were new since Time 1, and which were originally present at Time 1. Growth within a single leaf was defined as the new portion of lamina produced at the ligule over the year. Dieback was defined as the death of a previously live portion of lamina at Time 1. Tip loss was defined as the breaking off and loss of a portion of the lamina, which predominantly and naturally occurs to the oldest dead portion at the tip of the lamina. Lamina total length is defined as the sum of the live and dead portions for

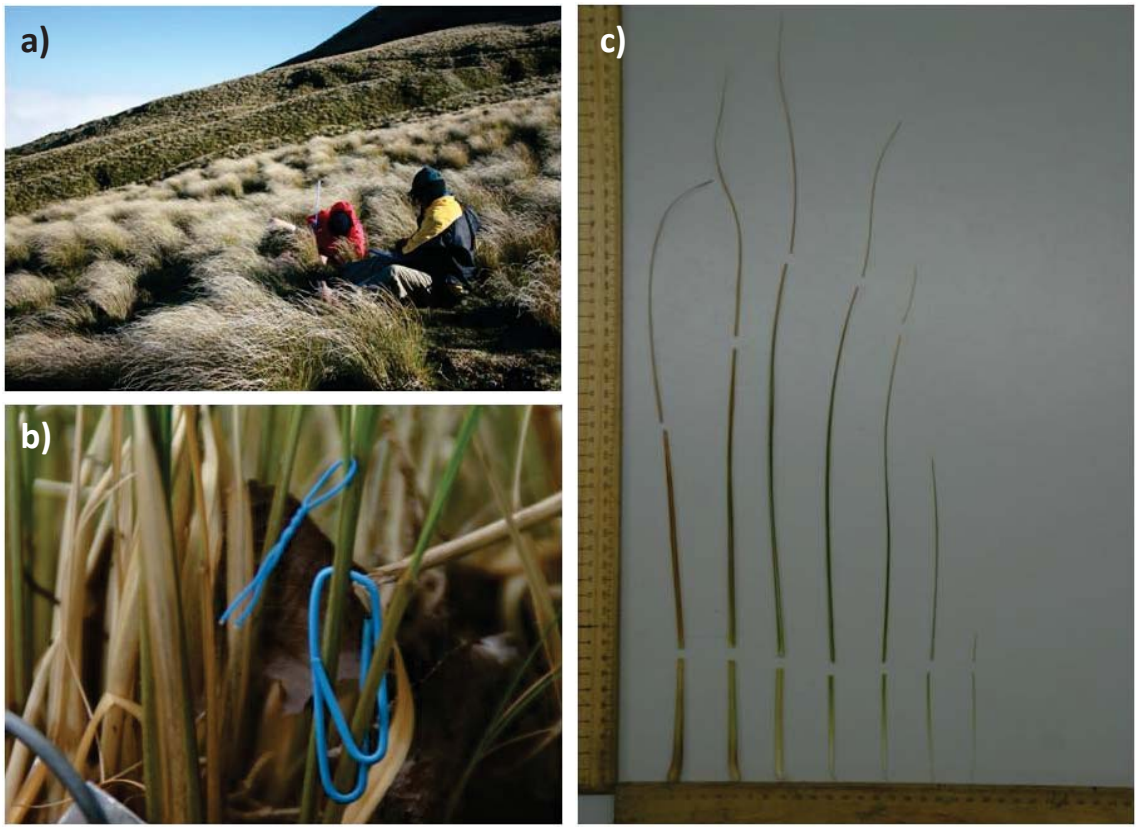


Figure 1: Measurement of tiller productivity via changes in leaf length. a) Lamina length measurements in the field at Time 1 on *C. rubra* tillers, Mt Taranaki. b) *C. pallens* tiller separated into leaf age classes, with leaves ordered from oldest to youngest, from left to right. Leaves are separated into sheaths (bottom), live lamina (middle), and dead lamina (top). c) Marking and identifying of individual leaves in the tiller, with all measured leaves inside paperclip and a young leaf marked with small twisted wire loop.

each individual lamina. Where leaves show no tip loss at either Time 1 (T1) or Time 2 (T2), growth is determined as:

$$\text{Growth}_{(mm)} = \text{Lamina Total Length}_{T2} - \text{Lamina Total Length}_{T1}$$

(Equation 1)

Where leaves had no tip loss at Time 1, but tip loss occurred at Time 2, growth was determined under *Assumption 1*: I.e., if tip loss had not occurred, each leaf would be capable of reaching the maximum leaf length in that tiller (*Tiller Max*). Therefore, growth is equal to:

$$\text{Lamina Growth}_{(mm)} = \textit{Tiller Max}\ \text{Lamina Total Length}_{(T1:T2)} - \text{Lamina Total Length}_{T1}$$

(Equation 2)

Where lamina tip loss was present at both Time 1 and Time 2, but there was no dead portion at Time 1, growth was determined using Equation 1, according to *Assumptions 2* and *3*. *Assumption 2* is that natural tip loss can only occur from the portion of the lamina that is already dead, and *Assumption 3* is that the live portion of a lamina cannot both die back and be lost within the same year, because tussocks are long-lived perennial plants.

Where lamina tip loss was present both a Time 1 and Time 2, and the lamina contained a dead portion at Time 1, growth was determined according to the above assumptions, and *Assumption 4*: I.e., the entire dead portion the lamina in each leaf with tip loss at Time 1, is lost at Time 2. Therefore growth is equal to:

$$\text{Lamina Growth}_{(mm)} = \text{Lamina Total Length}_{T2} - \text{Lamina Live Length}_{T1}$$

(Equation 3)

From these steps the length of new lamina for each leaf in the tiller was derived. To calculate the biomass of the new lamina portion, a length to biomass conversion was used. The length of the new portion of each lamina was multiplied by the weight to length ratio of the live portion from the same lamina (*Equation 4*). Where the lamina had died entirely after growth the same method was used, except the new lamina portion was instead multiplied by the length to weight ratio of the dead portion from the same lamina. This biomass conversion was made under the *Assumption 5*: I.e., that the lamina weight to length ratio is approximately uniform along the length of the live portion of the lamina, due to the long and thin shape of grass leaves.

$$\text{New Lamina Biomass}_{(g)} = (\text{Lamina Live Length}_{T2} / \text{Lamina Live Weight}_{T2}) \times \text{Lamina Growth}_{(mm)}$$

(Equation 4)

As the increase in sheath length was unable to be recorded, sheath productivity was estimated based on the productivity of the leaf's corresponding lamina, where the productivity of the sheath was equal to the productivity of the lamina multiplied by the sheath to lamina ratio (*Equation 5*). This was made under *Assumption 6*: I.e., that the leaf sheath biomass increases proportionally to the leaf lamina biomass, due to the fact that as a leaf increases in size the sheath must also increase in size to provide structural support.

$$\text{New Sheath Biomass}_{(g)} = (\text{Sheath Weight}_{T2} / \text{Lamina Weight}_{T2}) \times \text{New Lamina Biomass}$$

(Equation 5)

The total new biomass per tiller was equal to the sum of the lamina new growth biomass and sum of the sheath new growth biomass in the tiller, for each original leaf from Time 1, plus the biomass of any new leaves produced since Time 1, plus the biomass of any new tillers produced within the leaves of the existing tiller since Time 1.

Litter Production

Litter production was calculated using the same assumptions relating to die back and tip loss used above. Litter production is defined as the amount of dead material lost from the measured leaves of the plant over a year, involving the loss of entirely dead leaves and loss of dead portions from live leaves through tip loss. Where leaves at Time 1 were entirely lost at Time 2, litter production per lamina was assumed to be equivalent to the length at Time 1:

$$\text{Litter production}_{(mm)} = \text{Lamina Total Length}_{T1} - \text{Lamina Live Length}_{T2}$$

(Equation 6)

Where leaves were dead at Time 1 but persisted to Time 2, litter production per lamina was first taken as:

$$\text{Litter production}_{(mm)} = \text{Lamina Dead Length}_{T1} - \text{Lamina Dead Length}_{T2}$$

(Equation 7)

Where leaves were live at Time 1, and were entire at both Time 1 and Time 2, litter production is equal to zero. For all other leaf scenarios, litter production is equivalent to the lamina dead portion at Time 1 (*Equation 8*), which according to *Assumptions 2, 3, and 4* is lost at Time 2. Litter biomass is calculated in the same manner as new biomass (*Equation 5*), but instead uses the length to weight ratio of dead biomass from the corresponding leaf the litter was produced from. Where the entire leaf was lost, the dead portion length to weight ratio from the next youngest leaf was used.

Litter production per tiller is equivalent to the sum of litter production for all leaves in the tiller.

$$\text{Litter production} = \text{Lamina Dead}_{T1}$$

(Equation 8)

Productivity Measures

Productivity is expressed in multiple ways for each taxon to test for relationships across different scales. All productivity measures occur across a one-year time-frame, with biomass being equivalent to the weight of oven dry tissue. Tiller productivity is defined as mean new biomass produced per tiller. Plant productivity is defined as mean new biomass produced per plant, calculated by tiller number per plant. Productivity gram per gram of live biomass (g/g/yr) is defined as grams of new biomass produced per gram of live biomass at Time 1.

The length to dry weight ratio of leaves at Time 2 was calculated from the harvested material and the ratio used to estimate the biomass of the original leaves present at Time 1. To account differences in the ratio occurring with leaf age, leaves in tillers at Time 2 were categorised into age classes (*i*) starting from the oldest live leaf to youngest live leaf. The lamina biomass of a leaf in age class *i* at Time 1 was calculated as the lamina length at Time 1 multiplied by the lamina length to weight ratio of age class *i* (*Equation 9*). Where no corresponding age class between times was possible, the next nearest age class was used.

$$\text{Lamina Biomass}(i)_{T1} = \text{Lamina length}(i)_{T1} \times \text{Lamina Length}(i)_{T2} / \text{Lamina weight}(i)_{T2}$$

(Equation 9)

As no measurements of sheath lengths were possible at Time 1, the sheath biomass of a leaf at Time 1 is estimated using the sheath productivity calculated above instead of a length to weight ratio. The sheath biomass of a leaf at Time 1 is taken as the sheath biomass at Time 2 minus the productivity of the sheath (*Equation 10*).

$$\text{Sheath Biomass}_{T1} = \text{Sheath Biomass}_{T2} - \text{Sheath Productivity}$$

(Equation 10)

In addition to the rate of live biomass production (g/g/yr), two relative growth rates were calculated using Black's relative growth rate equation (Hoffmann and Poorter, 2002). A traditional relative growth rate per tiller, referred to as *RGR-L*, was calculated for each tiller using the live biomasses of each tiller at Time 1 and Time 2 (Equation 11). The mean RGR-L was reported for each taxon.

$$\text{RGR-L} = (\text{Ln} (\text{Tiller Live Biomass}_{T2}) - \text{Ln} (\text{Tiller Live Biomass}_{T1})) / T_2 - T_1$$

(Equation 11)

Due to the nature of grasses to turnover biomass within a tiller through leaf loss and leaf replacement, a second relative growth rate was calculated using the live biomass of the tiller at Time 1, and an estimation of live biomass at Time 2 if no leaf loss were to occur. The live biomass of the tiller at Time 2 was taken as the live biomass at Time 1 plus the productivity if not leaf loss were to occur (Equation 12).

$$\text{RGR-G} = (\text{Ln} (\text{Tiller Live Biomass}_{T1} + \text{Tiller Prod.}) - \text{Ln} (\text{Tiller Live Biomass}_{T1})) / T_2 - T_1$$

(Equation 12)

Litter production is expressed as g/g/yr, reporting grams of litter produced over the year per gram of live biomass at Time 1. Leaf C lability, a measure of leaf longevity, is also reported. Leaf C lability is reported here as the rate of leaf C production (g/g/yr) plus the rate of leaf C loss (g/g/yr), were high rates of leaf C production and leaf C loss both translate to increases C lability.

Analysis

To predict the influence of environmental and climatic variables on growth rate and productivity, general linear models were created using the same methods as described in *Chapter 2*. To help reduce the number of explanatory variables, a PCA ordination was also performed using the statistical program *Canoco* (Ter Braak and Smilauer, 2012) plotting the taxa sites according to their environmental and climate data. Highly correlating variables and variables with no clear link to productivity were not included in the models. Explanatory variables included in the models were: mean minimum winter temperature (*MinTW*), altitude (*Altm*), total annual rainfall (*Raint)*, mean maximum summer temperature (*MaxTS*), and soil organic C (*SoilC*).

Seven general linear models were created for each productivity measure using the statistical program *Systat* (Wilkinson, 1992). Five single variable models were created, as well as two combined models; one allowing for linear interactions between explanatory variables and one allowing for higher order interactions. The higher order parameters included *Raint*Altm*, *Raint*MaxTS*, *Raint*SoilC*, *Altm*MaxTS*, and *Raint*MaxTS*Altm* to take into account a strong east-west gradient occurring between taxa. Models were evaluated and ranked using the model evaluation criteria describe in *Chapter 2*.

To test for relationships between rates of productivity and the quality of litter produced, a Pearson's correlation matrix was performed using the statistical program R (Team, 2014). Significance values were adjusted for each of the productivity measures using the *Bonferroni* correction (Weisstein, 2004). Significance is reported to 0.05 and 0.10 *Bonferroni* adjusted levels. A Mantel test was also performed in the statistical program R (Team, 2014) using the *Vegan* package to test for correlations between genetic similarity and measures of productivity. First, a genetic similarity matrix was calculated by extracting phylogenetic branch distances for paired taxa using the *Ape* package in R. A similarity matrix for each productivity measure was calculated using the absolute value of the difference between paired taxa. Mantel tests were performed in R using Pearson's correlation and 999 permutations per test.

Results

Measures of Productivity

Variation in annual productivity was greatest between taxa at the productivity per plant scale, ranging from 2.2g per plant (*C. australis*) to 1543g (*C. flavescens* spp. *lupeola*), with a generic mean of 200g (*SE* = 70.5) (Table 1). Productivity per tiller showed similar trends, ranging from to 0.05g to 7.35g in the same respective taxa, with a generic mean of 1.36g (*SE* = 0.44). A more even spread of measures was seen in productivity per gram live biomass (g/g/yr), ranging from 0.50 (*C. australis*) to 1.34 (*C. rubra* ssp. *cuprea*) with a generic mean of 0.72 (*SE* = 0.04). Subspecies in the species groups *crassiuscula*, *pallens*, *rigida*, and *rubra* all showed intraspecific similarity in productivity per gram of live biomass, with the exception of *C. rubra* spp. *cuprea*

Table 1: Summary of mean annual productivity measurements for *Chionochloa*. Productivity is expressed as grams of oven dry biomass, and reported at the different scales of, grams per tiller, grams per plant, and grams per gram of live biomass at Time 1. *SI* = Stewart Island. Table 1 continues below.

Taxa	Productivity Measure		
	Per Tiller (g/yr)	**Per Plant (g/yr)**	**Per Live Biomass (g/g/yr)**
C. australis	0.05	2.2	0.50
C. conspicua ssp. *cunninghamii*	7.68	140.0	0.67
C. crassiuscula ssp. *crassiuscula*	0.36	3.7	0.59
C. crassiuscula ssp. *directa*	0.33	40.5	0.66
C. crassiuscula ssp. *torta*	0.15	3.7	0.68
C. defracta	0.29	106.6	0.54
C. flavescens ssp. *lupeola*	7.35	1543.7	0.90
C. juncea	0.27	13.1	0.82
C. lanaea	-	-	-
C. macra	0.27	28.3	0.62
C. pallens ssp. *cadens*	0.85	120.2	0.68
C. pallens ssp. *pallens*	0.74	24.6	0.67
C. pallens ssp. *pilosa*	0.94	135.0	0.82
C. rigida ssp. *amara*	2.41	618.7	0.51
C. rigida ssp. *amara* (*SI*)	1.24	214.7	0.55
C. rigida ssp. *rigida*	0.70	170.6	0.58
C. rubra ssp. *cuprea*	2.65	430.2	1.34
C. rubra ssp. *occulta*	1.36	243.7	0.73
C. rubra ssp. *rubra* var. *inermis*	0.69	80.2	0.73
C. rubra ssp. *rubra* var. *rubra*	0.50	27.6	0.63
C. spiralis	0.30	330.1	0.79
C. teretifolia	0.53	120.6	0.90
C. vireta	0.16	11.7	0.97
Mean	1.36	200.4	0.72
SE	0.44	70.5	0.04

(Table 1). However, this trend was less evident between the subspecies for productivity per tiller and per plant.

Relative growth rate in *Chionochloa* was highly variable between taxa, with a number of taxa decreasing in live biomass over the year (Table 1). RGR-L, relative growth rate calculated from change in live biomass over time, ranged from -0.34 (*C. australis*) to 0.52 in (*C. rubra* spp. *cuprea*), with a generic mean of 0.025 (*SE* = 0.04)

Table 1 continued: Summary of mean annual tiller relative growth rates RGR-L and RGR-G, and leaf turnover rates C lability and litter production. Leaf C lability and litter production are expressed as grams of oven dry biomass per gram of live biomass at Time 1. *SI* = Stewart Island.

Taxa	Productivity Measure			
	RGR-L	**RGR-G**	**Leaf C lability (g/g/yr)**	**Litter Production (g/g/yr)**
C. australis	-0.34	0.42	0.53	0.03
C. conspicua ssp. *cunninghamii*	0.18	0.56	0.72	0.05
C. crassiuscula ssp. *crassiuscula*	0.14	0.46	0.71	0.11
C. crassiuscula ssp. *directa*	0.03	0.51	0.83	0.17
C. crassiuscula ssp. *torta*	-0.01	0.50	0.84	0.16
C. defracta	-0.14	0.41	0.69	0.15
C. flavescens ssp. *lupeola*	0.08	0.64	1.09	0.19
C. juncea	0.33	0.61	1.00	0.18
C. lanaea	-	-	-	-
C. macra	-0.07	0.50	0.84	0.22
C. pallens ssp. *cadens*	-0.04	0.51	0.83	0.15
C. pallens ssp. *pallens*	-0.30	0.53	1.04	0.37
C. pallens ssp. *pilosa*	0.07	0.62	1.00	0.18
C. rigida ssp. *amara*	-0.02	0.42	0.60	0.08
C. rigida ssp. *amara* (SI)	-0.12	0.44	0.73	0.17
C. rigida ssp. *rigida*	-0.08	0.44	0.79	0.21
C. rubra ssp. *cuprea*	0.52	0.79	1.38	0.04
C. rubra ssp. *occulta*	0.11	0.55	0.85	0.12
C. rubra ssp. *rubra* var. *inermis*	0.09	0.54	0.85	0.12
C. rubra ssp. *rubra* var. *rubra*	-0.13	0.49	0.89	0.26
C. spiralis	0.15	0.59	0.91	0.13
C. teretifolia	-0.01	0.64	1.21	0.30
C. vireta	0.12	0.65	1.07	0.10
Mean	0.03	0.54	0.88	0.16
SE	0.04	0.02	0.04	0.02

(Table 1). Eleven of the taxa displayed negative RGR-L's, with six of these being only marginally negative (< -0.08). RGR-G (relative growth rate assuming no leaf loss), had a tighter spread ranging from 0.41 (*C. defracta*) to 0.79 (*C. rubra* ssp. *cuprea*), with a generic mean of 0.54 (SE = 0.02). C lability, a measure of leaf C turnover, ranged from 0.53g (*C. australis*) to 1.38g (*C. rubra* ssp. *cuprea*) with a generic mean of 0.88 (*SE*= 0.04). Litter production per gram of live biomass showed a similar trend ranging from

Table 2: Comparison of general linear models predicting the influence of environmental parameters on plant productivity. K= number of parameters in the model, including a constant. AICc = Akaike's Information Criteria. AICc Δi = difference in AICc score between model *i* and the best model out of the candidate model set. AICc W*i* = Akaike model weight. Only credible models are presented, with models with an AICc Δi < 10 and R^2 > 0.1 accepted as credible models. Strongest models within a candidate set (AICc Δi <2) are shown in bold. * Significance to 0.10 Bonferroni adjusted equivalent. ** Significance to 0.05 Bonferroni adjusted equivalent. Table 2 continues below

Productivity Measure	Model	K	P	r	R^2	AICc	AICcΔi	AICcw*i*
Tiller (g/yr)	MaxTS	2	0.036	0.45	0.20	31.4	5.19	0.053
	MinTW	2	0.113	0.35	0.12	33.5	7.30	0.018
	RainT + MaxTS	**3**	**0.004****	**0.67**	**0.44**	**26.2**	**0.00**	**0.705**
	MaxTS + MaxTS*RainT	4	0.003**	0.67	0.45	28.8	2.53	0.199
Plant (g/yr)	RainT	2	0.030	0.46	0.21	254.5	6.15	0.041
	RainT + Altm	3	0.028	0.56	0.31	254.2	5.85	0.048
	RainT*Altm + MaxTS*RainT	**3**	**0.002****	**0.69**	**0.48**	**248.4**	**0.00**	**0.894**
Live biomass (g/g/yr)	Altm	2	0.015*	-0.51	0.26	-76.2	4.71	0.074
	MaxTS	2	0.059	0.41	0.17	-73.4	7.44	0.019
	MinTW	2	0.135	0.33	0.11	-71.9	8.95	0.009
	SoilC	2	0.076	0.39	0.15	-73.0	7.92	0.015
	Altm + RainT + MaxTS	4	0.012*	0.67	0.45	-76.8	4.08	0.101
	Altm + MaxTS + MaxTS*Altm	**4**	**0.002****	**0.74**	**0.54**	**-80.9**	**0.00**	**0.779**

0.03g (*C. australis*) to 0.37g (C. *conspicua* spp. cunninghamii), with a generic mean of 0.16 (SE = 0.017).

Linear regressions between RGR-L and RGR-G, RGR-L and productivity per gram of live biomass, and RGR-G and productivity per gram of live biomass, showed a strong positive correlation between measures of relative growth rate (R^2 = 0.53, 0.54, 0.93 respectively). All groups of subspecies showed intraspecific similarities in leaf C

lability and RGR-G, with the exception of *C. rubra* spp. *cuprea*. This trend was less evident for RGR-L and litter production.

Linear regressions showed both plant productivity and tiller productivity to have moderate positive correlations with the size of each taxon (recorded as maximum tussock height) (R^2 = 0.33 and 0.39, respectively). Measures of relative growth rate g/g/yr, RGR-L, RGR-G, and turnover measures, leaf C lability and litter production all

Table 2 continued: Comparison of general linear models predicting the influence of environmental parameters on plant productivity. Only credible models are presented, with models with an AICc Δi > 10 and R2 < 0.1 rejected as non credible. Strongest models within a candidate set (AICc Δi <2) are shown in bold.

Productivity Measure	Model	K	P	r	R2	AICc	AICcΔ*i*	AICcW*i*
RGR-L	Altm	2	0.002**	-0.63	0.39	-80.5	3.06	0.170
	MinTW	2	0.007**	0.56	0.31	-77.8	5.80	0.043
	Altm + RainT + MaxTS	**4**	**0.001****	**0.59**	**0.35**	**-83.6**	**0.00**	**0.782**
RGR-G	Altm	2	0.061	-0.41	0.17	-104.1	3.98	0.067
	MaxTS	2	0.026	0.47	0.23	-105.7	2.39	0.149
	MaxTs + MinTW	**3**	**0.019**	**0.58**	**0.34**	**-106.6**	**1.49**	**0.232**
	Rain + MaxTS + Soil C	**4**	**0.009***	**0.68**	**0.47**	**-108.1**	**0.00**	**0.490**
Leaf C lability (g/g/yr)	**Altm**	**2**	**0.087**	**0.37**	**0.14**	**-70.7**	**0.00**	**0.329**
	MaxTS	**2**	**0.122**	**0.34**	**0.12**	**-70.1**	**0.60**	**0.243**
	SoilC	**2**	**0.116**	**0.35**	**0.12**	**-70.1**	**0.51**	**0.254**
Litter Production (g/g/yr)	No credible models							

showed no linear relationship to taxa size (R^2 = 0.05, 0.11, 0.04, 0.06, and 0.02 respectively).

A PCA ordination of taxa sampling sites based on environmental and climate data revealed a strong west-east gradient occurring along axis one (Figure 2). Axis one was the strongest explaining 96.9% of the variation, with axis 2 explaining a further 3.0%. The strongest parameters determining the west-east gradient, were total annual rainfall, mean summer soil moisture deficit, mean maximum summer temperature, and mean summer potential evapotranspiration. Western sites had greater rainfall and higher summer soil moisture levels, with eastern sites having higher mean maximum summer temperatures and mean summer potential evapotranspiration.

General Linear Models

The single best predictor of tiller productivity was *RainT + MaxTS*, with an Akaike weight (AICc W*i*) of 0.71 (Table 2), indicating a 71% chance this is the best model out of the candidate set (Symonds and Moussalli, 2011). Tiller productivity correlated positively with *RainT* (r = 0.31) and *MaxTS* (r = 0.45) individually. Plant productivity was best predicted by the higher order linear model, *RainT*Altm + MaxTS*RainT* (AICc W*i* = 0.89), individually having a positive correaltion with *RainT* (r = 0.46) and *MaxTS* (r = 0.13), and negative with *Altm* (r =-0.19). Productivity per gram of live biomass was also best predicted by a higher order linear model, *Altm + MaxTS + MaxTS*Altm* (AICc W*i* = 0.78), individually having a negative correlation with *Altm* (r = -0.51), and a positive correlation with *MaxTS* (r = 0.41).

RGR-L was best predicted by the linear model, *Altm + RainT + MaxTS* (AICc W*i* = 0.78), and individually showed positive, though weak, correlations with RainT (r = 0.15) and MaxTs (r = 0.34), and a negative correlation with Altm (r = -0.63). RGR-G was predicted equally well by two different models, *MaxTs + MinTW* (AICc W*i* = 0.23), and *RainT + MaxTS + Soil C* (AICc W*i* = 0.49), as both had an AIC_w of less than 2. RGR-G displayed a postive correlation with MinTW (r = 0.31), but shows positive correlations with MaxTS (r = 0.47), RainT (r = 0.13), and SoilC (r = 0.29). SoilC, Altm, and MaxTS showed to be equally good predictors of leaf C lability, however these were not significant, or good fits to the model (R^2 = < 0.14). None of the parameters used were good predictors of litter production, with only non-credible models (R^2 < 0.1) produced. Measures of productivity and growth rate were poorly correlated with genetic similarity (Table 3), with the exception of productivity per tiller (r = 0.312, p = 0.035).

Productivity and Litter Quality

Productivity was found to be related to certain litter quality parameters, though these relationships were not particularly strong (Table 3). Cellulose and the fibre measures, NDF and ADF, tended to show positive correlations with productivity, with cellulose significantly correlated (p = <0.05 Bonferroni correction) to tiller productivity. Total litter N also showed positive correlations with productivity per gram of live biomass, RGR-G, and leaf C lability. Rates of litter production and leaf C lability displayed significant negative correlations with Lignin to N ratio (p = <0.10 with Bonferroni correction), negative correlations with ADF, and positive correlations with hemicellulose. Litter production was also negatively correlated with litter lignin content.

Table 3: Summary of the strongest Pearson correlations for each productivity measure against litter quality parameters. P values are uncorrected, but significance for Bonferroni correction is indicated.* Significance to 0.1 Bonferroni adjusted equivalent. ** Significance to 0.05 Bonferroni adjusted equivalent

Productivity Measure	**Litter parameter**	***r***	***p***
Tiller (g/tiller/yr)	Cellulose	0.55	0.008**
	ADF	0.38	0.079
Plant (g/plant/yr)	Cellulose	0.46	0.031
Live biomass (g/g/yr)	N	0.41	0.059
RGR-L	NDF	0.38	0.081
RGR-G	N	0.36	0.100
Leaf C lability (g/g/yr)	Lignin:N	-0.51	0.016*
	Hemicellulose	0.47	0.028
	N	0.41	0.055
	ADF	-0.39	0.073
Litter production (g/g/yr)	Lignin:N	-0.43	0.016*
	Lignin	-0.45	0.037
	ADF	-0.52	0.073
	Hemicellulose	0.37	0.091

Table 4: Summary of Mantel test for correlation between genetic similarity and productivity measures. Significant (< 0.10) correlations highlighted in bold**.**

Productivity Measure	**Mantel statistic *r***	***p*-value**
Tiller	**0.312**	**0.035**
Plant	0.152	0.246
Live Biomass	-0.054	0.592
RGR-L	-0.022	0.595
RGR-G	0.010	0.452
Leaf C Lability	0.062	0.290
Litter Production	-0.001	0.473

Productivity and Genotype

A Mantel test showed poor correlations between measures of productivity (Table 4). All measures of productivity and litter turnover were found to have no relationship with genetic distance between taxa, with the exception of productivity per tiller, which was significantly correlated with genetic similarity.

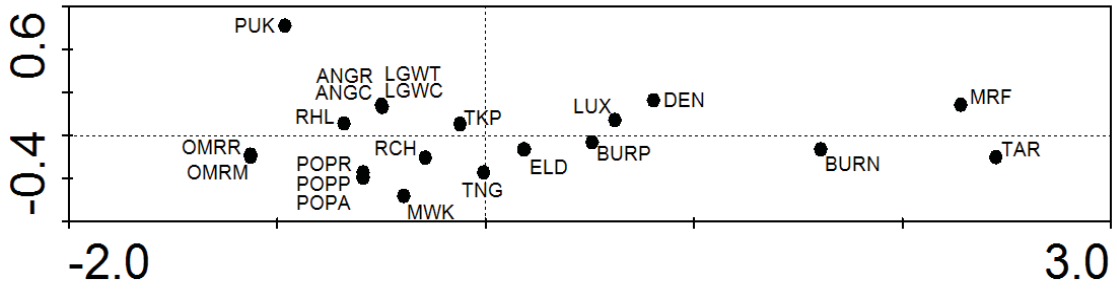


Figure 2: PCA ordination of sites (*n* = 22) by site environmental parameters described in *Chapter 2*. Sites are from left to right, Old Man Range *C. rigida* ssp. *rigida* (OMRR) and *C. macra* (OMRM), Pukerau *C. rubra* ssp. *cuprea* (PUK), Red Hills *C. defracta* (RHL), Poplars Range *C. australis* (POPA) and *C. pallens* ssp. *pilosa* (POPP) and *C. rubra* ssp. *occulta* (POPR), Mt Anglem *C. crassiuscula* ssp..*crassiuscula* (ANGC) and *C. rigida* ssp. *amara* (ANGR), Longwood *C. crassiuscula* ssp. *directa* (LGWC) and *C. teretifolia* (LGWT), Mangaweka *C. pallens* ssp. *pallens* (MWK), Richardson Range *C. vireta* (RCH), Takapari *C. conspicua* ssp. *cuninghamii* (TKP), Mt Tongariro *C. rubra* ssp. *rubra* (TNG), Mt Eldrig *C. crassiuscula* ssp. *torta* (ELD), Mt Burns *C. pallens* ssp. *cadens* (BURP), Mt Luxmore *C. spiralis* (LUX), Denniston *C. juncea* (DEN), Mt Burns *C. rigida* ssp. *amara* (BURN), Mt Rochfort *C. flavescens* ssp. *lupeola* (MRF), Mt Taranaki *C. rubra* ssp. *rubra* var. *inermis* (TAR).

Discussion

Productivity Measures

As expected, productivity varied greatly between taxa in the *Chionochloa* genus, as exemplified by these mid-range sites. This was most evident at the plant level, with an approximately 700 fold difference between the most productive and least productive taxa, whilst tiller productivity was less variable with a 150 fold difference approximately. Relative measures of growth were much closer between taxa, with approximately 2.7, 2.5, and 1.9 fold differences in productivity per gram of live biomass, RGR-L, and RGR-G respectively. In addition, taxon rank was also different between plant productivity, tiller productivity, and measures of relative growth rate, indicating the method and scale used to measure productivity influences the relationship

perceived. The productivities reported here are similar to other studies of *Chionochloa*, with Krna (2015) reporting similar values for *C. rubra* and *C. pallens* in productivity per plant, per tiller, and per gram of live biomass.

The larger range in productivity and differences in taxa rank occurring at the plant and tiller level are likely to have been caused by the differences between taxa in plant size and tussock structural make up, particularly due to differences in number of tillers per plant, and number of leaves per tiller. In addition, relationships between litter quality and productivity appear to be linked to the rate at which plant tissue is produced, as opposed to the quantity produced. Hence, relative rates of growth may provide a better measure for testing these relationships, particularly for litter quality which is thought to be related specifically to the rate of growth.

The negative RGR-L's recorded for a number of taxa indicate a reduction in live biomass over the period of measurement. However, all taxa displayed positive RGR-G's, indicating that growth did occur in all taxa. This infers that for taxa to achieve a negative RGR-L die-back must have been greater than new growth during the year. These negative results can be attributed to the nature of grasses to turnover leaves within a tiller. The strong correlations between RGR-L, RGR-G, and productivity per gram of live biomass suggest that plants that had greater die-back also had slower growth rates.

It is difficult to explain the negative rates of growth occurring for a number of taxa. It is possible that this negative growth indicates that the tillers are dying, however all taxa did produced some new biomass. Another possibility is that rates of dieback and growth are not always constant over time or in sync with each other. Payton and Mark (1979) found *Chionochloa* growth rates not to be constant, with large fluctuations in growth rate following disturbance. Lee *et al.* (2000) also found disturbance to influence growth rate, with these effects lingering long after the disturbance initially occurred. Thus, it is proposed to accurately detect the balance between growth and die-back in a tiller, measurements would need to be over a time period greater than one year.

Productivity and Environmental Stress

Productivity and relative growth rate were best predicted by altitude, temperature, and precipitation. These findings are consistent of with other studies of productivity and growth rate in the *Chionochloa* genus, and other global studies of alpine species (Kikvidze *et al.*, 2005; Michalet *et al.*, 2014). In *Chionochloa*, Mark

(1965), Williams (1977), Greer (1984), and Krna (2015) all report greater productivity for tussocks occurring at lower altitudes, reflecting plasticity in *Chionochloa* growth. Higher altitudes in montane systems are associated with lower plant productivity, due to a reduced growing season caused by longer periods of snow cover, reductions in temperature, as well as increased wind damage and desiccation (Callaway *et al.*, 2002; Larcher, 2003; Körner, 2007).

The positive correlation between mean maximum summer temperature and productivity found here is possibly due to an earlier snow melt and resulting in a longer growing season (Kirdyanov *et al.*, 2003). Higher temperatures also result in increased photosynthetic rates increasing production, where water and or radiation stress are not limiting (Nemani *et al.*, 2003). Scott (1970) found relative growth rates of both New Zealand indigenous and exotic grasses to greater at higher temperatures; however relative growth rates in two *Chionochloa* species were negatively affected at too higher temperature, suggesting high temperatures may hamper growth if the species is not adapted to those conditions. Whilst temperature is positively correlated with productivity across the genus, this suggests that taxa may be adapted for the temperature at the site they inhabit, and may not necessarily increase in productivity if warmer temperatures were to occur.

Due to geographic differences, New Zealand's grasslands are also known to vary greatly in climate and in rainfall (Percival *et al.*, 2000). In the models presented here, rainfall is individually a weak predictor of all productivity measures, but a strong positive predictor when combined with other parameters such as altitude and temperature. This may be due to the geographic difference described above. For example, semi-arid indigenous grasslands in central Otago have characteristically warm summer temperatures, but growth may be limited by low summer rainfall. In contrast, indigenous grasslands in Fiordland have characteristically high rainfall all year round, but growth may be limited by cool temperatures and low solar radiation.

Soil fertility, despite differences in soil N and soil C (*Chapter 4*), was a generally a poor predictor of productivity, with the exception of RGR-G, which included soil C as a predictor combination with climate parameters. This suggests that climate has more influence on productivity than soil fertility for *Chionochloa*. The high strength of prediction in these models should be interpreted with caution, due to the use of spatially modelled climate data instead of empirical data, and the resulting high correlation between modelled parameters (97% of variance explained in axis 1 of PCA

ordination). In addition, there may also be other parameters that influence productivity that were not included in the modelling; however the parameters that were used did result in strong and significant relationships At best, the modelled climate data are only indicative of the most likely climate at each site, and is unable to account for the influence of micro-climates.

Influence of Genotype on Productivity

Plant productivity is determined by a combination genotype and environment (Via and Lande, 1985). In this study, there is no evidence, with the exception of tiller productivity, to suggest genotype is greatly influencing the rate of productivity. The strong correlation between genetic dissimilarity and tiller productivity is again most likely explained by intra-specific similarities and inter-specific differences between taxa in tiller size and leaf number. A poor relationship between genetic similarity and similarity in productivity indicates productivity and relative growth rate are more likely to be controlled by climate and environmental conditions than phylogeny. This supports other findings, where environment often has a greater influence than genotype in determining plant phenotype, (Sultan, 2003).

Links between Productivity and Litter Quality

The rate at which plant material is produced has been suggested to influence the consequent litter quality (Wardle *et al.*, 2004). Relationships between litter quality and productivity were mixed, when compared to the evidence presented in the introduction of this chapter. Litter N was greater in taxa with higher relative growth rates for RGR-G and productivity per gram of live biomass; however other measures of productivity, i.e. per plant, per tiller, and RGR-L, did not correlate with litter N content.

A positive relationship between relative growth rate and leaf N content in grasses, as well as woody taxa, is well supported in the literature (Poorter *et al.*, 1990; Poorter and Bergkotte, 1992; Arendonk and Poorter, 1994; Mediavilla and Escudero, 2003). Greater leaf N content occurring in taxa with faster rates of growth have been linked to the role of N in photosynthesis (Evans, 1989; Wright *et al.*, 2004). High N content in fast-growing individuals also supports the C-S-R theory by Grime (1988), where N is recycled back into the soil through fast turnover of both leaf and litter, resulting in replenishment of N resources in the soil.

Litter fibre and structural compounds did not increase with decreasing productivity, as predicted. Poorter and Bergkotte (1992) reported increases in plant fibre and structural compounds, including cellulose, hemicellulose, lignin, and non soluble sugars with a reduction in relative growth rate In contrast to this, measures of litter fibre and associated structural components, such as cellulose, were found to be positively correlated with measures of productivity, although two of the three relative growth rates (RGR-G and live biomass productivity g/g/yr) reported no relationship with fibre or structural compounds.

The higher measures of litter fibre and structural compounds associated with greater productivity reported here, is most likely due to the most productive plants being larger and taller, and thus requiring a greater quantity of structural components to support an maintain an upright structure. This is supported by a moderate correlation between plant productivity, and taxon maximum tussock height, as well as tiller productivity and taxon maximum tussock height. Also supporting this is a positive correlation occurring between plant height and cellulose. Climatic stress may still be responsible for some structural compounds in plants with greater environmental stress; however these results suggest the relationship between plant height and structural compounds is likely to outweigh this.

Litter quality parameters were better correlated with rates of litter production and leaf C lability, than with productivity and relative growth rate. Litter production and C lability, were strongly and negatively correlated with litter lignin, lignin:N, and ADF, which is likely to result in a reduction in the addition of litter C to soil through the persistence in long lived material on the plant. Similarly, leaf lifespan and longevity have also been found to correlate with greater levels of structural components in other studies (Chapin III *et al.*, 1986; Williams *et al.*, 1989; Takashima *et al.*, 2004; Kitajima *et al.*, 2012). Conversely, in direct contrast to the studies above, hemicellulose in this study was positively correlated with rates of litter production and leaf C lability. This may be explained by a negative correlation between hemicellulose and lignin, suggesting that lignin has a greater influence on leaf longevity than hemicellulose. Similarly, lignin has a stronger correlation with litter production and leaf C lability than ADF (which is lignin plus cellulose), suggesting lignin also has a greater influence on leaf longevity than cellulose.

Increased leaf lifespan and longevity has been suggested to be a result of slow growth rate in stress tolerating plants (Chapin III *et al.*, 1986). This slow growth rate,

and associated accumulation of secondary plant metabolites, allows for plant persistence and survival in its environment through protection from abiotic and biotic stresses (Coley, 1988; Reich *et al.*, 1992; Grime, 2006). The results presented here find no relationship between relative growth rate and leaf longevity, as indicated by litter production and leaf C lability. Again, this may be due to an increase in structural compounds associated with increased plant height in more productive habitats. In addition, results from *Chapter 2* suggest structural compounds lignin, hemicellulose, and ADF display a phylogenetic component, which may further complicate detecting any climatic stress - leaf longevity relationships.

Conclusion and Implications

Productivity and relative growth rates in this congeneric group were best predicted by a combination of altitude, temperature, and rainfall, with increased stress resulting in a reduction in productivity and relative growth rates. However, in general the corresponding plant growth rates did not correlate strongly with litter quality, with the exception of litter N. The strong link between litter N and litter decomposition indicates that fast growing plants in the *Chionochloa* genus may be producing a litter that decomposes at a greater rate. However, other litter quality variables, particularly leaf structural components, did not respond to relative growth rate as expected, and may oppose or cancel out the influence of N on litter decomposition. This hypothesis is tested in the following chapter. If greater leaf N concentration, as found in plants with faster growth rates, results in greater rates of litter decomposition, this could possibly result in reduced rates of C sequestration in faster growing plants.

References

Aerts, R. & Chapin Iii, F. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research,* 30**,** 1-67.

Ahuja, I., De Vos, R. C., Bones, A. M. & Hall, R. D. 2010. Plant molecular stress responses face climate change. *Trends in plant science,* 15**,** 664-674.

Arendonk, J. & Poorter, H. 1994. The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Plant, Cell & Environment,* 17**,** 963-970.

Bradley, K. L. & Pregitzer, K. S. 2007. Ecosystem assembly and terrestrial carbon balance under elevated CO 2. *Trends in Ecology & Evolution,* 22**,** 538-547.

Callaway, R. M., Brooker, R., Choler, P., Kikvidze, Z., Lortie, C. J., Michalet, R., Paolini, L., Pugnaire, F. I., Newingham, B. & Aschehoug, E. T. 2002. Positive interactions among alpine plants increase with stress. *Nature,* 417**,** 844-848.

Chapin, F. S. 1991. Integrated responses of plants to stress. *BioScience,* 41**,** 29-36.

Chapin, F. S., Bloom, A. J., Field, C. B. & Waring, R. H. 1987. Plant responses to multiple environmental factors. *Bioscience,* 37**,** 49-57.

Chapin Iii, F., Mckendrick, J. & Johnson, D. 1986. Seasonal changes in carbon fractions in Alaskan tundra plants of differing growth form: implications for herbivory. *The Journal of Ecology***,** 707-731.

Coley, P. 1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia,* 74**,** 531-536.

Coley, P. D., Bryant, J. P. & Chapin, F. S. 1985. Resource availability and plant antiherbivore defense. *Science,* 230**,** 895-899.

Cornelissen, J. H. C., Quested, H. M., Gwynn-Jones, D., Van Logtestijn, R. S. P., De Beus, M. a. H., Kondratchuk, A., Callaghan, T. V. & Aerts, R. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology,* 18**,** 779-786.

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

Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia,* 78**,** 9-19.

Greer, D. H. 1984. Seasonal changes in photosynthetic activity of snow tussocks (Chionochloa spp.) along an altitudinal gradient in Otago, New Zealand. *Oecologia,* 63**,** 271-274.

Grime, J. P. 1988. The CSR model of primary plant strategies—origins, implications and tests. *Plant evolutionary biology.* Springer.

Grime, J. P. 2006. *Plant strategies, vegetation processes, and ecosystem properties*, John Wiley & Sons.

Hoffmann, W. A. & Poorter, H. 2002. Avoiding bias in calculations of relative growth rate. *Annals of Botany,* 90**,** 37-42.

Kikvidze, Z., Pugnaire, F. I., Brooker, R. W., Choler, P., Lortie, C. J., Michalet, R. & Callaway, R. M. 2005. Linking patterns and processes in alpine plant communities: a global study. *Ecology,* 86**,** 1395-1400.

Kirdyanov, A., Hughes, M., Vaganov, E., Schweingruber, F. & Silkin, P. 2003. The importance of early summer temperature and date of snow melt for tree growth in the Siberian Subarctic. *Trees,* 17**,** 61-69.

Kitajima, K., Llorens, A. M., Stefanescu, C., Timchenko, M. V., Lucas, P. W. & Wright, S. J. 2012. How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. *New Phytologist,* 195**,** 640-652.

Körner, C. 2007. The use of 'altitude'in ecological research. *Trends in ecology & evolution,* 22**,** 569-574.

Krna, M. A. 2015. *Productivity, decomposition and carbon sequestration of Chionochloa species across altitudinal gradients in montane tussock grasslands of New Zealand.* PhD, Massey Univeristy, New Zealand.

Lambers, H. & Poorter, H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences.

Larcher, W. 2003. *Physiological plant ecology: ecophysiology and stress physiology of functional groups*, Springer Science & Business Media.

Lee, W., Fenner, M., Loughnan, A. & Lloyd, K. 2000. Long-term effects of defoliation: incomplete recovery of a New Zealand alpine tussock grass, Chionochloa pallens, after 20 years. *Journal of Applied Ecology,* 37**,** 348-355.

Macarthur, R. & Wilson, E. 1967. The theory of island biography. *Princeton, New jersey*.

Mark, A. 1965. The environment and growth rate of narrow-leaved snow tussock, Chionochloa rigida, in Otago. *New Zealand journal of botany,* 3**,** 73-103.

Mediavilla, S. & Escudero, A. 2003. Relative growth rate of leaf biomass and leaf nitrogen content in several Mediterranean woody species. *Plant Ecology,* 168**,** 321-332.

Michalet, R., Schöb, C., Lortie, C. J., Brooker, R. W. & Callaway, R. M. 2014. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. *Functional Ecology,* 28**,** 75-86.

Nemani, R. R., Keeling, C. D., Hashimoto, H., Jolly, W. M., Piper, S. C., Tucker, C. J., Myneni, R. B. & Running, S. W. 2003. Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *science,* 300**,** 1560-1563.

Niemann, G. J., Pureveen, J. B., Eijkel, G. B., Poorter, H. & Boon, J. J. 1992. Differences in relative growth rate in 11 grasses correlate with differences in chemical composition as determined by pyrolysis mass spectrometry. *Oecologia,* 89**,** 567-573.

Payton, I. & Mark, A. 1979. Long-term effects of burning on growth, flowering, and carbohydrate reserves in narrow-leaved snow tussock (Chionochloa rigida). *New Zealand journal of botany,* 17**,** 43-54.

Percival, H. J., Parfitt, R. L. & Scott, N. A. 2000. Factors Controlling Soil Carbon Levels in New Zealand Grasslands Is Clay Content Important? *Soil Science Society of America Journal,* 64**,** 1623-1630.

Poorter, H. & Bergkotte, M. 1992. Chemical composition of 24 wild species differing in relative growth rate. *Plant, Cell & Environment,* 15**,** 221-229.

Poorter, H., Remkes, C. & Lambers, H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology,* 94**,** 621-627.

Poorter, H. & Villar, R. 1997. The fate of acquired carbon in plants: chemical composition and construction costs.

Reich, P., Walters, M. & Ellsworth, D. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecological monographs,* 62**,** 365-392.

Scott, D. 1970. Relative growth rates under controlled temperatures of some New Zealand indigenous and introduced grasses. *New Zealand journal of botany*, 8**,** 76-81.

Sultan, S. E. 2003. Phenotypic plasticity in plants: a case study in ecological development. *Evolution & development,* 5**,** 25-33.

Symonds, M. R. & Moussalli, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology,* 65**,** 13-21.

Takashima, T., Hikosaka, K. & Hirose, T. 2004. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous Quercus species. *Plant, Cell & Environment,* 27**,** 1047-1054.

Team, R. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-0.

Ter Braak, C. J. & Smilauer, P. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0.

Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution***,** 505-522.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Van Der Putten, W. H. & Wall, D. H. 2004. Ecological linkages between aboveground and belowground biota. *Science,* 304**,** 1629-1633.

Weisstein, E. W. 2004. Bonferroni correction.

Wilkinson, L. 1992. *SYSTAT for Windows: statistics, graphics, data, getting started, version 5*, Systat.

Williams, K., Field, C. B. & Mooney, H. A. 1989. Relationships among leaf construction cost, leaf longevity, and light environment in rain-forest plants of the genus Piper. *American naturalist***,** 198-211.

Williams, P. 1977. Growth, biomass, and net productivity of tall-tussock (Chionochloa) grasslands, Canterbury, New Zealand. *New Zealand journal of botany,* 15**,** 399-442.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. & Diemer, M. 2004. The worldwide leaf economics spectrum. *Nature,* 428**,** 821-827.

Chapter 4

Is litter quality the determining factor in litter decomposition within the genus *Chionochloa*?

A test under controlled conditions

Recently dead litter, ground litter, and litter amended soil – M. Dickson

Introduction

Decomposition and C sequestration

The process of decomposition is of equal importance in C cycling to that of productivity, as it plays an important role in controlling the release and storage of Carbon (C) and nutrients in the soil. The soil forms a major component of the terrestrial biosphere, effectively forming the "epidermis" of our planet, and providing the basis for the recycling of resources in the terrestrial biosphere (Lavelle and Spain, 2001). Hence, decomposition plays an important role not just in C sequestration and C cycling, but consequently in the functioning of the entire terrestrial biosphere.

To quantify and predict rates of C sequestration it is crucial to understand the process of decomposition and the factors that control it. Decomposition includes the breakdown of all organic material; however, in most terrestrial ecosystems the majority of net primary production enters the soil as plant litter (Cadisch and Giller, 1997). Decomposition of plant organic matter occurs through physical fragmentation, leaching of soluble organic C, microbial metabolism of organic matter, and the storage of C in the soil through the formation of stable humus. Physical break down of litter is caused by environmental processes such as wet-dry, shrink-swell, and hot-cold cycles, as well as other environmental conditions such as wind stress. Biological break down occurs through both micro- and macro-organisms, by chewing and fragmentation, ingestion, and digestion (Berg and McClaugherty, 2008). Microbial decomposition results in the production of carbon dioxide, allowing for the transfer of C back to the atmosphere, whilst also transforming organic matter into mineral forms now accessible for plant use (Adl, 2003).

Transformation of organic matter occurs through two processes, mineralization and humification (Zech *et al.*, 1997). Mineralisation is the catabolic conversion of elements contained in organic matter into inorganic forms accessible for use and uptake by living organisms, and subsequently determines the fluxing of available nutrients for both plants and microorganisms (Bernal *et al.*, 1998). In contrast, humification is the anabolic process in which organic molecules are condensed into degradation resistant organic polymers. These organic polymers may persist in the soil for decades or centuries, and therefore determine the subsequent rate of accumulation of stabilised organic matter in the soil, resulting in C sequestration (Zech *et al.*, 1997). Both litter quality and soil physical and chemical conditions influence the rate and total amount of

mineralisation and humification occurring during decomposition (Cadisch and Giller, 1997).

Factors Determining Decomposition

Decomposition is well known to be controlled predominantly, and in decreasing order, by climate, litter quality, and soil microorganisms, as well as other physical soil properties (Aerts, 1997; Zhang *et al.*, 2008; Prescott, 2010). Climate influences soil temperature and moisture regimes, with microbial respiration well known to be negatively correlated with soil moisture and positively correlated with temperature (Berg *et al.*, 1993). Litter influences the rate and total amount of decomposition occurring through its chemistry and structure, with certain litter characters being more resistant to physical and microbial breakdown than others (Berg and McClaugherty, 2008).

Soil organisms also directly control decomposition processes and rates (Lavelle and Dickinson, 1987). While soil animals and soil microbes both play important roles in litter decomposition, the primary decomposers in soil systems tend to be microorganisms, encompassing both bacteria and fungi (Berg and McClaugherty, 2008). Greater rates of microbial biodiversity and biomass have also been reported to correlate with greater rates of decomposition (McGuire and Treseder, 2010). In addition to soil microorganisms, the soil structure may influence the rate of decomposition with physical aggregates, such as clay minerals, known to physically inhibit and prevent decomposers from accessing resources.

Warm temperatures, high moisture content, high oxygen availability, and high palatability of litter to decomposers all favour greater rates of microbial activity and greater rates of litter decomposition. Conversely cooler temperatures, moisture limitation, low oxygen content, and poor litter quality all result in lower rates of litter decomposition, and the build up of organic C in the upper soil profile (Cadisch and Giller, 1997).

Litter Quality Parameters and Decomposition

Litter quality and its influence on decomposition have been well studied, with some general trends in litter decomposition being identified. The best general indicators of litter quality are listed as total N, the C:N ratio, total lignin, and the lignin:N ratio (Melillo *et al.*, 1982; Cadisch and Giller, 1997; Heal *et al.*, 1997). Lignin is probably the

most recalcitrant of all naturally produced organic chemicals, persisting in the soil and often forming humic compounds (Cadisch and Giller, 1997). Whilst lignin and lignocelluloses can eventually be broken down, their prolonged persistence in the soil can result in the long term net storage of C. Depolymerisation of large polymers such as lignin is restricted to certain types of fungi and bacteria such as 'white rot fungi', and in the absence of such microorganisms lignin and associated plant compounds may remain intact and persist in the soil (Guerriero *et al.*, 2016). The Lignin:N ratio of litter has also be found to be a good indicator of long-term litter decomposition, with an negative linear relationship proposed between the two, thought to be due to the retarding effect of N on lignin-degrading microorganisms (Melillo *et al.*, 1982; Carreiro *et al.*, 2000).

Similarly, in the absence of lignin, nutrient and element deficiencies in litter may limit microbial activity, resulting in reduced, limited, or suspended rates of decomposition. Nutrient deficiency occurs when the C to Nutrient ratio of the litter is high compared with that of the living microorganisms attempting to decompose the plant material (Lavelle and Spain, 2001). Nitrogen is one of the most common factors limiting litter decomposition, as it directly influences the growth and turnover of microbial biomass mineralizing the organic C (Cadisch and Giller, 1997). The C:N ratio has historically been, and still is, considered one of the best indices of litter quality (Eiland *et al.*, 2001). The C:N ratio is a good predictor of the initial rate of litter decomposition, with a reduction in decomposition associated with increasing C:N, again due to microbial N limitation (Cadisch and Giller, 1997).

The structural carbohydrates cellulose and hemicellulose, along with lignin, make up the majority of plant fibre, and are a major component of the plant biomass. The fibre fraction of litter is more difficult to decompose, when compared to the rapidly decomposing soluble fraction. Cellulose and hemicellulose levels tend to decompose slowly over the initial phases of decomposition, whereas the latter phases of decomposition are dominated by lignin (Melillo *et al.*, 1989). Soluble litter compounds are generally made up of metabolic carbohydrates, amino acids, and phenolics, and are readily leached from plant material. They also generally serve as an energy rich source for microorganisms and are the first components of leaf litter to decompose, with their abundance being an important determinant in the early phases of litter mass loss and carbon mineralisation (Collins *et al.*, 1990; Soong *et al.*, 2015). Physical litter parameters, such as leaf waxes, pubescence, and physical toughness, can also provide an

accurate indication of litter quality, though this may be a reflection of their chemical properties (Pérez-Harguindeguy *et al.*, 2000).

Phenols appear to play varying roles in litter decomposition. Some phenols are easily leached with rain-fall, whilst others contain high levels of oxidising enzymes which result in quick destruction during leaf-fall. However, it appears a significant proportion of the phenolic fraction does remain in the leaf litter for some years, particularly that bound to protein or the cell wall (Lavelle and Spain, 2001). Phenolics can be used as a C substrate by decomposers, but many phenols can inhibit the growth or function of decomposing organisms by binding to enzymes, or chemically binding to N leaving it unusable to decomposers (Martin and Haider, 1980; Waterman and Mole, 1994). Phenolics may play an important role in the formation of recalcitrant soil organic N, and hence reduce litter decomposition rates. Lignin and phenolic compounds have similar influences on decomposition, though different processes are involved (Lavelle and Spain, 2001).

Hypotheses and Aims

As outlined and examined in *Chapters 2* and *3*, litter quality is influenced by phylogeny and environmental conditions, which in turn influence growth rate and plant strategy. Wardle *et al.* (2004) propose that higher plants as the primary producers in ecosystems will not only influence predators through the production of secondary metabolites in leaf material, but also the decomposers of that leaf material, suggesting a link between productivity and decomposition. Aerts (1997) also proposes a triangular relationship between climate, litter quality, and decomposition. This suggests that decomposition rates in *Chionochloa* litter should correspond to its litter quality measures (*Chapter 2*), which in turn should be influenced by the environment and rate at which they were produced (*Chapter 3*). This chapter aims to investigate if the variability in *Chionochloa* litter quality, attributed to environment, growth rate and phylogeny, translates into detectable trends in litter decomposition.

In this experiment, *Chionochloa* litter varying in quality and chemical composition is decomposed in a controlled environment to assess the influence of litter quality on decomposition. To isolate the influence of litter quality on litter decomposition, soil temperature, soil moisture, soil characteristics, and soil microbial community are controlled in an *ex-situ* incubation experiment on a common, i.e. shared, alpine soil. In a second trial, litters are also decomposed in an identical controlled *ex-*

situ incubation experiment; however this time incubation occurs on their corresponding home-site soil to assess the combined influence of soil characteristics and litter quality on the rate of litter decomposition.

The aim of this experiment is to determine the rates of decomposition occurring in litters of varying quality and chemistry, and to identify any correlations between litter quality and decomposition. It is hypothesised that rates of decomposition will correlate with key indicators of litter quality, as described above. This experiment also aims to determine the influence of soil type and soil characteristics on litter decomposition, with soils with greater moisture content and increased microbial activity hypothesised to correlate with increased litter decomposition.

Methods

Location, Species Sampled, and Litter Collection and Preparation

Sampling locations, plots, taxa, litter collection and preparation are as described in previous methods in *Chapter 2*.

Experimental Design

Carbon mineralisation during soil and litter decomposition was measured by trapping of respired carbon dioxide carbon (CO_2-C) from incubated soils in an alkaline solution (i.e. alkali trapping). Methods used were based on the static incubation method described in Alavoine *et al.* (2008), and closed compartment CO_2 absorption by Hopkins (1993). Two litter incubation experiments were conducted for 23 taxa, the first consisting of the incubation of litter on a common soil, and the second the incubation of litter on the corresponding home site soil of that litter.

Soil Collection

Soil samples were collected for an *ex situ* laboratory decomposition experiment for the corresponding taxa and site plots, as described in *Chapter 2*. Collection of soil samples occurred at the same time as the litter samples were collected, during the second visit to the sites in February 2014. At each sampling plot, soil cores (n=10) from the top 10cm of the soil profile in and directly adjacent to each plot were collected using a soil corer (diameter 2.8cm) and bulked. Live vegetation, stones, and fresh litter not

part of the initial humus layer were removed and not counted as part of the soil profile. The bulked soil samples were immediately sieved in the field to 4mm to remove large roots, other vegetation material, stones, and to homogenise the soil. The soils were then kept "field fresh", by storage in sealed polyethylene bags in a dark chiller (within 4 days of collection) at 4˚C until incubation. Soil samples for organic C and total N were collected as described in *Chapter 2.*

Soil Preparation and Analysis

Before soil incubation, the field moisture content and water holding capacity of each soil was calculated. Water holding capacity (WHC) was calculated by saturation on a ceramic pressure plate by means of a water bubble tower with 50cm of suction at 5kpa (Klute, 1986). Uniform volumetric water content for all soils was then achieved by adjusting the water content to 70% of WHC through addition of distilled water to dry soils, and removal of excess water in wet soils through forced air drying in a dark chiller at 4˚C. A WHC of 60% has be recommended as an ideal moisture content for carbon mineralisation, (Hopkins, 1993); however due to the high field moisture content of most collected soils, and high rainfall in New Zealand alpine grassland environments (Percival *et al.*, 2000), a water content of 70% WHC was chosen. Soil samples collected for organic C and total N were analyzed using the same methods described previously in *Chapter 2*.

Incubation Chambers

Soils were incubated in one litre glass Agee jars (95mm deep x 175mm high). Agee jars were sealed with an air tight screw on lids and contained a PVC plastic alkali trap stand (55 mm x 70 mm), a plastic alkali trap (45 mm x 55 mm), and a plastic water container (45mm D x 30mm H) within the trap stand (Figure 1: d). The equivalent of 30 grams of oven dry soil, with a moisture content set to70% of its WHC, was added to each jar. These jars were then amended with 1.5 grams of prepared litter (a rate of 5%) and incubated at 25˚C in a dark, temperature-controlled room. 10 ml of deionised water was added to each jars water container and placed within the trap stand to help prevent loss of soil moisture loss evaporation. 30 ml of 1 Molar NaOH solution was placed in each alkali trap on the trap stand within the jar to absorb CO_2 accumulating in the head space for measurement. The jars were sealed air tight, and NaOH solution collected and

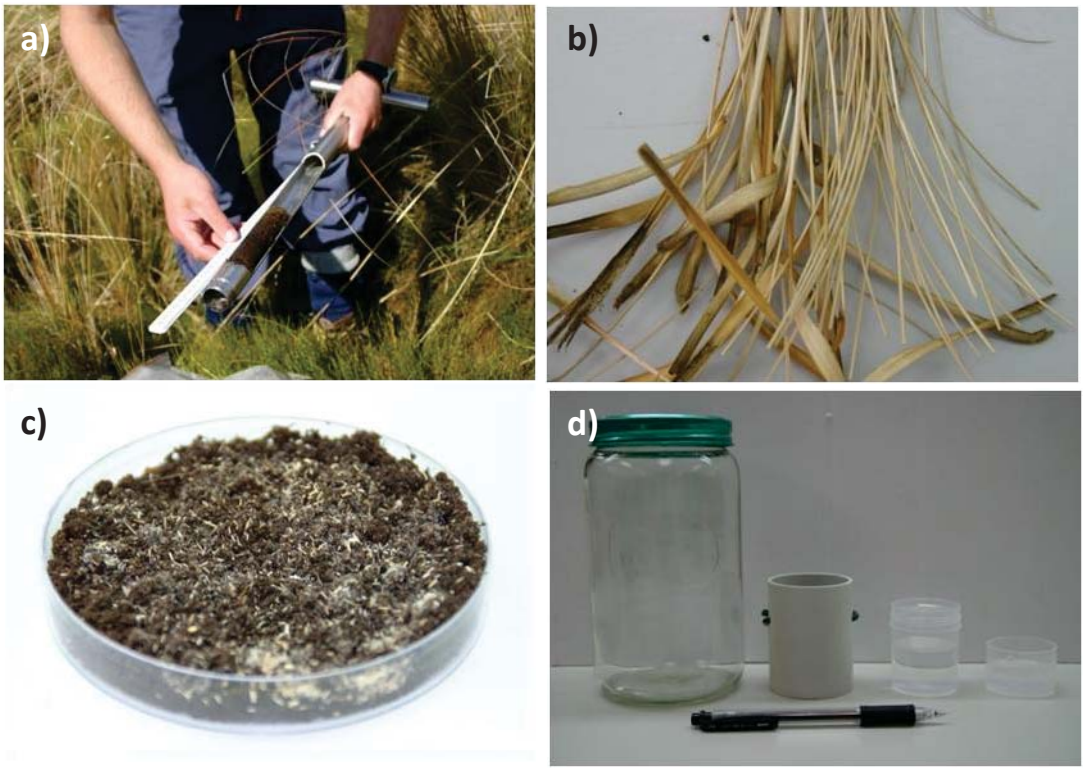


Figure 1: Experimental set-up for litter decomposition through controlled soil incubation. a) Collection of soil from the top 10cm of the soil profile in *C. rubra* ssp. *cuprea* grassland, Pukerau. b) Recently dead attached litter collected from *C. rubra* ssp. *rubra* var. *rubra*, Mt Tongariro. c) Example of a sieved soil amended with mixed in ground *Chionochloa* litter. d) Soil and litter incubation jar components. From left: 1L Agee jar with screw top seal lid; Alkali trap stand; Alkali trap with 30ml NaOH; water vial with 10ml of deionised water. In jar set, the water vial was placed within the trap stand at bottom of jar, the Alkali trap on top of the trap stand, and soil placed around the outside of the trap stand.

replaced at set time periods. To replenish oxygen content within the jar, jars were opened and aerated for 5 minutes at each NaOH trap change.

Titration

CO_2 absorbed in the alkali trap was measured by titration of a 5ml aliquot of the NaOH solution against 0.2 Molar HCl, using phenolphthalein as an indicator to determine residual NaOH in the trap. Before titration, 5ml of 1 Molar $BaCl_2$ solution was added to 5ml aliquot of NaOH to precipitate out any carbonates (Hesse, 1971). As two moles of NaOH reacts with one mole of CO_2, the total grams of CO2-C within a jar is calculated as the total moles of NaOH reacted divided by 2, and multiplied by the molar mass of C. The litter C released as CO_2-C (g) through decomposition is calculated as the sum of the litter amended jar CO_2-C, minus the mean basal soil jar CO_2-C, minus the mean control jar CO_2-C. The decomposition of soil C in the basal jars is calculated as the sum of the mean basal soil jar CO_2-C, minus the mean control jar CO_2-C.

Common Soil Incubation

A standardised alpine soil, referred to as the common soil, was used as a controlled variable for litter incubation to test the relationship between litter chemistry (independent variable), and litter CO_2-C production (dependant variable). The soil, a Firm Brown alpine soil (Hewitt, 1998), was collected from above the tree line in *Chionochloa pallens* tussock grassland at 1000m in elevation near Field Hut in the Tararua range (Latitude -40.9147 S, Longitude 175.2643 E). An alpine soil was chosen over a standard potting mix soil or standard lowland soil to minimise any major physical differences or differences in microbial communities between alpine and lowland soils, as the majority of *Chionochloa* are alpine taxa. The alpine Firm Brown Tararua soil was chosen as it is geographically isolated from the sampling sites of other taxa (closest site: Takapari, Ruahine Ranges), and because Brown soils are listed as one of the most common and abundant soil in New Zealand (Hewitt, 1998). The common soil was collected and prepared under the same methods as the home-site soils.

A total of 29 jars were incubated, 23 jars containing common soil amended with an individual taxon litter, 3 basal jars containing common soil only, and 3 blank jars containing no soil or litter, which were incubated as a control. Jars were incubated for a total of 156 days, with sampling occurring on days 1 - 8, 12, 16, 24, 32, 43, 53, 63, 77, 115, and 156, with intervals between sampling times selected to reflect the decreasing rate of decomposition over time. Litter decomposition was measured as gram per gram loss of C and by the rate of exponential decay, indicated by the decomposition constant *k* (Berg and McClaugherty, 2008).

Site Soil Incubation

Home site soils from each of the 23 taxa were incubated as in the methods above. Two jars were incubated for each taxon; the first, a basal jar containing just the home-site soil, and the second, a jar containing home-site soil amended with litter from its corresponding taxon. A further three blank jars were incubated as controls. Jars were incubated for a total of 150 days with sampling occurring on days 1 - 8, 11, 15, 21, 28, 37, 46, 61, 75, 90, 114, and 150, with intervals between sampling times selected to reflect the decreasing rate in decomposition over time.

Analysis

To predict the influence of litter quality and soil characteristics on litter C and soil C decomposition, general linear models were created using the same methods as described in *Chapter 2*. To display relationships between litter quality variables and soil characteristics, a PCA ordination was performed using the statistical program *Canoco* (Ter Braak and Smilauer, 2012). In the case of the common soil, where all soil characters were standardised, the litter quality variables described in *Chapter 2* where used to create 10 individual linear models, and combined using the forward stepwise method to create 1 combined linear model.

To predict litter decomposition in the home-site soils, the soil characters, total soil N (SON), total organic C (SOC), C:N ratio (SOCN), soil water holding capacity (WHC), and rate of soil C decomposition (SODgg) were used as model explanatory variables, in addition to the strongest litter quality variables from the common soil models. Seven general linear models were created for home-site soil decomposition using the statistical programme *Systat* (Wilkinson, 1992); six single variable linear models and one combined linear model. Similarly, a set of general linear models was also created using soil characters to predict soil C decomposition. Models were evaluated and ranked using Akaike's Information Criterion as described in the methods of *Chapter 2*. To test for relationships between the soil characters used in the models, a Pearson correlation matrix test was performed using the statistical program R (Team, 2014).

Results

Decomposition Substrate

Total litter decomposition gram per gram (g/g) and rate of litter decomposition (*k*), were both on average greater on home-site soil compared to litter decomposition on the common soil (Table 1). Total litter decomposition (g/g) on the common soil ranged from 0.083 to 0.204 with a mean of 0.145 (*SE* = 0.006), compared to litter decomposition on the home-site soil, which ranged from 0.026 to 0.423, with a mean of 0.204 (SE = 0.022). Litter *k* on common soil ranged from 0.0006 to 0.0051, with a mean

Table 1: Comparison of total litter C loss (Litter CO_2-C) g/g of litter C added occurring during decomposition, for litter incubated on a common soil, and on its corresponding home-site soil. *k* indicates the decomposition constant over the incubation. The taxa legend corresponds to the following Figures 3 and 4. SI = Stewart Island.

Legend	Taxa	Litter on Common Soil		Litter on Home Soil	
		Litter C lost (g/g)	*k*	Litter C lost (g/g)	*k*
	C. australis	0.083	0.0006	0.357	0.0029
	C. conspicua ssp. *cunninghamii*	0.179	0.0013	0.130	0.0009
	C. crassiuscula ssp. *crassiuscula*	0.097	0.0007	0.044	0.0003
	C. crassiuscula ssp. *directa*	0.140	0.0010	0.026	0.0008
	C. crassiuscula ssp. *torta*	0.107	0.0007	0.105	0.0007
	C. defracta	0.137	0.0010	0.347	0.0028
	C. flavescens ssp. *lupeola*	0.118	0.0008	0.184	0.0014
	C. juncea	0.136	0.0009	0.174	0.0012
	C. lanea	0.134	0.0009	0.204	0.0015
	C. macra	0.152	0.0011	0.265	0.0020
	C. pallens ssp. *cadens*	0.143	0.0010	0.176	0.0012
	C. pallens ssp. *pallens*	0.171	0.0013	0.227	0.0018
	C. pallens ssp. *pilosa*	0.162	0.0012	0.179	0.0013
	C. rigida ssp. *amara*	0.135	0.0010	0.160	0.0011
	C. rigida ssp. *amara* (SI)	0.143	0.0010	0.239	0.0019
	C. rigida ssp. *rigida*	0.162	0.0012	0.213	0.0015
	C. rubra ssp. *cuprea*	0.169	0.0013	0.094	0.0007
	C. rubra ssp. *occulta*	0.151	0.0011	0.242	0.0017
	C. rubra ssp. *rubra* var. *inermis*	0.162	0.0012	0.295	0.0026
	C. rubra ssp. *rubra* var. *rubra*	0.204	0.0015	0.120	0.0008
	C. spiralis	0.111	0.0008	0.423	0.0038
	C. teretifolia	0.144	0.0010	0.106	0.0008
	C. vireta	0.190	0.0014	0.384	0.0032

of 0.0011 (*SE* = 0.5 x 10^{-4}), compared to litter *k* on the home site soils which ranged from 0.0003 to 0.0038, with a mean of 0.0016 (SE = 0.19 x 10^{-4}). However, greater litter decomposition on site soil was not the case for all taxa litters, with 7 of the 23 litters having a lower total decomposition and rate of decomposition on their home site soil compared to decomposition on the common soil.

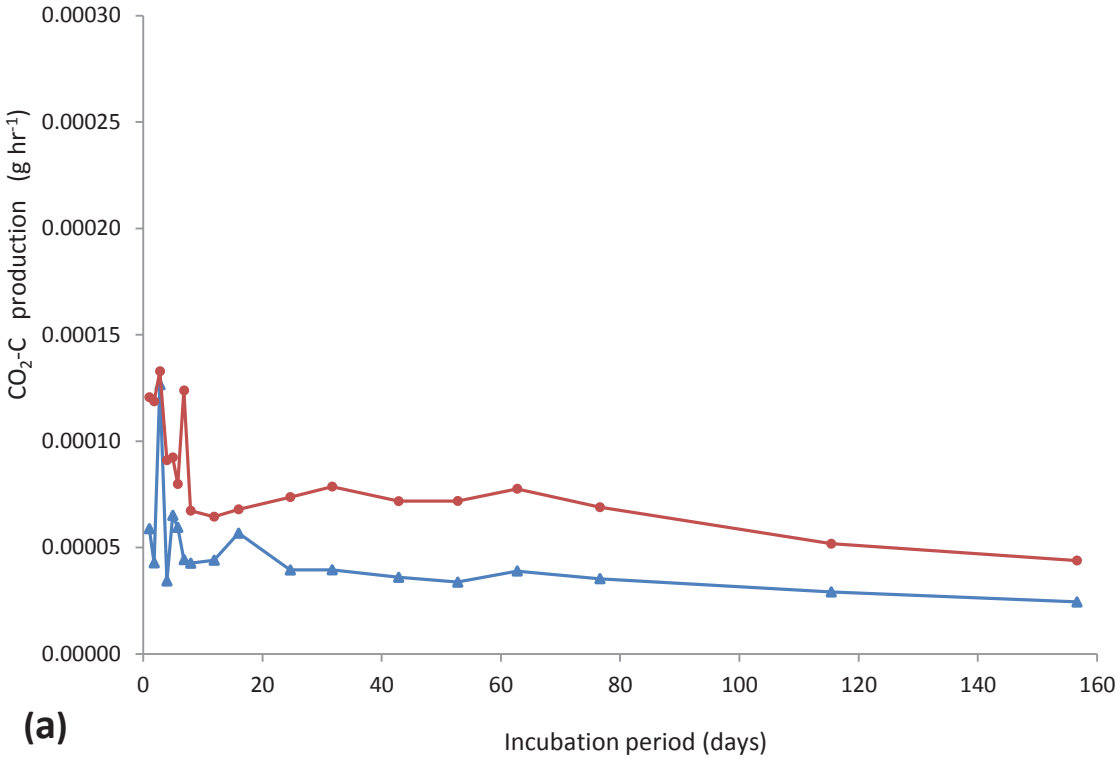
Temporal Trends in Decomposition

Despite these differences, overall there were similar trends in common soil and home site soil decomposition over time (Figure 2). Rate of soil CO_2 production from the basal and litter-amended soils was greatest in both trials in the very immediate stages of decomposition, i.e. during days 1 and 2. Both trials showed fluxing in CO_2 production over days 1 to 10, with basal and litter-amended soils showing matching peaks and falls. Rates of decomposition appeared to stabilise after approximately days 10 to 20 for both trials, and continued to steadily decrease for the rest of the incubation in both the basal and litter amended soils of both trials. However, this rate of decrease was greater in the home-site soil compared to the common soil, as explained by larger mean litter *k* in the site soil trial. Although both trials have similar trends, CO_2 production was both initially and consistently higher over the entire incubation period for the home site basal and litter amend soils (Figure 2; b), when compared to the common soils (Figure 2; a). This was particularly evident in initial fluxing from days 1 to 10, with the site basal and litter amended soils being approximately two times greater than both the common basal and litter amended soils over the incubation period.

Rates of Litter Decomposition

The decomposition trend described above was fairly consistent for all taxa on the common soil trial (Figure 3; a). Variation in decomposition rate between litter types was greatest during fluxing, which occurred over the initial 10 days of incubation. Later, two slight peaks in rate of decomposition are apparent in most of the litters, occurring at approximately day 30 and day 60 of the incubation. Litter decomposition rates on the common soil reduce in variability from approximately day 30 of the incubation onwards, with litters showing greater similarity, and a clear rank in the rate of decomposition from day 60 onwards. From approximately day 100 of the incubation, differences in rate litter decomposition are greatly reduced, though remain consistent between the different taxa.

Litter decomposition occurring on its home-site soil (Figure 3; b) is much more variable in comparison to decomposition on a common soil. Initially, variability occurs in fluxing between days 1 and 10; however from here onwards litters vary greatly in rate of decomposition over time. After approximately day 10, litters on home-site soils generally had a single peak of maximum decomposition rate, with rate of litter decomposition falling away after this maximum peak. In contrast to the common soil,



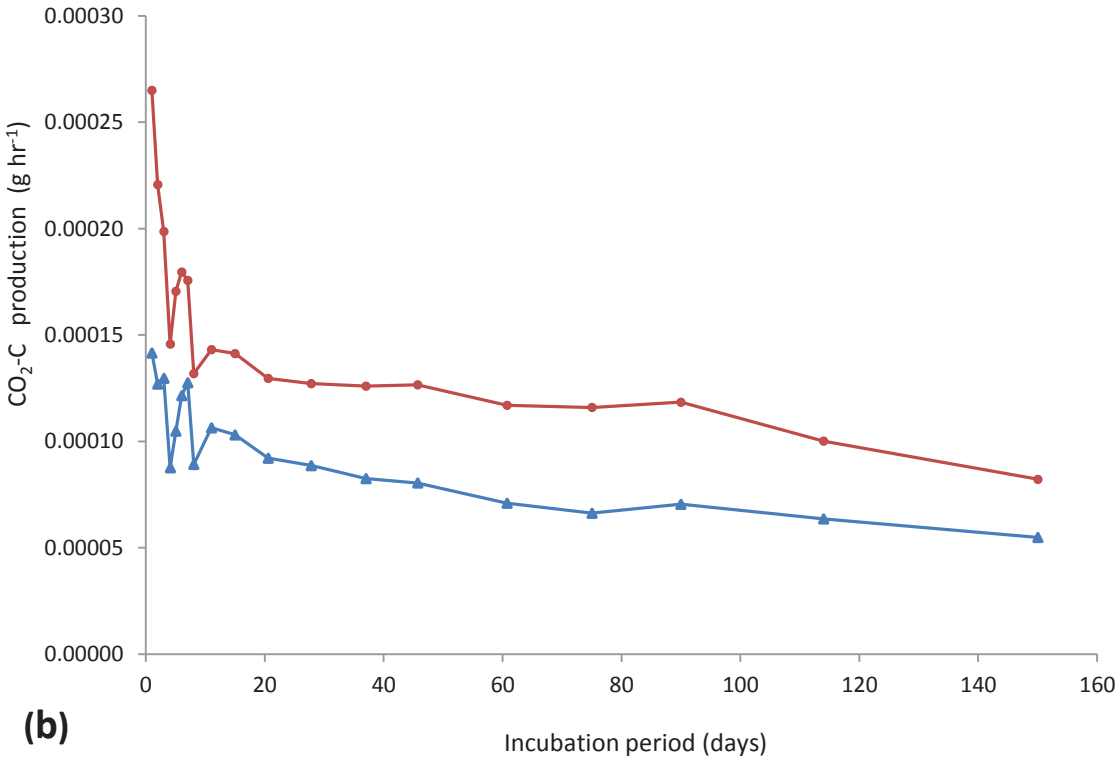


Figure 2: Mean CO_2-C production for all litter-amended soils (Red + Dot) and un-amended basal soils (Blue + Triangle) for: **(a)** incubation of individual litters on a common soil, and **(b)** incubation of individual litters on corresponding home-site soil.

patterns in the rate of litter decomposition over time were highly variable between taxa on the home site soils. These differences included variability in the initial start time of the main peak of litter decomposition, variability in the size of the peak, as well as variability in the duration of these peaks (Figure 3; b).

Litter decomposition rates appear to be converging at the end of the incubation period on the home-site soils; however this is much more variable compared to the same point of time in the common soil trial. The majority of litters appear to be decreasing in rate of decomposition from 100 days of incubation onwards, however at 150 days of incubation not all litters appeared to have reached a stable rate of decomposition, with some still decreasing greatly, some effectively having ceased decomposition, and others showing increasing rates of decomposition. The negative rate in litter decomposition of *C. crassiuscula* ssp. *directa*, from day 100 onwards results from a negative balance between CO_2 production from the litter-amended soil and the basal soil, due to a continued decrease in the litter amended soil respiration below the basal soil respiration rate.

Cumulative litter carbon loss

These differences in the rate of litter decomposition can be seen when comparing cumulative litter carbon loss between trials (Figure 4). The same trend as above is seen for cumulative litter carbon loss occurring on the common soil (Figure 4; a), with taxa maintaining their relative rank in litter carbon loss over time. Overall, rates of litter carbon loss on the common soil show an even spread, though differences between litter carbon loss increases with the duration of the incubation. Litter carbon loss is still occurring for each taxon at 150 days of incubation.

In contrast, cumulative litter carbon loss occurring on home-site soil is much more variable compared to the common soil trial (Figure 4; b). In addition, there is greater variation in rate of litter carbon loss at 150 days of incubation in the home site soil. At this point on the home-site soils, litter carbon loss is decreasing in some, has ceased in others, and is increasing in others.

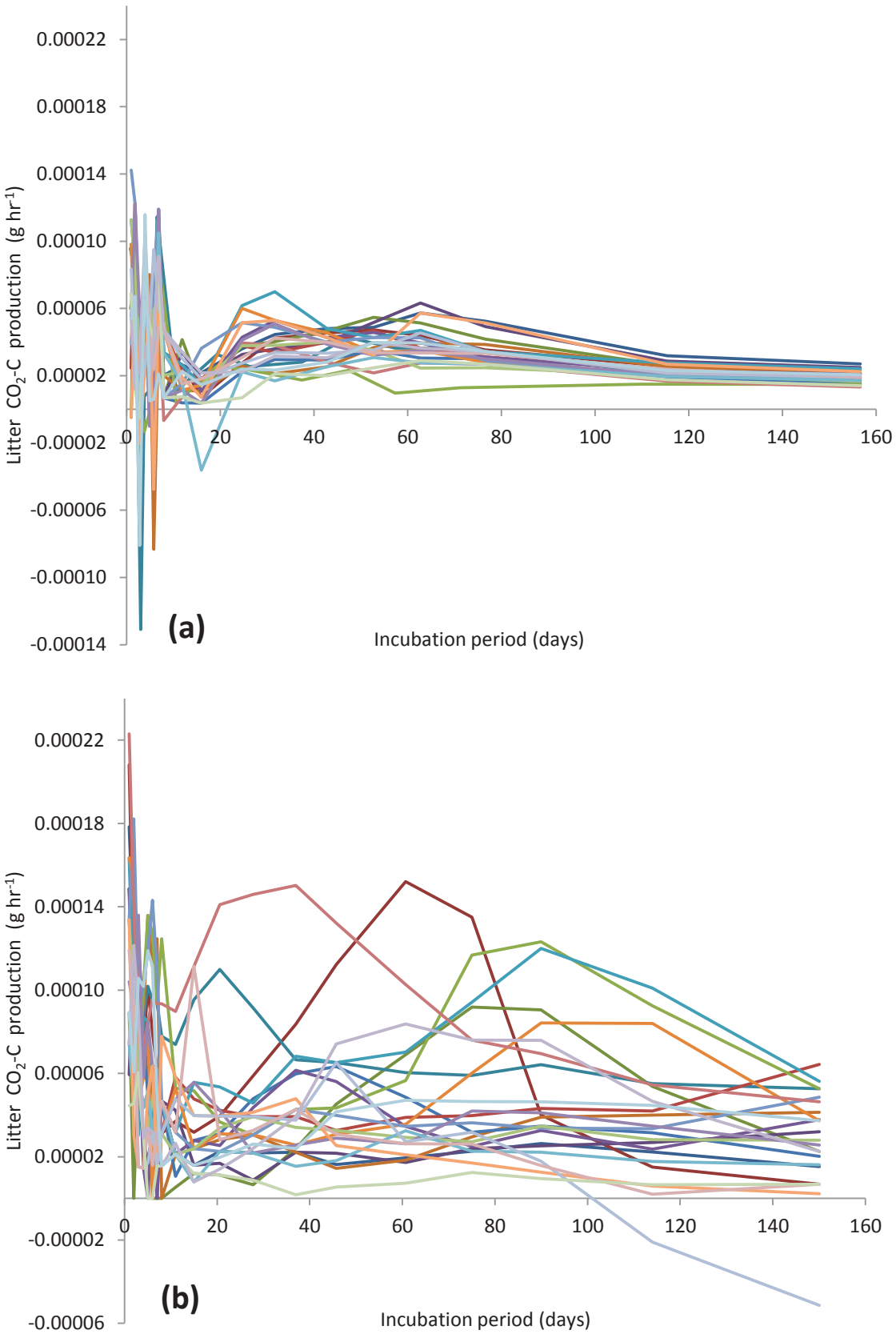


Figure 3: Summary display of the rate of litter CO_2-C production over time for each of the 23 taxa for; **(a)** incubation of individual litters on a common soil, and **(b)** incubation of individual litters on their corresponding home site soil. Taxa are identifiable from the legend in Table 1.

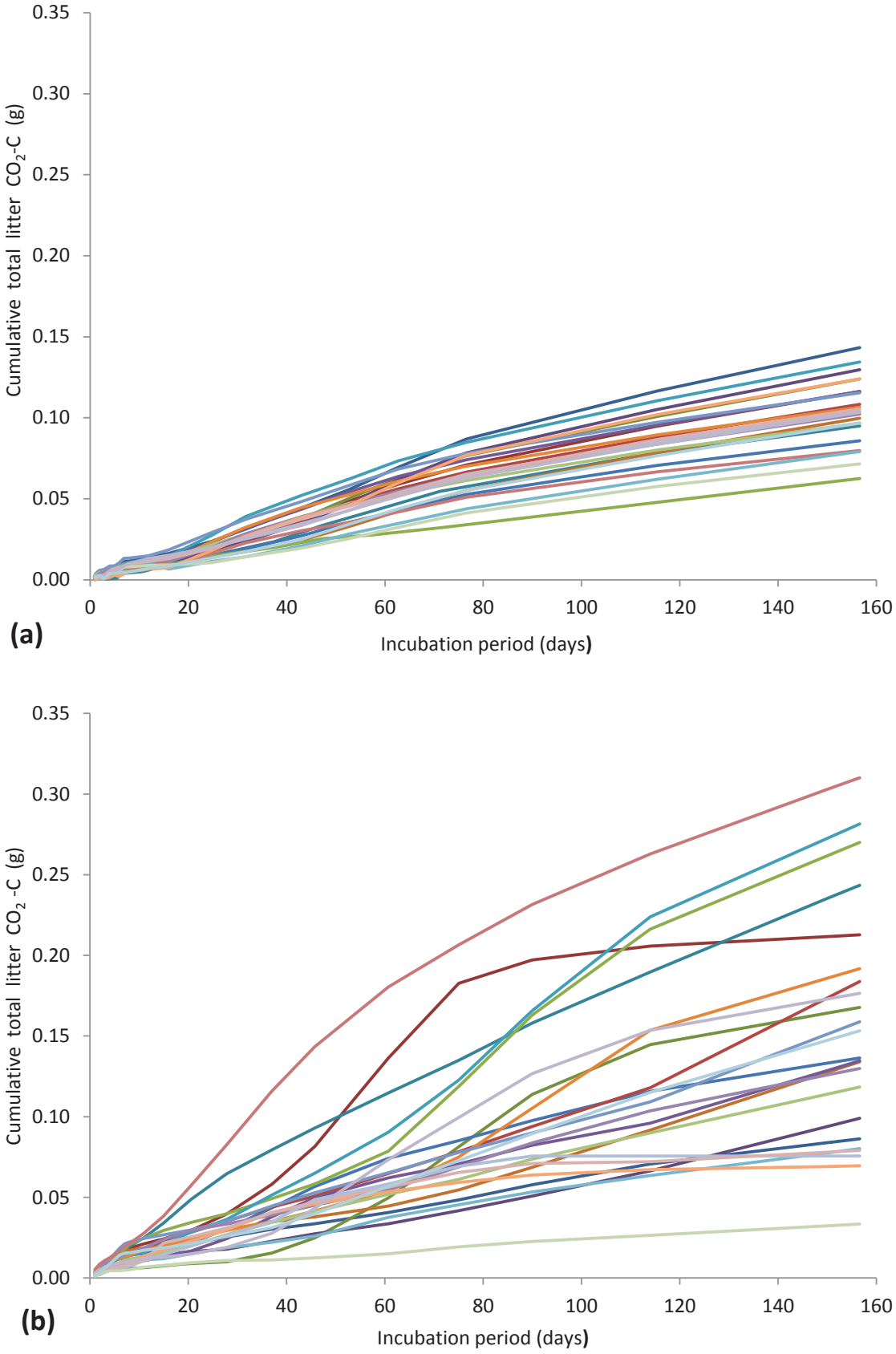


Figure 4: Summary display of the cumulative total litter CO_2-C produced over time for each of the 23 taxa for; **(a)** incubation of individual litters on a common soil, and **(b)** incubation of individual litters on their corresponding home site soil. Individual taxa are not indicated, but legend is present in Table 1.

General Linear Models of Litter Decomposition

A PCA ordination of litter quality variables and soil incubation variables displayed a strong litter N gradient occurring along axis 1 (Figure 5). Axis 1 explained 82% of the variation in the data, and axis 2 a further 13%. Litter N was the strongest parameter driving axis 1, with litter N opposed to litter C:N, with a strong negative correlation (R^2 = 0.89) occurring between the two. The remaining variables are distributed predominantly along axis 2, with the strongest variables driving axis 2 being soil C and N, and Soil water holding capacity, which are opposed by soil C decomposition.

Variables included in the models of common soil litter decomposition were the litter quality measures described in detail in *Chapter 2*. Using the general linear models, the strongest predictors of total litter decomposition on the common soil were litter lignin content and litter lignin:N ratio (Table 2). Lignin and lignin:N both had strong negative correlations (r = -0.60 and -0.62, respectively) with the total decomposition g/g. As general linear models, lignin and lignin:N were both strong and significant predictors of litter decomposition (AICc Δi <2, p = 0.003 & 0.002, respectively). Individually, acid detergent fibre (ADF), neutral detergent fibre (NDF), and C also showed strong to moderate correlations with litter decomposition rates (Table 2), with ADF and NDF positively correlating with decomposition, and C negatively correlating with decomposition rates.

Variables selected for inclusion in the models of home-site soil litter decomposition were soil C, Soil N, Soil C:N ratio, soil water holding capacity, soil C decomposition g/g, as well as the strongest litter quality parameter, the Lignin: N ratio (Figure 5). The resulting models showed Litter lignin:N to be a poor predictor of litter decomposition in the home-site soil trial.

Soil N was negatively correlated with home-site soil litter decomposition (r = -0.47) and was a moderate to strong predictor of total litter decomposition g/g. Soil C, the Soil C:N ratio, soil WHC, and soil C decomposition g/g were all individually strong and significant predictors of soil decomposition (R^2 = 0.31, 0.40, 0.36, & 0.33 respectively, p-values < 0.01; Table 2). Soil C, soil C: N, and WHC were all found to be negatively correlated with litter decomposition g/g. In addition, litter decomposition g/g was also found to be positively correlated with the soil C decomposition g/g. The strongest predictor model out of the candidate models for home-site soil litter decomposition was soil C:N in combination with soil C decomposition g/g (R^2 = 0.75,

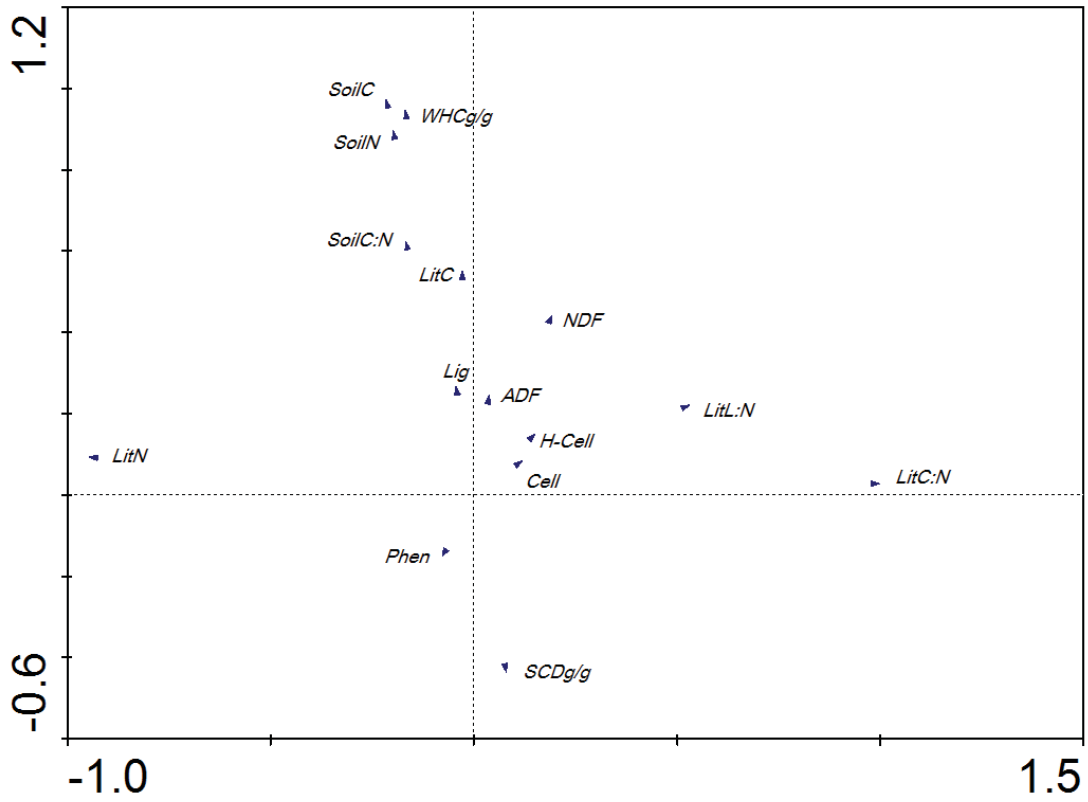


Figure 5: PCA ordination of litter quality parameters and soil quality parameters for each taxa and site. Litter parameters include: Total N (*Lit N*), C content (Lit C), Lignin (*Lig*), Acid detergent fibre (*ADF*), neutral detergent fibre (*NDF*), Cellulose (*Cell*), total phenolics (*Phen*), Hemicellulose (*H-cell*), Lignin to N ratio (*Lit L:N*), and C to N ratio (*Lit C:N*). Soil Parameters include: soil C (*Soil C*), Soil N (Soil N), Soil C to N ratio (*Soil C:N*), soil water holding capacity (*WHC*), and total g/g soil C decomposition (*SCD g/g*).

p = 0.0001), with an AICcW*i* of 0.93, indicating a 93% chance this is the most likely model for predicting home-site soil litter decomposition

General Linear Models for Soil C Decomposition

Soil nutrient levels were the strongest predictors of basal soil C decomposition in the home-site soils, with Soil C and N were found to be negatively correlated with soil C decomposition (Table 3). Both were equally good candidate models, each having a 42% probability that either model is the best model out of the candidate set, given the data used. The soil N model had a greater significance level than the soil C model (p = 0.02 & 0.03, respectively) and was a slighter better fit (R^2 = 0.232 & 0.205, respectively). Soil WHC also correlated negatively with soil C decomposition, and proved to be a potential predictor model, with a 12% probability it is the most likely

Table 2. Comparison of general linear models predicting the influence of litter quality on litter decomposition, and the influence of litter quality + soil type on litter decomposition. K= number of parameters in the model including the constant; AICc = Akaike's Information Criteria; AICc Δ*i* = difference in AICc score between model *i* and the best model out of the candidate models; AICc W*i* = Akaike model weight. All models generated are presented in the table, including non credible models. Strongest models within a candidate set (AICc Δ*i* <2) are shown in bold. * = *Bonferroni* significance to 0.1. ** = *Bonferroni* significance to 0.05.

Decomposition Substrate	Model	K	P	r	R^2	AICc	AICc Δ*i*	AICc W*i*
Common Soil (g/g)	NDF	2	0.079	0.37	0.14	-162.6	6.62	0.016
	ADF	2	0.011	0.52	0.27	-165.7	3.55	0.074
	HCEL	2	0.229	0.26	0.07	-161.2	8.01	0.008
	CELL	2	0.533	0.14	0.02	-159.9	9.33	0.004
	LIG	**2**	**0.003****	**-0.60**	**0.36**	**-169.2**	**0.00**	**0.435**
	PHEN	2	0.603	0.12	0.01	-159.9	9.33	0.004
	Lit C	2	0.081	-0.37	0.17	-162.6	6.62	0.016
	Lit N	2	0.446	0.17	0.03	-159.9	9.33	0.004
	Lit C:N	2	0.41	-0.18	0.03	-159.9	9.33	0.004
	Lit L:N	**2**	**0.002****	**-0.62**	**0.38**	**-169.2**	**0.00**	**0.435**
Home -Site Soil (g/g)	L L:N	2	0.890	0.03	0.00	-99.8	18.27	0.000
	SOC	2	0.006**	-0.56	0.31	-108.4	9.64	0.008
	SON	2	0.024	-0.47	0.22	-105.5	12.57	0.002
	SOCN	2	0.001**	-0.63	0.40	-111.3	6.74	0.032
	WHC	2	0.002**	-0.60	0.36	-110.1	7.94	0.018
	SODG	2	0.004**	0.57	0.33	-109.0	9.09	0.010
	SOCN+SODG	**3**	**0.0001****	**0.77**	**0.60**	**-118.0**	**0.00**	**0.931**

Table 3: Comparison of general linear models predicting the influence of soil characteristics on soil C decomposition. K= number of parameters in the model including the constant; AICc = Akaike's Information Criteria; AICc Δi = difference in AICc score between model i and the best model out of the candidate models; AICc Wi = Akaike model weight. All models generated are presented in the table, including non credible models. Strongest models within a candidate set (AICc Δi <2) are shown in bold.

Model	K	P	r	R2	AICc	AICc Δ*i*	AICc W*i*
Soil C	**2**	**0.030**	**-0.452**	**0.205**	**-175.86**	**0**	**0.417**
Soil N	**2**	**0.020**	**-0.482**	**0.232**	**-175.86**	**0**	**0.417**
Soil C:N	2	0.327	-0.21	0.458258	-171.24	4.62	0.042
WHC	2	0.072	-0.38	0.616441	-173.44	2.42	0.124

predictor model out of the model candidate set.

Soil C, N and WHC were all found to be highly correlated. In a Pearson correlation matrix test, soil C was strongly and positively correlated with soil N (r = 0.93, p = 0.0001) and with soil WHC (r = 0.95, p = 0.0001), as was soil N with soil WHC (r = 0.87, p = 0.0001; Table 4).

Table 4: Pearson's correlation matrix with corresponding p-values for site soil variables influencing decomposition. Significant relationships are indicated in bold, with additional significance for Bonferroni correction indicated. Soil carbon is equivalent to total organic C, whilst Soil N is equivalent to total soil N, both expressed as % oven dry mass. Water holding capacity (WHC) is expressed as g water retention per g of oven dry soil, whilst soil C decomposition is equal to basal soil C loss during incubation. * Significance to 0.1 Bonferroni adjusted equivalent** Significance to 0.05 Bonferroni adjusted equivalent

	Soil C (%)	Soil N (%)	Soil C:N	WHC (g/g)
	Pearson's *r*			
Soil N (%)	0.93			
Soil C:N	0.61	0.34		
WHC (g/g)	0.95	0.87	0.63	
Soil C Decomposition (g/g)	-0.45	-0.48	-0.21	-0.38
	p-value			
Soil N (%)	**0.0001****			
Soil C:N	**0.002****	0.1134		
WHC (g/g)	**0.0001****	**0.0001****	**0.0014****	
Soil C Decomposition (g/g)	**0.0303**	**0.0200***	0.3268	**0.0723**

Discussion

The temporal patterns observed here in the rate of litter decomposition are similar to those reported in other experiments incubating and amending soils with litter (Ross *et al.*, 1995; Saggar *et al.*, 1996; Ross *et al.*, 2003). The initial maximum spike in microbial activity occurring in both amended and un-amended common soils can be explained by disturbance to the soil when the litter was initially added. This mixing is likely to result in the addition of oxygen to the soil as well as turning over any organic matter, resulting in increased microbial activity. Fluxes in litter CO_2-C produced in the following days are likely due the metabolism of the litter soluble fraction, which is the

most readily decomposable fraction of the litter (Berg and McClaugherty, 2008), after which the rate of litter decomposition appears reduce.

Common Soil Litter Decomposition

In the common soil, taxa show two latter peaks in litter decomposition after initial fluxing at approximately days 30 and 60, before reaching a relatively constant rate of litter decomposition. These latter peaks are possibly explained by the eventual decomposition of the structural compounds cellulose and hemicellulose, which follows the initial rapid decomposition of the soluble fraction (Berg and Staaf, 1980). This change in the relative proportion of different litter components over time, results in a exponential decay curve for the litter (Berg and McClaugherty, 2008). Mass loss from the litter was still occurring after 150 days of incubation, indicating the litters have not reached their asymptotical limit values, as described by Berg *et al.* (2010).

Chionochloa litter was found to have a very slow and constant rate of decomposition on the common soil. After 156 days (5.2 months) of incubation in conditions ideal for microbial decomposition, mass loss of *Chionochloa* litter C only ranged from 8 - 20%. Taylor *et al.* (1989) found that mass loss of varying of types of montane litter, when incubated for 4 months at a constant 26˚C, was equivalent to 1.5 – 2 years of mass loss in the field. As *Chionochloa* litter here was incubated at a similar temperature (25˚C), it is suggested the approximately 5.2 months of incubation may be equivalent to a much longer time period under field conditions, potentially 2-3 years using the scalar above. Many *Chionochloa* taxa also inhabit alpine environments, which is also likely to further lower rates of field decomposition relative to the laboratory incubation, due to much cooler temperatures occurring at these sites in both summer and winter.

In a field decomposition experiment, Williams *et al.* (1977) found similar trends in the decomposition of *C. rigida* and *C macra*, with slow rates of decomposition occurring in the field, and estimated leaf C turnover times in *C. rigida* litter ranging from 6.7 years to up to 21 years. Krna (2015) found litter mass loss to decline over time in *C. rubra* and *C. pallens* during decomposition, with mass loss of 46% in *C. rubra* over 3 years, and mass loss in *C. pallens* of 20 to 30% over two years, again suggesting the incubation of *Chionochloa* litter in this experiment could be equivalent to 2-3 years of field decomposition.

Litter Quality as a Predictor of Litter Decomposition

Of the litter quality variables measured, only a few were found to have an influence on litter decomposition on the common soil. Despite large variation between taxa in leaf N content, C:N, and total phenolics, as described in *Chapter 2*, none of these litter quality measures were found to influence the rate of litter decomposition. Contrary to what was hypothesized, litter fibre measures NDF and ADF showed moderate positive correlations with litter decomposition, despite being associated with increased leaf toughness and reduced decomposition (Ruffo and Bollero, 2003). However, components of plant fibre, cellulose and hemicellulose, can be broken down into simpler sugars by microorganisms (Pérez *et al.*, 2002) then and utilized as an energy source during the secondary phase of decomposition (Berg and McClaugherty, 2008).

Lignin proved to be a strong and significant predictor of litter decomposition in the genus *Chionochloa*, as also found in many other litter decomposition experiments (Meentemeyer, 1978; Melillo *et al.*, 1982; Aerts, 1997). Furthermore, of all the litter quality measures recorded, lignin and lignin:N were the only significant predictors of litter decomposition in the *Chionochloa* genus. These results may be explained by the particularly low litter N content for all *Chionochloa* taxa, unalleviated by apparent differences in their C:N ratios. Taylor *et al.* (1989) found C:N ratios to be better predictors of decomposition in litters with low lignin content and high nutrient content, and conversely Lignin:N ratios to be better predictors of decomposition in litters with high levels of lignin content. These results suggest lignin is the dominant controller of litter decomposition in *Chionochloa*, possibly as a result of the generically low litter N content found in *Chionochloa*.

In addition to lignin, the lignin:N ratio was also a significant predictor of the rate of litter decomposition. This may be due to the strong positive correlation between lignin and lignin:N, suggesting the decomposition relationship is predominantly driven by lignin. However, there is evidence for interactions between lignin and N during litter decomposition. Litter with greater litter N has been found to increase rates of litter decomposition in the initial phase of decomposition (Melillo *et al.*, 1982; Hobbie, 2005) and in some cases increase lignin decomposition in the early stages as well (Hobbie, 2000). Thus, it is possible lower leaf N may have resulted in lower rates of lignin decomposition, and thus lower overall decomposition.

Home-Site Soil Litter Decomposition

Litter decomposition on its corresponding home-site soil showed a similar trend in initial litter CO_2-C production, as describe above for litter decomposition on the common soil. However, litter decomposition rate between taxa was highly variable after this, with no common pattern observable between taxa. Notable differences from decomposition on the common soil occurred in rate of litter CO_2-C production over time, with some taxa showing early rapid rates of decomposition followed by a rapid reduction in decomposition and other taxa appearing to reach their asymptotical limit value for decomposition by the end of the incubation. In contrast some taxa appear to have a relatively constant rate of decomposition, with others still increasing in rate of litter decomposition at 150 days of incubation. The rapid litter decomposition occurring in some soils is likely due to ideal conditions for decomposition, which may include a suitable microbial community, adequate soil moisture, and adequate resources for microorganisms (Couteaux *et al.*, 1995).

Notably, for most taxa, litter decomposition on its home site soil was also greater than on the common soil. The common soil basal rate of soil C decomposition was close to the median of the home-site soils, with a decomposition *k* (0.0003) similar to the mean *k* (0.0004, SE= 0.00003). This would suggest that the increase in litter decomposition for most litters when incubated on their home site soil may not necessarily be due to decomposition occurring on a soil with more microbial activtiy, but due to some other characteristic associated with home-site soils. This may be due to relationships between site litter and site soil microbial community. Strickland *et al.* (2009) found that microbial communities that shared a common history with a litter type had greater rates of decomposition compared to those microbial communities foreign to a litter type, suggesting that microbial communities may be adapted to their site litter source and have a functional significance. When these results are compared with decomposition of corresponding litters on the common soil, it is clear that soil characteristics appear to have a large influence not only on the amount of total decomposition, but also on the rate of decomposition over time.

Soil Characteristics as Predictors of Litter Decomposition

Whilst litter quality measures could be used to predict the rate of litter decomposition on the common soil (where soil characteristics were standardised), they were found not to influence litter decomposition on the home-site soils (where soil

characteristics were variable). Instead, soil characteristics were found to be the strongest predictors of litter decomposition, likely due to a large range in soil types resulting from the wide geographic range samples were collected from, and differences in habitat between taxa in *Chionochloa* (Connor, 1991).

Whilst lignin and lignin:N levels in *Chionochloa* litter appear to determine the baseline rate of litter decomposition, as in the common soil trial, soil characteristics appear to have a dominant and superseding influence over litter quality in litter decomposition. These findings are supported by other reports in the literature, where litter quality has been suggested to have the potential to influence the rate of litter decomposition, but rates of litter decomposition are instead largely determined by variability in abiotic and biotic conditions resulting from environmental heterogeneity (Sariyildiz and Anderson, 2003; Sariyildiz *et al.*, 2005; Rejmánková and Houdková, 2006).

For home-site soil litter decomposition, organic soil C, soil N, soil C:N, soil WHC, and basal soil C decomposition were all strong and significant predictors of litter decomposition. The positive correlation found to occur between basal soil C decomposition and litter C decomposition suggests that soils with greater rates of microbial activity also had greater rates litter decomposition. This is supported by McGuire and Treseder (2010) who found increased microbial biomass to be linked to increased rates of decomposition. Organic soil C, soil N, soil C: N, and soil WHC were all found to negatively influence rates of litter decomposition. These results are contrasting to those expected, as greater soil organic C and N should provide greater available resources for microorganisms, increasing microbial biomass and activity, and hence increasing decomposition (McGuire and Treseder, 2010).

In this experiment, as the moisture content of each soil was set to 70% of its WHC, soils with a larger WHC will thus have been incubated with a higher soil water content. The negative relationships occurring above may be due to the strong positive correlations between soil WHC (indicating soil water content), and soil organic C and N. These can be explained by the potential of soil organic matter to greatly increase the WHC of that soil (Hudson, 1994). Reduced litter decomposition is thus not the result of microbial resource and nutrient limitation, but rather through possible unfavourable conditions associated with increased water content. Similarly, organic soil C, soil N, and soil WHC were all strongly and significantly correlated with the basal rate of soil C

decomposition in home site soils. Thus, it is not surprising that the strongest predictive model for litter decomposition was a combination of soil C:N and soil C decomposition.

It is proposed that the decrease in both basal soil C and litter C decomposition associated with increasing WHC is due to an increase in soil water content, resulting in anoxic conditions for microorganisms. Whilst soil moisture can be limiting in decomposition (Aerts, 1997), an excess of water can lead to greatly reduced rates of decomposition through the production of anoxic conditions (Laiho, 2006). Tate *et al.* (2000) also attributed relatively slow rates of litter decomposition in *C. pallens* tussock grassland to a high water holding potential in the grassland soils, and resulting anoxic conditions. A positive feedback may also be occurring, where greater soil water content results in reduced decomposition, resulting in build up organic matter in the soil, which results in the potential to hold more water in the soil.

Conclusions, Implications, and Limitations

Results from the common soil experiment suggest that when soil characteristics are similar, litter quality determines the rate of litter decomposition. In the *Chionochloa* genus, lignin was the primary determinant of litter decomposition. The relatively slow and constant rates of decomposition in *Chionochloa* litter are most likely due to low leaf N content, and the control of decomposition rates by the recalcitrant polymer lignin. Whilst litter quality has the potential to influence rates of litter decomposition, soil characteristics were ultimately found to have a greater and overriding influence on litter decomposition. Soil characters that found to have the largest influence on litter decomposition were soil WHC (i.e. water content) and organic matter, as well as soil microbial activity, indicated by increased basal soil C decomposition. These results suggest that litter decomposition and consequent C storage in the soil is more heavily influence by site characters and environment than by litter quality.

It may be difficult to apply these results to litter decomposition in the field, due to larges differences in temperature, moisture, climate, and disturbance regimes between a controlled laboratory experiment and field conditions. However, comparisons with rates of decomposition measured by Krna (2015) for corresponding taxa suggest decomposition from the incubation in this experiment may be equal to 2-3 years decomposition in field conditions. Decomposition in the field is likely to be lower higher altitudes relative to lowland sites, due to a longer duration of snow lie and winter conditions and cooler summer temperatures. In addition, the WHC of a soil may not be

a true measure of the soils field moisture regimes, as some sites may be considerably wetter, or drier, than others, which is further likely to influence decomposition.

To accurately budget for C sequestration it is recommended multi-year field decomposition experiments be used, due to the slow rate of decomposition in *Chionochloa* and to take into account the influence of soil characteristics and climate on litter decomposition. However, the results from this experiment give a measure of the differences in rates of litter decomposition occurring between closely related taxa, and the relative influences of both litter quality and soil characteristics on these rates of litter decomposition. It is suggested that *Chionochloa* has the potential to sequester C, due to the production of a slowly decomposing litter. These results also suggest that litter quality is likely to determine the rate of decomposition in environments that have similar climates and soil characteristics, whilst in environments with greater variability in climate and soil characteristics, decomposition will instead be dominated by these characteristics.

References

Adl, S. M. 2003. *The ecology of soil decomposition*, CABI.

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos*, 439-449.

Alavoine, G., Houlbert, J.-C. & Nicolardot, B. 2008. Comparison of three methods to determine C decomposition of organic materials in soils under controlled conditions. *Pedobiologia,* 52, 61-68.

Berg, B., Davey, M., De Marco, A., Emmett, B., Faituri, M., Hobbie, S., Johansson, M.-B., Liu, C., Mcclaugherty, C. & Norell, L. 2010. Factors influencing limit values for pine needle litter decomposition: a synthesis for boreal and temperate pine forest systems. *Biogeochemistry,* 100, 57-73.

Berg, B. & Mcclaugherty, C. 2008. Plant litter. *Decomposition, humus formation, carbon sequestration. 2nd Ed Springer*.

Berg, B., Mcclaugherty, C. & Johansson, M.-B. 1993. Litter mass-loss rates in late stages of decomposition at some climatically and nutritionally different pine sites. Long-term decomposition in a Scots pine forest. VIII. *Canadian Journal of Botany,* 71, 680-692.

Berg, B. & Staaf, H. 1980. Decomposition rate and chemical changes of Scots pine needle litter. II. Influence of chemical composition. *Ecological Bulletins*, 373-390.

Bernal, M., Sanchez-Monedero, M., Paredes, C. & Roig, A. 1998. Carbon mineralization from organic wastes at different composting stages during their incubation with soil. *Agriculture, Ecosystems & Environment,* 69, 175-189.

Cadisch, G. & Giller, K. E. 1997. *Driven by nature: plant litter quality and decomposition*, CAB international.

Carreiro, M., Sinsabaugh, R., Repert, D. & Parkhurst, D. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology,* 81, 2359-2365.

Collins, H., Rickman, R., Elliott, L., Bezdicek, D. & Papendick, R. 1990. Decomposition and interactions among wheat residue components. *Soil Science Society of America Journal,* 54, 780-785.

Connor, H. 1991. Chionochloa Zotov (Gramineae) in New Zealand. *New Zealand journal of botany,* 29, 219-282.

Couteaux, M.-M., Bottner, P. & Berg, B. 1995. Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution,* 10**,** 63-66.

Eiland, F., Klamer, M., Lind, A.-M., Leth, M. & B♣th, E. 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microbial Ecology,* 41**,** 272-280.

Guerriero, G., Hausman, J. F., Strauss, J., Ertan, H. & Siddiqui, K. S. 2016. Lignocellulosic biomass: Biosynthesis, degradation, and industrial utilization. *Engineering in Life Sciences,* 16**,** 1-16.

Heal, O., Anderson, J. & Swift, M. 1997. Plant litter quality and decomposition: an historical overview. *Driven by nature: plant litter quality and decomposition*.

Hesse, P. R. 1971. A textbook of soil chemical analysis.

Hewitt, A. E. 1998. *New Zealand soil classification*, Manaaki Whenua Press.

Hobbie, S. E. 2000. Interactions between litter lignin and nitrogenitter lignin and soil nitrogen availability during leaf litter decomposition in a Hawaiian montane forest. *Ecosystems,* 3**,** 484-494.

Hobbie, S. E. 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. *Ecosystems,* 8**,** 644-656.

Hopkins, D. W. 1993. Carbon Mineralization. *Soil Sampling and Methods of Analysis.* 2nd Edition ed.

Hudson, B. D. 1994. Soil organic matter and available water capacity. *Journal of Soil and Water Conservation,* 49**,** 189-194.

Klute, A. 1986. Water retention: laboratory

methods. *Methods of soil analysis. Part 1. Physical and mineralogical methods.* 2nd Edition ed.

Krna, M. A. 2015. *Productivity, decomposition and carbon sequestration of Chionochloa species across altitudinal gradients in montane tussock grasslands of New Zealand.* PhD, Massey Univeristy, New Zealand.

Laiho, R. 2006. Decomposition in peatlands: Reconciling seemingly contrasting results on the impacts of lowered water levels. *Soil Biology and Biochemistry,* 38**,** 2011-2024.

Lavelle, P. & Dickinson, R. 1987. Biological processes and productivity of soils in the humid tropics. *The Geophysiology of Amazonia: Vegetation and Climate*.

Lavelle, P. & Spain, A. 2001. *Soil ecology*, Springer Science & Business Media.

Martin, J. & Haider, K. 1980. Microbial degradation and stabilization of 14C-labeled lignins, phenols, and phenolic polymers in relation to soil humus formation. *Lignin biodegradation: microbiology, chemistry, and potential applications.*

Mcguire, K. L. & Treseder, K. K. 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biology and Biochemistry,* 42**,** 529-535.

Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecology,* 59**,** 465-472.

Melillo, J. M., Aber, J. D., Linkins, A. E., Ricca, A., Fry, B. & Nadelhoffer, K. J. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. *Ecology of Arable Land—Perspectives and Challenges.* Springer.

Melillo, J. M., Aber, J. D. & Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology,* 63**,** 621-626.

Percival, H. J., Parfitt, R. L. & Scott, N. A. 2000. Factors Controlling Soil Carbon Levels in New Zealand Grasslands Is Clay Content Important? *Soil Science Society of America Journal,* 64**,** 1623-1630.

Pérez-Harguindeguy, N., Díaz, S., Cornelissen, J. H., Vendramini, F., Cabido, M. & Castellanos, A. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. *Plant and Soil,* 218**,** 21-30.

Pérez, J., Munoz-Dorado, J., De La Rubia, T. & Martinez, J. 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *International Microbiology,* 5**,** 53-63.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry,* 101**,** 133-149.

Rejmánková, E. & Houdková, K. 2006. Wetland plant decomposition under different nutrient conditions: what is more important, litter quality or site quality? *Biogeochemistry,* 80**,** 245-262.

Ross, D., Tate, K. & Newton, P. 1995. Elevated CO2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoaquept soil. *Plant and soil,* 176**,** 37-49.

Ross, D., Tate, K., Newton, P. & Clark, H. 2003. Carbon mineralization in an organic soil, with and without added grass litter, from a high-CO 2 environment at a carbon dioxide spring. *Soil Biology and Biochemistry,* 35**,** 1705-1709.

Ruffo, M. L. & Bollero, G. A. 2003. Residue decomposition and prediction of carbon and nitrogen release rates based on biochemical fractions using principal-component regression. *Agronomy Journal,* 95**,** 1034-1040.

Saggar, S., Parshotam, A., Sparling, G., Feltham, C. & Hart, P. 1996. 14 C-labelled ryegrass turnover and residence times in soils varying in clay content and mineralogy. *Soil Biology and Biochemistry,* 28**,** 1677-1686.

Sariyildiz, T. & Anderson, J. 2003. Interactions between litter quality, decomposition and soil fertility: a laboratory study. *Soil Biology and Biochemistry,* 35**,** 391-399.

Sariyildiz, T., Anderson, J. & Kucuk, M. 2005. Effects of tree species and topography on soil chemistry, litter quality, and decomposition in Northeast Turkey. *Soil Biology and Biochemistry,* 37**,** 1695-1706.

Soong, J. L., Parton, W. J., Calderon, F., Campbell, E. E. & Cotrufo, M. F. 2015. A new conceptual model on the fate and controls of fresh and pyrolized plant litter decomposition. *Biogeochemistry,* 124**,** 27-44.

Strickland, M. S., Lauber, C., Fierer, N. & Bradford, M. A. 2009. Testing the functional significance of microbial community composition. *Ecology,* 90**,** 441-451.

Tate, K., Scott, N., Ross, D., Parshotam, A. & Claydon, J. 2000. Plant effects on soil carbon storage and turnover in a montane beech (Nothofagus) forest and adjacent tussock grassland in New Zealand. *Soil Research,* 38**,** 685-697.

Taylor, B. R., Parkinson, D. & Parsons, W. F. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology,* 70**,** 97-104.

Team, R. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-0.

Ter Braak, C. J. & Smilauer, P. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Van Der Putten, W. H. & Wall, D. H. 2004. Ecological linkages between aboveground and belowground biota. *Science,* 304**,** 1629-1633.

Waterman, P. G. & Mole, S. 1994. *Analysis of phenolic plant metabolites*, Blackwell Scientific.

Wilkinson, L. 1992. *SYSTAT for Windows: statistics, graphics, data, getting started, version 5*, Systat.

Williams, P., Nes, P. & O'connor, K. 1977. Macro-element pools and fluxes in tall-tussock (Chionochloa) grasslands, Canterbury, New Zealand. *New Zealand journal of botany,* 15**,** 443-476.

Zech, W., Senesi, N., Guggenberger, G., Kaiser, K., Lehmann, J., Miano, T. M., Miltner, A. & Schroth, G. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma,* 79**,** 117-161.

Zhang, D., Hui, D., Luo, Y. & Zhou, G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology,* 1**,** 85-93.

Appendix 1: Summary of the soil characters for site soils and the common soil used in litter incubation. Organic C and N are expressed as percent oven dry mass, whilst water holding capacity (WHC) is equivalent to grams of water retention per g of oven dry soil. Soil C loss is the equivalent to g soil C decomposition per g of soil C added, whilst soil *K* indicates the rate of soil C decomposition.

Taxa	Organic C (%)	Total N (%)	C:N	WHC (g/g)	Soil C loss (g/g)	Soil *K*
C. australis	8.2	0.57	14.2	1.37	0.097	0.00069
C. conspicua ssp. *cunninghamii*	16.9	0.82	20.7	1.59	0.048	0.00033
C. crassiuscula ssp. *crassiuscula*	22.7	1.22	18.6	3.43	0.057	0.00039
C. crassiuscula ssp. *directa*	50.6	1.55	32.6	6.79	0.040	0.00027
C. crassiuscula ssp. *torta*	20.8	1.22	17.0	3.43	0.053	0.00037
C. defracta	15.6	1.12	14.0	1.70	0.043	0.00029
C. flavescens ssp. *lupeola*	12.5	0.66	18.9	1.97	0.062	0.00042
C. juncea	4.7	0.22	21.2	1.21	0.098	0.00066
C. lanaea	7.3	0.35	20.7	0.75	0.053	0.00037
C. macra	3.7	0.23	15.7	0.46	0.068	0.00046
C. pallens ssp. *cadens*	16.1	0.97	16.5	2.26	0.044	0.00030
C. pallens ssp. *pallens*	24.8	1.19	20.8	2.77	0.056	0.00038
C. pallens ssp. *pilosa*	8.4	0.50	16.9	0.95	0.069	0.00047
C. rigida ssp. *amara*	12.3	0.75	16.5	1.58	0.039	0.00026
C. rigida ssp. *amara (SI)*	15.0	0.69	21.8	1.98	0.074	0.00052
C. rigida ssp. *rigida*	5.2	0.30	17.2	0.73	0.056	0.00038
C. rubra ssp. *cuprea*	49.2	2.18	22.6	4.64	0.030	0.00021
C. rubra ssp. *occulta*	7.9	0.47	17.0	0.82	0.047	0.00030
C. rubra ssp. *rubra* var. *inermis*	23.6	1.14	20.8	2.93	0.099	0.00072
C. rubra ssp. *rubra* var. *rubra*	7.7	0.29	26.5	1.16	0.056	0.00039
C. spiralis	5.5	0.40	13.9	0.99	0.066	0.00045
C. teretifolia	25.5	1.34	19.0	3.53	0.042	0.00029
C. vireta	6.4	0.40	16.1	0.88	0.124	0.00089
Common soil	8.3	0.52	16.1	2.43	0.050	0.00034
Mean	15.8	0.80	19.0	2.10	0.061	0.0004
SE	2.56	0.10	0.86	1.48	0.005	0.0000

Chapter 5

Synthesis and Discussion:

Are low-producing plants sequestering carbon at a greater rate than high-producing plants?

Chionochloa australis in full flower, Poplars Range – M. Dickson

Introduction

Carbon (C) sequestration within an ecosystem is determined by the balance between rates of productivity and decomposition (Bradley and Pregitzer, 2007). C sequestration, or storage of C in the soil, occurs when the ratio of the total C fixed (productivity) to total C released (decomposition), within a system over a set time period, is greater than one (Krna and Rapson, 2013). As discussed in the previous chapters, there is evidence that the processes of plant productivity and litter decomposition are interrelated. Whilst there is evidence for a positive correlation between rates of productivity and litter decomposition (Coley *et al.*, 1985; Poorter *et al.*, 1990; Poorter and Bergkotte, 1992; Cornelissen *et al.*, 2004), the magnitude of this relationship and its influence on C sequestration is not as well understood. In particular, if litter decomposition is greatly reduced relative to productivity in low-producing plants, then there is the potential for low-producing plants to sequester C at equal or greater rates relative to higher producing plants.

This chapter aims to summarise and synthesise the relationships between productivity and decomposition in the genus *Chionochloa*. Ratios of productivity to decomposition are created to investigate the relative influence of productivity on C sequestration. It is hypothesised litter decomposition will be positively correlated with the rate at which litter is produced. As a result, the rate of C sequestration in low-producing plants could be equal to, or potentially greater than, that occurring in high-producing plants.

Synthesis of Findings in Chionochloa

A review of the literature in previous chapters reported strong links between environment, productivity, litter quality, and decomposition for a number of different taxa. The results found here in the genus *Chionochloa* show environment to be a strong predictor of productivity. Higher values of productivity were correlated with warmer temperatures, greater rainfall, and lower altitudes. Similarly, some measures of litter quality in *Chionochloa* could also be predicted by the environment in which the litter was produced. Litter N content was found to be lower at higher altitudes, whilst NDF, cellulose, and litter C were all greater at sites with warmer temperatures. Furthermore, Leaf N, cellulose, and NDF were also found to directly correlate with measures of productivity. Other measures of litter quality showed no relationship to environment or productivity, though some, i.e. hemicellulose, lignin and ADF, could be explained by

genetic relatedness. These measures of litter quality all correlated strongly with rates of leaf turnover, with hemicellulose positively correlated with leaf C lability and litter production, and lignin and ADF displaying negative correlations with both leaf C lability and litter production.

Whilst environment and productivity were found to influence litter quality, this did not translate to differential rates in litter decomposition. Instead, litter decomposition was strongly and negatively correlated with lignin content and the lignin:N ratio, which were found to be related to phylogeny. This suggests phylogeny has a greater influence on litter decomposition than the environment in which, and rate at which, the litter was produced. While lignin was a strong predictor of litter decomposition on the common soil, when decomposition occurred on home-site soils lignin had no significant influence on the rate of litter decomposition, suggesting soil characteristics have a greater and overruling influence on decomposition. These results show that environment, productivity, and phylogeny are able to influence plant litter quality, and that this litter quality then determines the rate of litter decomposition, when soil characteristics are similar.

P:D Ratios and C Sequestration

To test for relationships between productivity and decomposition, measures of productivity were first correlated against their equivalent measure of decomposition (Table 1). The total amount of decomposition per tiller and per plant for each taxon was taken as the mean total litter production per tiller or plant, multiplied by its corresponding rate of litter decomposition.

The rates of carbon sequestration for each of the taxa were calculated using productivity to decomposition (P:D) ratios (Kirschbaum, 2000; Krna, 2015). These P:D ratios were then plotted against each of their corresponding productivity measures to test the proposed relationship between productivity and C sequestration. Linear regressions were fitted to display the strength of these relationships. Where curvature occurred, second order polynomials were also fitted. As leaf C lability and litter production were calculated in grams per gram of live biomass, they were correlated against the P:D ratios calculated for the live biomass productivity measure (Table 1).

Table 1: Productivity measures and their corresponding measures of decomposition used to calculate P:D ratios. Incubation time (*t*) is equal to 156 days of incubation for common and home site soil decomposition. RGR-L = relative growth rate of live biomass. RGR-G = relative growth rate assuming no dieback. Productivity measures are fully described in *Chapter 3.*

Productivity Measure	Units	Decomposition Measure	Units
Tiller	g / tiller / year	C mass loss	g / tiller / *t*
Plant	g / plant / year	C mass loss	g / plant / *t*
Per Live Biomass	g / g live biomass / year	C mass loss	g / g live biomass/ *t*
RGR-L	-	*k*	-
RGR-G	-	*k*	-
Leaf C Lability	g / g live biomass / year	C mass loss	g / g live biomass/ *t*
Litter Production	g / g live biomass / year	C mass loss	g / g live biomass/ *t*

Relationships between Productivity and Decomposition

Litter decomposition per tiller and per plant was found to increase linearly with productivity per tiller and productivity per plant (Figure 1; a-d). This correlation was stronger for both of these productivity measures when decomposition occurred on the common soil (R^2 = 0.71 and 0.88 respectively) as opposed to decomposition on the site soil (R^2 = 0.51, and 0.82 respectively). Conversely, relationships between rates of productivity and decomposition were much weaker, with poor correlations occurring for all measures (Figure 1; e-j). When decomposed on the common soil, higher productivity per gram of live biomass and higher RGR-G's appeared to be correlated with higher rates of decomposition, whilst the lowest rates of decomposition were found to occur in taxa with the slowest rates of growth rates (Figure 1; e & i). However, overall these correlations were weak (R^2 = 0.08 and 0.10, respectively). When considering litter decomposition on home site soils, relationships between decomposition and measures of growth rate were even weaker, with a greater spread in P:D ratios occurring and no distinguishable relationships (Figure 1; f, h & j).

Relationships between measures of leaf turnover (leaf C lability and rate of litter production) and litter decomposition were similar to those described for relative growth rate. For the common soil trial, higher rates of leaf C lability and litter production generally resulted in the higher rates of decomposition (Figure 1; k & m), though overall these relationships were weak (R^2 = 0.15 and 0.08, respectively). On home site

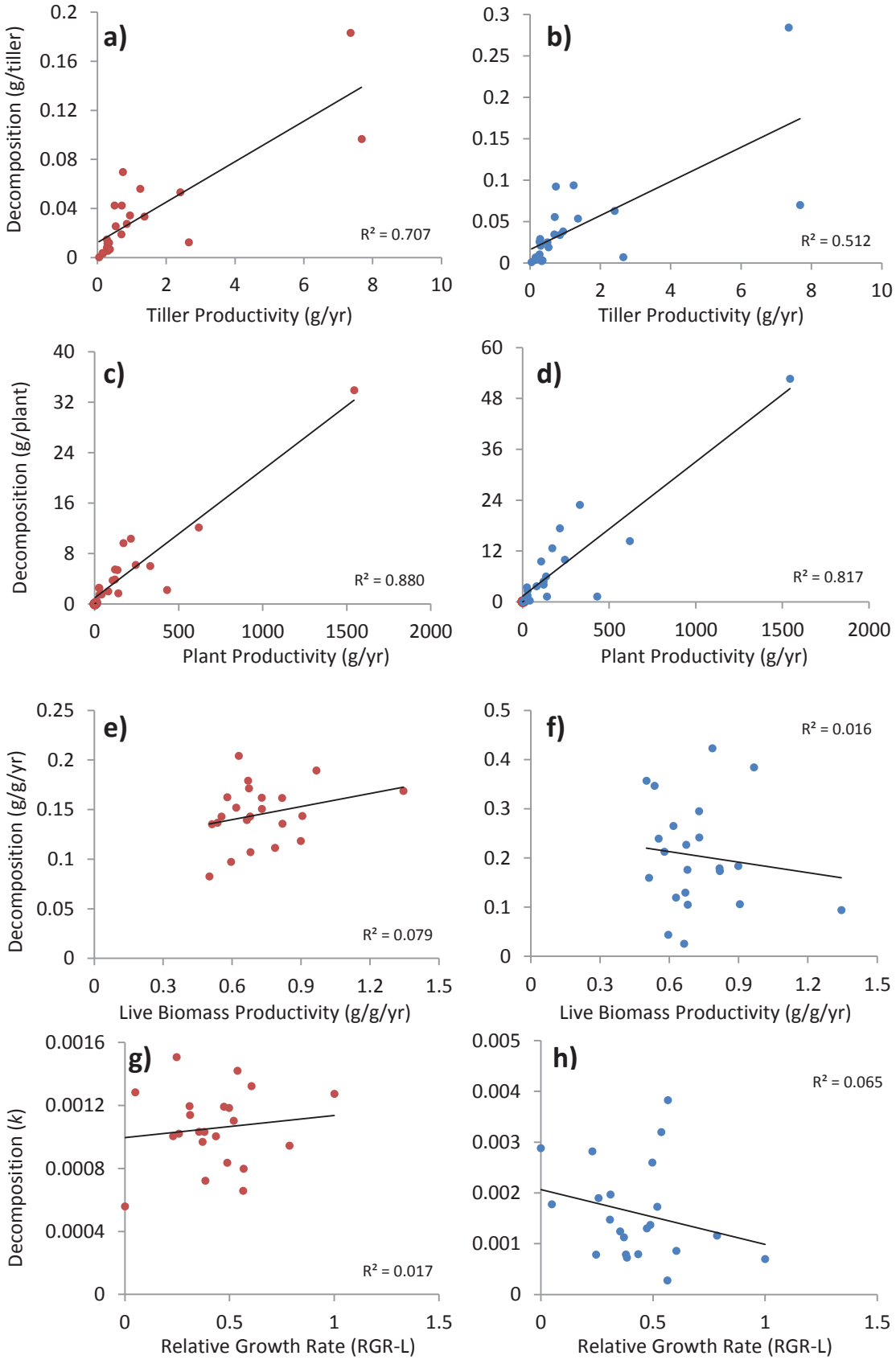


Figure 1: Correlations between productivity and decomposition for taxa in the genus *Chionochloa* (n = 22). Relationships are reported for all measures of productivity and leaf turnover (as outlined in Table 1). Litter decomposition on common soil is displayed in the left column (Red dots), with litter decomposition on home-site soils displayed in the right column (Blue dots). Figure 1 (i – n) continued below.

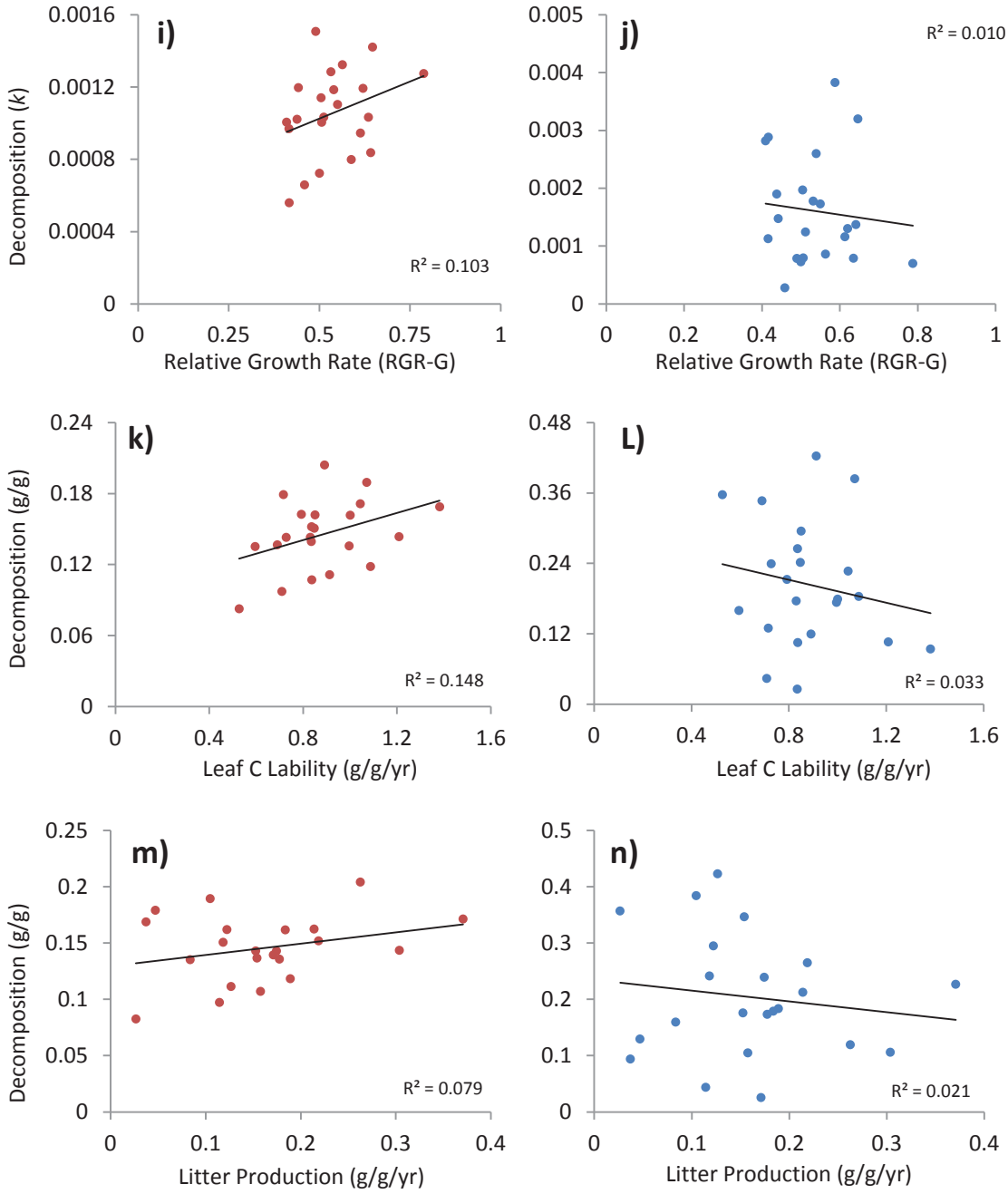


Figure 1 continued: Litter decomposition on common soil is displayed in the left column (Red dots), with litter decomposition on home-site soils displayed in the right column (Blue dots).

soils, these trends were negative, though these relationships even more variable and weaker when compared to the common soil (R^2 = 0.03 and 0.02 respectively).

Relationships between Productivity and C sequestration

The positive linear correlations between productivity and decomposition per tiller and per plant resulted in similar rates of C sequestration for both low- and high-producing tillers and plants (Figure 2; a-d). These relationships were true for

decomposition occurring on both the common soil and home site soils, However there were notable outliers in each case. In the common soil trial, the two outliers were *C. rubra* ssp. *cuprea* and *C. australis* (Figure 2; a & c). For *C. australis,* the high P:D ratio on the common soil can be attributed to a combination of a slow rate of decomposition as well as low litter production per tiller and per plant. The low litter production in *C. australis* may be attributed to the retention of dead leaves on the tiller for longer than a year, and thus were not recorded as litter fall in this study. *C. rubra* ssp. *cuprea* also had a much greater P:D, though not due to a slow rate of decomposition, but solely due to much lower litter production relative to other taxa. Again, the low litter production in *C. rubra* ssp. *rubra* may be due to dead leaves persisting on the tiller for longer than a year, resulting in an apparently low annual litter production, as measured here.

In the home-site soils, *C. rubra* ssp. *cuprea* was again an outlier (Figure 2; b & d), due to low total litter decomposition associated with low litter production. The P:D ratio for *C. australis* on its home site soil was similar to the majority of other taxa, despite low litter production, due to an increase in the rate of litter decomposition on its home site soil, relative to the common soil. This increase in decomposition on its home site soil may be due the functional significance of the soil microbial community in litter decomposition. Strickland *et al.* (2009) found that microbial communities that shared a common history with a litter type had greater rates of decomposition compared to those foreign to a litter type, suggesting microbial communities may be specific to certain litter types.

For a few taxa (*C. crassiuscula* ssp. *directa*, *C. crassiuscula* ssp. *crassiuscula,* and *C. conspicua*) P:D ratios were notably greater on home site soil than on the common soil, indicating reduced rates of decomposition on their home site soils relative to the common soil. These reduced rates of decomposition may be linked to greater soil water content occurring in these home site soils (soils ranked 1, 3, and 7 in water content, respectively), resulting in anoxic conditions for microorganisms.

Both tiller and plant productivity appear to have no relationship with C sequestration (Figure 2; a-d), due to the strong positive correlations between tiller/plant productivity and total decomposition (Figure 1; a-d). Measures of growth rate were found to be positively correlated with P:D ratios, on the common soil (Figure 2; e-j). Productivity per gram of live biomass, RGR-L, and RGR-G all showed moderate to weak positive correlations with P:D ratios on the common soil (R^2 = 0.44, 0.78, and

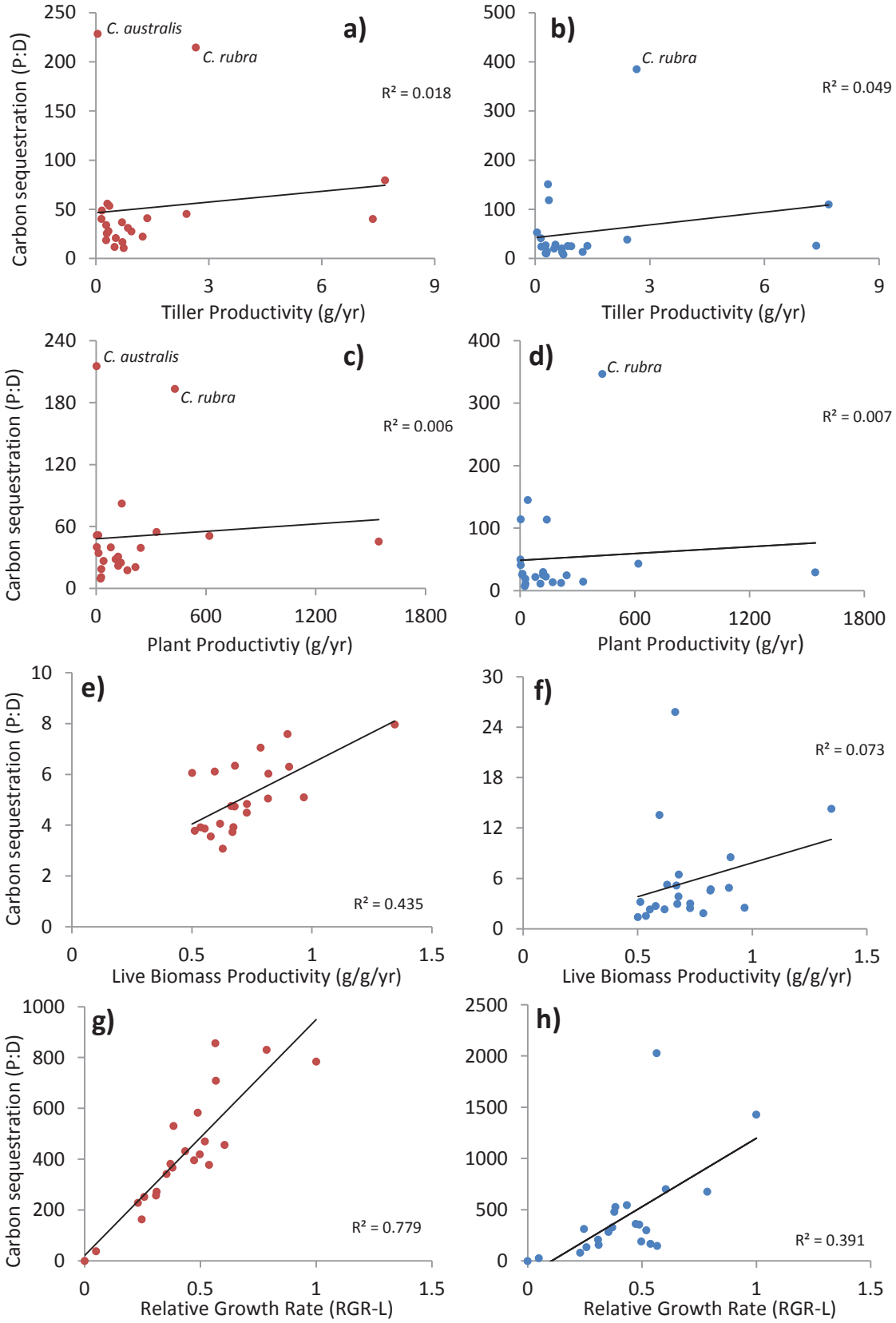


Figure 2: Correlations between productivity and C sequestration, as indicated by a P: D ratio for each *Chionochloa* taxon (n = 22). Measures of productivity and equivalent decomposition measure used to created P:D ratios are listed in Table 2. Litter decomposition on common soil is displayed in the left column (Red dots), with litter decomposition on home-site soils displayed in right column (Blue dots).

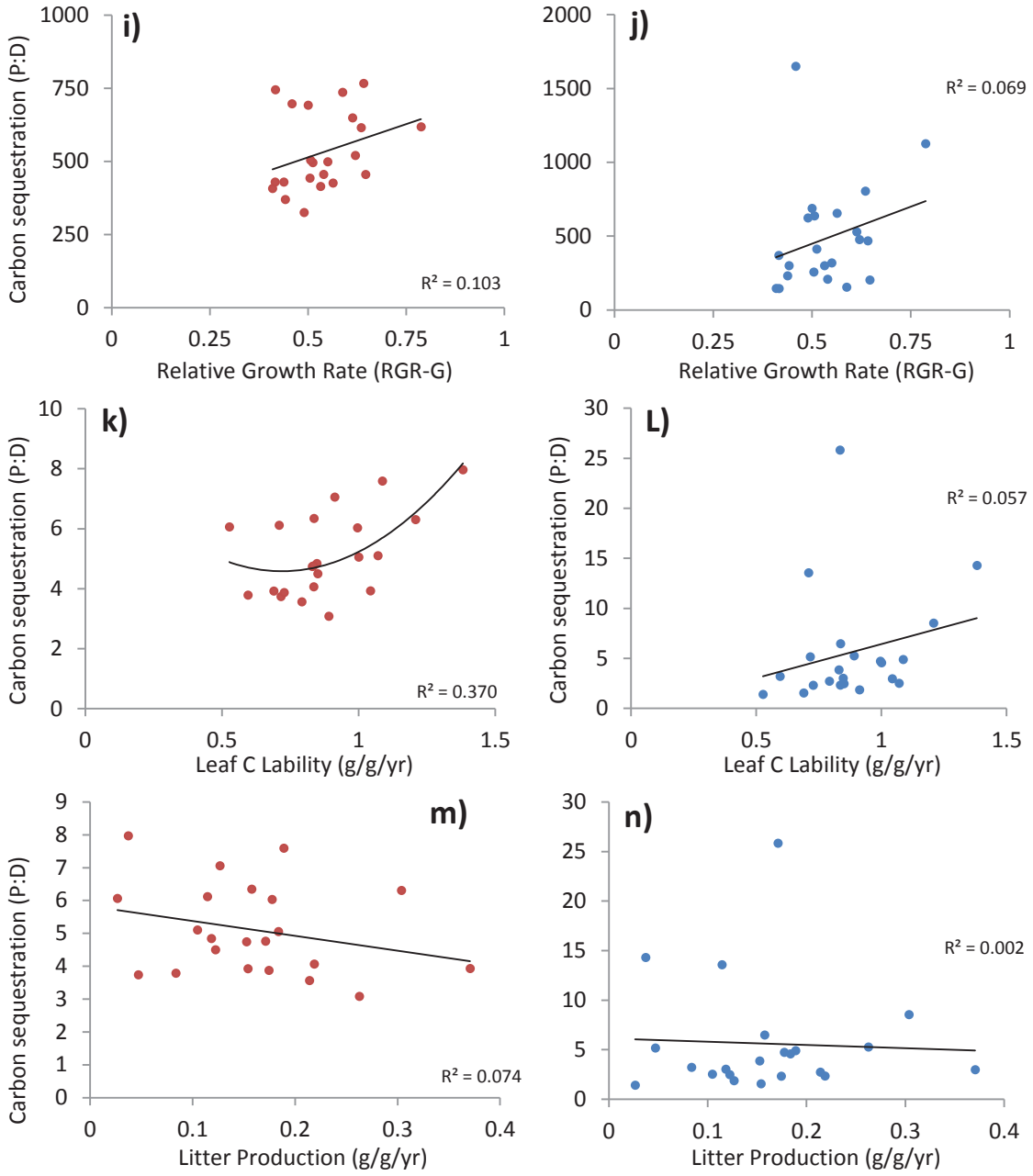


Figure 2 continued: Litter decomposition on common soil is displayed in the left column (Red dots), with litter decomposition on home site soils displayed in right column (Blue dots).

0.10, respectively); however these relationship were much weaker when considering decomposition on home site soil (R^2 = 0.07, 0.39, and 0.07, respectively).

Leaf C lability (i.e. leaf C rate of turnover) showed a curvilinear relationship with rates of C sequestration, showing greater rates of C sequestration in leafs with low C lability than expected relative to higher rates of C lability (Figure 2; k). However, again, this relationship was not evident when considering decomposition on home site soil (Figure 2; L). There was no relationship between litter production and rates of C sequestration when litter was decomposed on both the common soil and home site soils,

though results were even more sporadic when decomposition occurred on the home site soil (Figure 2; m & n).

Rate of Productivity, Litter Quality, and C Sequestration

The positive correlation between total litter decomposition and productivity per tiller and per plant indicates that higher-producing tillers and plants have greater total decomposition. However, as mentioned above this increase in total decomposition is not necessarily due to an increase in the rate of decomposition, but rather due to increased litter production associated with higher productivity. From this it appears that the rate of productivity has little influence on the rate of litter decomposition.

When considering just the influence of litter quality on decomposition, i.e. decomposition on the common soil, rates of decomposition appear to be slightly greater in tussocks with greater rates of productivity. However, any small increases in decomposition and C loss resulting from this are not great enough to counter the corresponding C gain associated with increase in productivity. As a result, and contrary to the hypothesis of this research, rates of C sequestration are greater in plants with greater rates of productivity compared to plants with lower rates of productivity. This is occurs though greater rates of C fixation in higher producing plants, with the rate of C loss effectively remain constant for all taxa independent of the rate of productivity. This raises the question as to what might be responsible for this relatively slow and constant rate of litter decomposition found for all *Chionochloa* taxa.

The relatively slow and constant rate of litter decomposition between taxa on the common soil, may be due to the chronically low leaf N content found in *Chionochloa* tussocks in this study and in others (Craine and Lee, 2003). Another explanation for the slow rate of decomposition is the relatively high fibre and lignin content in *Chionochloa* leaves. Whilst there was variation in litter quality, only lignin (which was related to phylogeny, as opposed to growth rate and environment) was found to influence decomposition.

The nonlinear relationship between C lability and rate C sequestration on the common soil, showed taxa with low C lability to be sequestering C at a greater rate than would be expected relative to taxa with higher C lability. This is most likely due to the strong negative correlations between leaf C lability, and lignin and ADF, where increased lignin and ADF result in a tougher litter. This is in support of other findings, where increased leaf longevity (i.e. how long a leaf persists on plant) has been

associated with increased leaf toughness (Wright and Cannon, 2001; Osunkoya *et al.*, 2008; Kitajima *et al.*, 2012) and reduced rates of decomposition (Cornelissen and Thompson, 1997) . However, this study expands on this showing that low leaf C lability can also result in increased rates of C sequestration.

Despite large differences between *Chionochloa* taxa in environment and rate of productivity, there was little variation in litter quality, as confirmed by decomposition on the common soil. This suggests that the environment and growth rate are not likely to significantly influence rates of C sequestration through influences on litter quality. In addition, for this group of closely related taxa, the strongest determinant of litter decomposition, lignin, was found to be correlated with phylogeny. From this it can be could concluded that changes in phylogeny and genotype are more likely to increase variation in litter quality, and hence influence C sequestration, than changes in environment. This is supported by Bradley and Pregitzer (2007) who also suggest that changes in plant communities, and hence phylogeny, are more likely to affect C sequestration than changes in climate.

Soil Characteristics and C Sequestration

Whilst high-producing plants may sequester C at a greater rate when soil characteristics are standardized, this is not the case when decomposition occurs on different soil types, as occurs in the field for *Chionochloa*. When decomposed on the home-site soil, the relationship between productivity and C sequestration was weak. This is likely due in some part to variation between sites in water content, soil characteristics, and microbial biomass and biodiversity. These results are similar to those of other studies (Vesterdal, 1999; Prescott *et al.*, 2000; Prescott, 2010), where soil and environmental characters were found to have a greater and overriding influence over litter quality in decomposition. In support of this, here soil characteristics were not only found to be better predictors of litter decomposition than productivity, but also better predictors of C sequestration than productivity.

This is shown by the strong correlation between the rate of C sequestration and soil water content, indicated by soil water holding capacity (WHC) (Figure 3a). This is attributed to reduced rates of decomposition in soils with greater water content, due to the production of anoxic conditions for microorganisms. Similarly, reduced rates of microbial activity were generally found to correlated with increased rates of C sequestration (Figure 3b), though this relationship was not as strong ($R^2 = 0.24$). This is

also supported by the negative correlation between microbial activity (indicated by soil C decomposition) and soil water content found in *Chapter 4*.

Whilst there are difficulties in applying laboratory results to natural ecosystems (Eller *et al.*, 2005), decomposition of litter on home-site soils is likely to be more representative of actual *in-situ* field decomposition compared to common soil decomposition, as it accounts for the differences that occur between taxa in soil moisture, soil microbial biomass and diversity, and home-site soil characters. When considering this, low-producing plants do have the potential to sequester C at rates greater than high-producing plants; however this is not related to their litter production, but rather due to the dominating influence of soil characteristics on litter decomposition. For example, some of the lowest producing and slowest grow taxa had the highest rates of C sequestration out of all taxa when decomposition occurred on home-site soils (Figure 2).

As temperature and water content were controlled in this experiment, it could be expected that P:D ratios could actually be larger for a number of low producing taxa in field conditions, where temperatures may be considerably lower and soil water content may be higher. This is supported by the fact that a number of home site soils were saturated above their calculated soil WHC when collected from the field in summer. This suggests that some *Chionochloa* taxa may inhabit environments that are poorly drained, possibly with excess water all year round. Furthermore, according to the results of this research, decomposition at these wet sites is likely to be further reduced, as well as at higher altitude sites where temperatures are lower and sites under snow cover for a longer period of the year.

These results are in support of other studies on C sequestration in indigenous *Chionochloa* tussock grassland. Tate (1992) found increased soil C storage for a number of indigenous tussocks, including *Chionochloa*, to occur at sites that were wetter, cooler, and at higher altitudes, with much lower soil C storage for tussocks occurring at semi-arid sites with dry soils. Similarly, Tate *et al.* (2000) attributed enhanced C sequestration in *Chionochloa* tussock grassland, relative to directly adjacent *Nothofagus* beech forest, to greater soil water retention occurring in the tussock grassland. It was suggested recalcitrant soil C was linked to soil pore-size distribution and percentage of water fill space, where greater soil water retention resulted anaerobic conditions for microorganisms, and the build up of recalcitrant organic C in the soil.

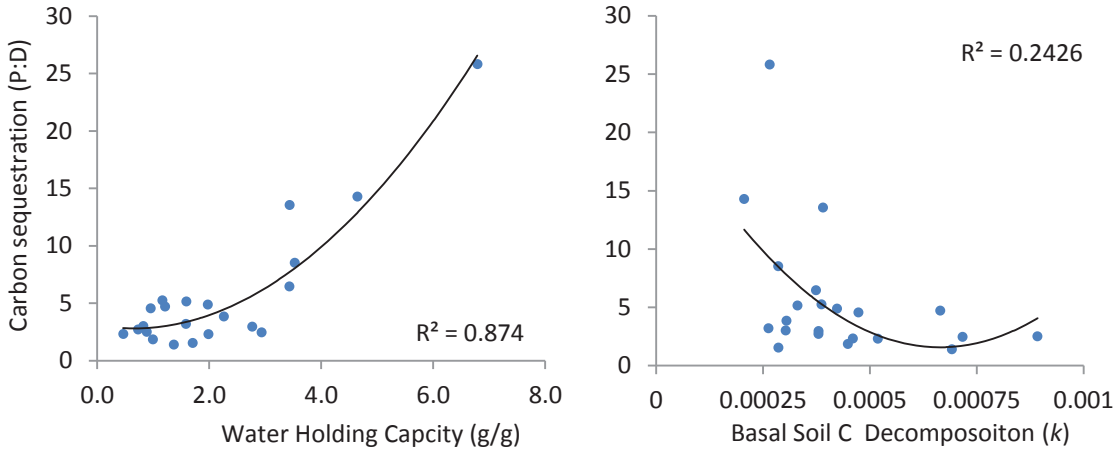


Figure 3: Relationships between rates of C sequestration and, a) soil water holding capacity, and b) soil C decomposition, indicating soil microbial activity.

Greater soil water retention found by Tate *et al.* (2000) in the tussock grassland was attributed to lower transpiration rates, greater snow cover in winter, greater snow melt in spring and summer, and more precipitation reaching the soil due to relatively low amounts of canopy evaporation. Thus, soil drainage may be a better predictor of recalcitrant soil C accumulation than other environmental measures, particularly if litter quality is relatively constant.

C Sequestration in Chionochloa *Grassland*

Chionochloa grasslands have been found to have relatively high rates of soil C sequestration, and are considered to store more C annually than other grasslands according to global average estimates (Tate *et al.*, 1995). Soil C storage in *Chionochloa* tussock grassland has also been shown to be greater than that of adjacent mature *Nothofagus* beech forest (Ross *et al.*, 1996; Tate *et al.*, 2000), with markedly slower soil C turnover times for the grassland (50 years) compared to the forest (27 years), despite almost identical climatic conditions. Greater C sequestration in the *Chionochloa* tussock grassland was attributed to greater annual net primary productivity, greater root productivity, and reduced soil decomposition as a result of greater water retention in the tussock grassland soils.

In addition, the annual P:D ratios reported in this research may be underestimated, as it is likely the laboratory decomposition here is equivalent to much more than one year of field decomposition. Taylor *et al.* (1989) found that mass loss of varying types of litter incubated for 4 months at a constant 26˚C was equivalent to 1.5 – 2 years of mass loss in the field. This suggests the approximately 5 months of laboratory

incubation at 25˚C in this research could be equivalent to a much longer decomposition time in the field, possibly 2-3 years, which would further increase the *Chionochloa* P:D ratios found here. Thus, *Chionochloa* tussock grasslands have the potential to sequester large quantities of C due to relatively high productivity, low-quality litter, wet cold montane environments, along with potentially high soil water retention, and consequently reduced levels of decomposition.

Other research has also found grasslands, particularly undisturbed indigenous grasslands, to have the potential for large amounts of soil C sequestration, perhaps being almost as important as forests (Minami *et al.*, 1993; Scurlock and Hall, 1998; Ross *et al.*, 2000; Tate *et al.*, 2000; Conant, 2010). Whilst the majority of C sequestration in forests occurs through temporary storage in woody somatic tissue, C sequestered in grasslands is stored in the soil through leaf litter fall, as no woody tissue is produced (Krna and Rapson, 2013). The long term storage of C sequestered in the soils of indigenous grasslands is thus dependent on these soils and ecosystems remaining intact, placing greater importance on the protection of these grasslands, supporting the claim by Mark (2012).

Climate Change and C Sequestration

Although climate change is likely to increase productivity through an increase in global temperatures, this increase in temperature will also increase the rate of decomposition in both plant litter and organic soil C (Kirschbaum, 2000; Davidson and Janssens, 2006). If climate change is to influence plant litter quality as suggested (Melillo *et al.*, 1993), the results presented here suggest climate change is unlikely to influence litter quality through the production of a more recalcitrant litter, but rather though changes in litter quality associated with shifts in ecosystem assembly and phylogeny (Bradley and Pregitzer, 2007).

Krna (2015), using an altitudinal gradient as a proxy for climate change, suggests that increases in temperature are likely to increase C sequestration in New Zealand's indigenous tussock grasslands, due to a greater increase in productivity relative to decomposition. However, the above study does not take into account changes in precipitation associated with climate change and its influence on decomposition and C sequestration. Increased precipitation leading to wetter soils could result in reduced decomposition and increases in C sequestration, whilst reductions in precipitation leading to drying out of soils could not only result in increased rates of litter

decomposition, but also the release of organic C currently stored in the soil (Tate *et al.*, 2000). However, the responses of decomposition to climate change mentioned above would need to be measured against the corresponding response of productivity to determine if net C sequestration or net C loss were to occur.

Limitations and Future Research

The advantages of laboratory decomposition include the control of variables not possible in a field decomposition experiment, such as constant temperature and water content. Measurement of decomposition in the laboratory through CO_2 trapping is likely to be more accurate than traditional mass-loss measurements using mesh bags in the field, as CO_2 trapping allows the detection of actual litter C release, whilst mesh bags only account for mass loss of C from the bag, and not C release as CO_2. In field decomposition, a small mesh size can also stop macro-organisms from accessing litter (Bradford *et al.*, 2002), whilst alternatively too large a mesh size can result in intact litter falling out of the bag.

For accurate C budgeting and research into the influence of environment on litter decomposition, *in situ* CO_2 trapping as used by Tate *et al.* (1993) would give a more accurate account of litter decomposition in the field over time. However this would be labour intensive and costly, as litter decomposition needs to be measured over a sufficient duration, in the case of *Chionochloa*, over years.

This research only takes into account above ground productivity, decomposition, and C sequestration. Differences in shoot-to-root ratios occurring between plants, caused by differences in growth strategies and environmental conditions (Wilson, 1988), may result in differences in rates of above- and below-ground C sequestration between taxa. The shoot-to-root ratio has been reported to be negatively correlated with temperature (Wilson, 1988), implying higher-altitude tussocks in colder climates may allocated more biomass below-ground, and consequently sequester a greater relative portion of C below-ground compared to high-producing plants. Thus research into below ground allocation of C and C sequestration would be further beneficial in predicting C sequestration in tussock grasslands.

Conclusions and Implications

Whilst plant growth rate has been linked to litter quality both here and in other studies, this did not translate to a strong relationship between tissue growth rate and

litter decomposition. When considering decomposition on the common soil, any small increases in decomposition associated with increased growth rate were small in comparison to the corresponding increase in productivity. Thus, plants with higher growth rates were found to be sequestering C at a greater rate than plants with slower growth rates. However, when decomposition occurred on home site soils, this relationship was not apparent; with soil water content strongly correlated with reduced rates of decomposition and increased C sequestration.

In the genus *Chionochloa* there was no great difference between the closely related taxa in litter quality, as measured by decomposition on the common soil. This was despite large differences occurring between taxa in environmental and climatic conditions, as well as differences in relative growth rates. From this it is concluded that any large differences in litter quality are thus more likely to result from differences in phylogeny and functional group than environmental and climatic conditions.

In the genus *Chionochloa*, relatively high-productivity rates, poor quality litter, wet and cool montane environments, and the occurrence of wet and poorly drained soils for a number of taxa, suggest that *Chionochloa* grasslands may be significant C sinks. This highlights the need for the protection and enhancement of New Zealand's indigenous grasslands and peat lands, not only to allow continued C sequestration, but also to prevent loss of soil C stored in these environments. Climate change, particularly change in precipitation regimes, could result in increased C sequestration if grasslands become wetter and cooler, or decreased C sequestration if grasslands become drier and warmer.

Overall, low-producing plants have the potential to sequester C at a greater rate than high-producing plants; however, this is determined by the influence of environmental conditions on decomposition, rather than influence of litter quality on decomposition.

References

Bradford, M. A., Tordoff, G. M., Eggers, T., Jones, T. H. & Newington, J. E. 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos,* 99**,** 317-323.

Bradley, K. L. & Pregitzer, K. S. 2007. Ecosystem assembly and terrestrial carbon balance under elevated CO 2. *Trends in Ecology & Evolution,* 22**,** 538-547.

Coley, P. D., Bryant, J. P. & Chapin, F. S. 1985. Resource availability and plant antiherbivore defense. *Science,* 230**,** 895-899.

Conant, R. T. 2010. *Challenges and opportunities for carbon sequestration in grassland systems,* FAO.

Cornelissen, J. & Thompson, K. 1997. Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist,* 135**,** 109-114.

Cornelissen, J. H. C., Quested, H. M., Gwynn-Jones, D., Van Logtestijn, R. S. P., De Beus, M. a. H., Kondratchuk, A., Callaghan, T. V. & Aerts, R. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology,* 18**,** 779-786.

Craine, J. & Lee, W. 2003. Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand. *Oecologia,* 134**,** 471-478.

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

Eller, G., Krüger, M. & Frenzel, P. 2005. Comparing field and microcosm experiments: a case study on methano-and methylo-trophic bacteria in paddy soil. *FEMS microbiology ecology,* 51**,** 279-291.

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

Kitajima, K., Llorens, A. M., Stefanescu, C., Timchenko, M. V., Lucas, P. W. & Wright, S. J. 2012. How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. *New Phytologist,* 195**,** 640-652.

Krna, M. A. 2015. *Productivity, decomposition and carbon sequestration of Chionochloa species across altitudinal gradients in montane tussock grasslands of New Zealand.* PhD, Massey Univeristy, New Zealand.

Krna, M. A. & Rapson, G. L. 2013. Clarifying 'carbon sequestration'. *Carbon Management,* 4**,** 309-322.

Mark, A. F. 2012. Recent progress with the conservation and protection of temperate indigenous grasslands in New Zealand. *Parks,* 18**,** 37.

Melillo, J. M., Mcguire, A. D., Kicklighter, D. W., Moore, B., Vorosmarty, C. J. & Schloss, A. L. 1993. Global climate change and terrestrial net primary production. *Nature,* 363**,** 234-240.

Minami, K., Goudriaan, J., Lantinga, E. & Kimura, T. 1993. Significance of grasslands in emission and absorption of greenhouse gases.

Osunkoya, O. O., Daud, S. D. & Wimmer, F. L. 2008. Longevity, lignin content and construction cost of the assimilatory organs of Nepenthes species. *Annals of Botany,* 102**,** 845-853.

Poorter, H. & Bergkotte, M. 1992. Chemical composition of 24 wild species differing in relative growth rate. *Plant, Cell & Environment,* 15**,** 221-229.

Poorter, H., Remkes, C. & Lambers, H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology,* 94**,** 621-627.

Prescott, C., Zabek, L., Staley, C. & Kabzems, R. 2000. Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures. *Canadian Journal of Forest Research,* 30**,** 1742-1750.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry,* 101**,** 133-149.

Ross, D., Tate, K. & Feltham, C. 1996. Microbial biomass, and C and N mineralization, in litter and mineral soil of adjacent montane ecosystems in a southern beech (Nothofagus) forest and a tussock grassland. *Soil Biology and Biochemistry,* 28**,** 1613-1620.

Ross, D., Tate, K., Newton, P., Wilde, R. & Clark, H. 2000. Carbon and nitrogen pools and mineralization in a grassland gley soil under elevated carbon dioxide at a natural CO2 spring. *Global Change Biology,* 6**,** 779-790.

Scurlock, J. & Hall, D. 1998. The global carbon sink: a grassland perspective. *Global Change Biology,* 4**,** 229-233.

Strickland, M. S., Lauber, C., Fierer, N. & Bradford, M. A. 2009. Testing the functional significance of microbial community composition. *Ecology,* 90**,** 441-451.

Tate, K. 1992. Assessment, based on a climosequence of soils in tussock grasslands, of soil carbon storage and release in response to global warming. *Journal of Soil Science,* 43**,** 697-707.

Tate, K., Parshotam, A. & Ross, D. 1995. Soil carbon storage and turnover in temperate forests and grasslands-a New Zealand perspective. *Journal of biogeography***,** 695-700.

Tate, K., Ross, D., O'brien, B. & Kelliher, F. 1993. Carbon storage and turnover, and respiratory activity, in the litter and soil of an old-growth southern beech (Nothofagus) forest. *Soil Biology and Biochemistry,* 25**,** 1601-1612.

Tate, K., Scott, N., Ross, D., Parshotam, A. & Claydon, J. 2000. Plant effects on soil carbon storage and turnover in a montane beech (Nothofagus) forest and adjacent tussock grassland in New Zealand. *Soil Research,* 38**,** 685-697.

Taylor, B. R., Parkinson, D. & Parsons, W. F. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology,* 70**,** 97-104.

Vesterdal, L. 1999. Influence of soil type on mass loss and nutrient release from decomposing foliage litter of beech and Norway spruce. *Canadian Journal of Forest Research,* 29**,** 95-105.

Wilson, J. B. 1988. A review of evidence on the control of shoot: root ratio, in relation to models. *Annals of Botany,* 61**,** 433-449.

Wright, I. & Cannon, K. 2001. Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora. *Functional Ecology,* 15**,** 351-359.